THE BIOPHYSICAL PROPERTIES OF PLANTAR CALLUS AND THE RELATIONSHIP BETWEEN PRESSURE AND CALLUS DEVELOPMENT AND REGRESSION

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‘Next time someone complains that you have made a mistake, tell him that may be a good thing. Because without imperfection, neither you nor I would exist.’

Stephen W. Hawking, 2010.

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Acknowledgements

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Preface: Funding and my role as a PhD student

My PhD was sponsored by Reckitt Benckiser who own Scholl foot care brand. The sponsorship is part of a prior research contract concerned with better understanding of corns, callus and heel fissures, and the efficacy of Scholl treatments for these skin conditions. Scholl involvement in the PhD has been at the level of conceiving the general topic of the PhD, i.e. the relationship between pressure and callus, and the need to better understand the properties of callus.

During my PhD I have been involved in all aspects of all of the studies contained in the thesis. The work detailed in Chapters 3, 4 and 7 were undertaken as part of larger projects and involved other researchers. My role in the instrument reliability study (Chapter 3) was protocol development, data collection, processing and analysis. My role for skin characterisation study (Chapter 4) was assisting in protocol development, some data collection, and all data processing and analysis. Some of the data was collected by a HPC registered podiatrist to ensure effective delivery of the contract research work by the research group. However, I processed and analysed everything used in this thesis. I undertook all aspects of the work described to design and implement the loading device (Chapter 5), supported in the device design and production by Mr Paul Busby (he completed CAD diagrams and advised on technical aspects and manufacturing). I am responsible for all aspects of the skin loading pilot study (Chapter 6). In the final study which concerned pressure reduction using orthotic insoles (Chapter 7), I worked with Dr Daniel Parker who designed the insoles used. I was involved in protocol design and collected all of the skin data and some of the in-shoe pressure data. I also processed and analysed all data for this chapter.

At the time of writing, work undertaken for the reliability study (Chapter 3) and skin characterisation study (Chapter 4) have been published (Hashmi et al., 2015a, Hashmi et al., 2015b). The publications include the callus work detailed in these chapters but also work on corns and heel fissures which were included in the Reckitt Benckiser research contract. In addition two further papers have been submitted for publication which detail clinical trials concerning the treatment of callus (Hashmi et al., under review) and heel fissures (Hashmi et al., under review) comparing standard podiatric treatment with off-the-shelf home treatments. This work extended from the characterisation study.

Abstract

Plantar calluses are common skin lesions which often require professional treatment by podiatrists. They commonly present under prominent areas such as the metatarsal heads and can cause significant discomfort during ambulation. Furthermore, they are one of the known risk factors for ulceration in individuals with systemic conditions such as diabetes mellitus. Anecdotal evidence suggests that mechanical factors contribute to calluses and there are numerous studies linking callus to increased peak plantar pressure. However, whether callus is a result of increased pressure or vice versa remains unclear. Skin on other areas of the body has been shown to respond to external loading forces, but no research has specifically investigated the relationship between callus and pressure.

A critical review of the literature explored the methods used for skin profiling through biophysical skin measurement. Skin hydration, distensibility and topography were revealed to be useful measurement parameters to characterise plantar skin and for this study, three devices were chosen for testing these parameters. However, as these devices have not received much attention for testing plantar callus in previous research, the first study investigated their repeatability on normal and callused plantar skin. These devices were shown to provide adequate measures of skin properties so they were then used in a larger scale study investigating the biophysical characteristics of normal and callused plantar skin. It was found that callused skin was less hydrated, less distensible, and rougher in texture than normal plantar skin.

Work was then undertaken to develop a device that could apply loads to plantar skin in a safe manner so that the skin's response to external loading could be assessed. A subsequent pilot study was conducted to assess whether normal plantar skin in individuals prone to callus would display callus-like skin changes as a result of increased vertical pressure applied by the skin loading device over a minimum period of six weeks. After the skin loading period, no effect could be observed in normal plantar skin properties. The reasons for this are explored in depth. A study was then undertaken in order to assess the effect of plantar pressure reduction in callused skin over a period of 12 weeks. Pressure reduction was achieved by using customised insoles worn by the study participants. No change in callused skin properties was observed and the reasons for this are explored in depth. These studies provide a strong starting point in understanding the link between pressure and callus and provide a foundation for further research.

Chapter 1: Introduction

Plantar calluses are hyperkeratotic skin lesions which commonly present under areas of pressure such as the metatarsal heads (Singh, 1996). They are extremely common, reportedly affecting up to 68% of individuals (White and Mulley, 1989). The lesions are around two to three times thicker than normal plantar skin (Thomas et al., 1985) and can be very painful (Helfand, 2003) which can lead to mobility problems in elderly individuals (Menz and Lord, 2001). In individuals suffering from diabetes, calluses can also increase the risk of ulceration (Murray et al., 1996) which subsequently decreases quality of life (Firth et al., 2011).

It is believed that external insults, such as poorly fitting shoes, contribute to callus development (Singh, 1996) and there is sufficient evidence to link calluses to increased peak pressures under the forefoot (Menz et al., 2007). However little is known about this relationship and whether callus causes increased pressures or vice versa. While hyperkeratotic-like skin responses have been observed as a result of applying loads to human skin (Rubin, 1949), no studies have been conducted on the foot which specifically show increased loading to be a precursor to callus development. This area of study is important because until more is known about the relationship between callus and loading, preventative strategies cannot progress.

In order to investigate the relationship between callus and loading, researchers must first know about the properties of normal and callused skin so the skin's response to loading can be quantified. However, knowledge in this area is also scant. Therefore, the aims of this thesis are to (1) investigate the biophysical properties of normal and callused skin; and (2) quantify the skin's response to increases and decreases in external loads.

The work undertaken for this thesis satisfies the criteria for a PhD as outlined in the Descriptor For A Higher Education Qualification At Level 8: Doctoral Degree, published as part of the framework for higher education qualifications in England, Wales and Northern Ireland (QAA, 2008, p.23). The first section of this thesis (Chapter 2) critically reviews the available literature on plantar callus to identify more precisely the research gaps. The review also examines different biophysical devices available that are designed to measure skin properties and will discuss potentially useful devices to study important skin parameters. Work undertaken for this chapter demonstrates the acquisition of a substantial knowledge base essential for doctoral candidates. Chapter 3 describes a study which aimed to assess the reliability of biophysical instruments for use on callused and normal skin

types, and demonstrates research techniques and knowledge of academic enquiry. Chapter 4 presents a larger scale project to characterise normal and callused skin using these devices, adding new knowledge through an original research project. Chapter 5 describes the development of a device which was designed to apply external loads to the foot. Chapter 6 details a pilot study which assessed whether it was possible to generate a callus-like skin response through load application using the device developed in Chapter 5. Chapter 7 describes a study that was conducted to investigate any changes in callus properties following reduction of plantar pressures using orthotic insoles. Chapters 5–7 are examples of an ability to design, implement, and evaluate research, making informed judgements on research issues, problem solving and tying together skills and attributes gained during the earlier stages of the PhD. Finally, Chapter 8 discusses the work in this thesis as a whole and how it contributes to future research, and clinical practice; and thus the contribution to knowledge made through this PhD thesis.

Chapter 2: Background and critical review of the literature

Introduction

The aims of this critical review of the literature are to provide an overview of plantar callus including its prevalence, consequences and treatments. Literature on skin anatomy, callus histology and biochemistry; and vertical and shear pressures will also be reviewed and the research gaps highlighted. The second part of the review will deal with different devices that are designed to measure the biophysical properties of the skin and will consider the devices that might be most helpful in plantar skin research. The gaps in the literature relating to these devices will also be discussed.

Part A. Plantar callus

A.1 Introduction

Plantar callus and corns are hyperkeratotic lesions which commonly present under areas of pressure such as the metatarsal heads. Hyperkeratosis refers to increased thickening of the stratum corneum, and it has been suggested that in normal skin this is a physiological mechanism to protect underlying soft tissues from mechanical stress (Thomas et al., 1985, Singh, 1996), but in plantar skin these lesions can become symptomatic and as a result cause problems. Calluses can be differentiated from corns as they normally occupy a greater surface area and are diffuse, while corns are well defined and circular with a central visible core (Singh, 1996, Freeman, 2002). The most common areas for callus development on the forefoot include the first, second and fifth metatarsal heads respectively but 'roll-off' calluses are also common on the medial aspect of the first metatarsal head and interphalangeal joint (Woodburn and Helliwell, 1996, Potter and Potter, 2000a, Springett et al., 2003, Menz et al., 2007, Spink et al., 2009).

Corns and callus are a common problem and historically presented to NHS podiatry (Farndon et al., 2009), accounting for up to 68% of foot complaints reported in the literature (Figure A.1) (Ebrahim et al., 1981, Black and Hale, 1987, White and Mulley, 1989, Robbins et al., 1996, Helfand et al., 1998, Dunn, 2004, Spink et al., 2009). The highest rates have been observed in community (White and Mulley, 1989, Dunn, 2004, Spink et al., 2009) and workplace surveys (Springett et al., 2003), and those attending foot screening programmes (Robbins et al., 1996); while the lowest were observed in hospital patients or those attending hospital clinics (Ebrahim et al., 1981, Black and Hale, 1987)

and those recruited in retirement homes (Helfand, 2003). It could be hypothesised given the potential link between the development of these lesions and pressure that there is a relation to levels of activity. Therefore the populations investigated in hospitals, clinics and care homes may be less active than those populations in community and workplace settings.

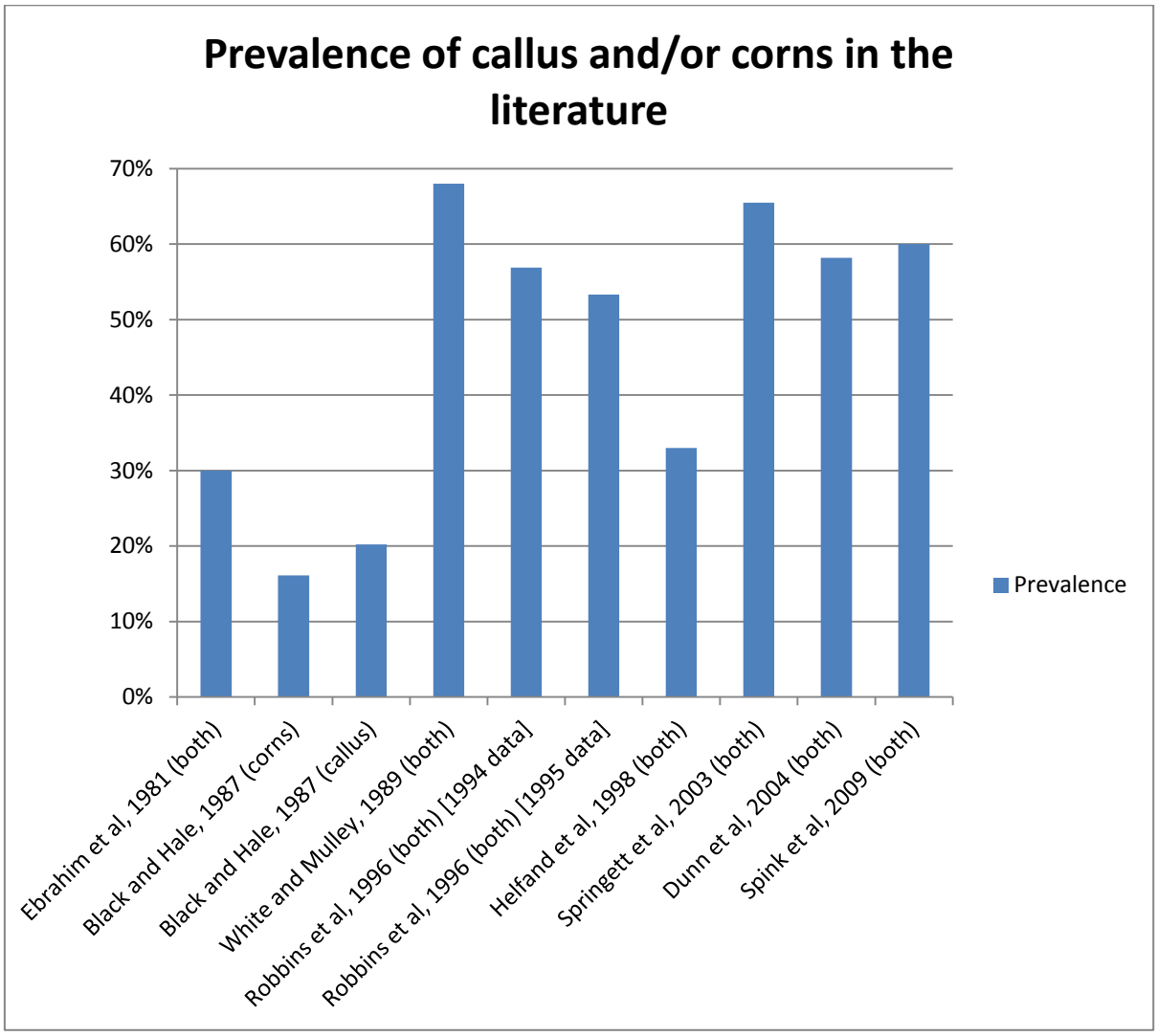


Figure A.1 – Prevalence of corns and callus reported in literature

Most of the literature into prevalence of these lesions has focussed on the elderly population (Ebrahim et al., 1981, Black and Hale, 1987, White and Mulley, 1989, Helfand et al., 1998, Dunn, 2004, Spink et al., 2009), presumably due to an increased prevalence of foot problems with age. However other groups such as the working population, athletes, and the homeless population also have a high prevalence (Robbins et al., 1996, Adams, 2002, Springett et al., 2003). Gender differences have been reported in several studies

(Figure A.2) with females showing a higher prevalence of corns and callus, as well as general non-traumatic foot problems (Gorter et al., 2000). The reasons for this could stem from choice of footwear, for example studies have shown that wearing high heels increases forefoot peak pressures (Snow et al., 1992, Mandato and Nester, 1999, Yung-Hui and Wei-Hsien, 2005) and shifts pressure from the lateral to the medial forefoot (Snow et al., 1992, Yung-Hui and Wei-Hsien, 2005).

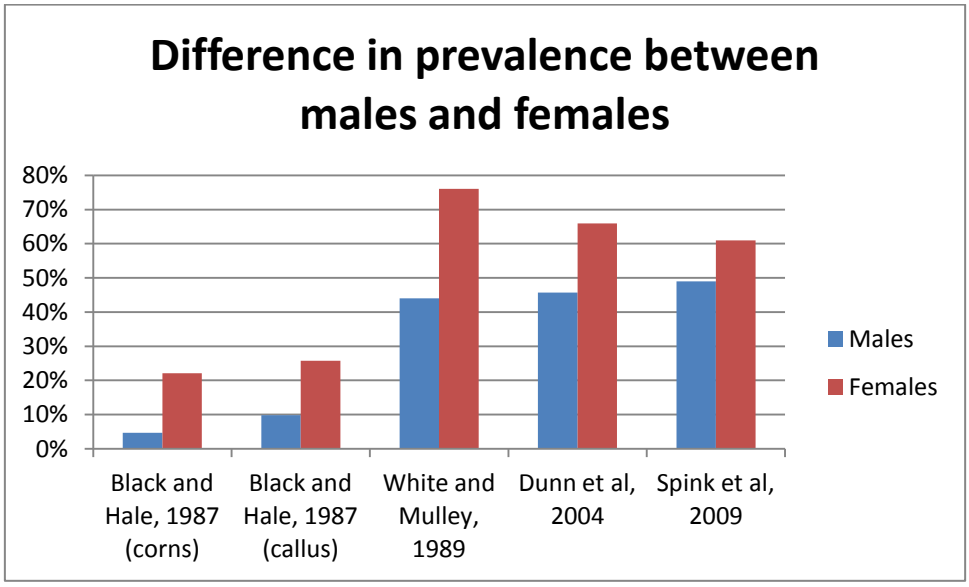


Figure A.2 – Gender differences reported in the literature

A.2 Consequences of callus and corns

The primary consequence of plantar hyperkeratoses in otherwise healthy individuals have been listed as pain, burning, and tenderness during gait (Helfand, 2003) which is troublesome as the lesions are located at weight bearing areas. Foot pain has been shown to have a whole host of consequences of its own; a negative impact on balance, walking, and climbing stairs (Menz and Lord, 2001), and it increases the risk of falls (Benvenuti et al., 1995, Mickle et al., 2010). Aside from pain and associated disability, the presence of hyperkeratotic lesions in individuals with diabetes can greatly increase the risk of ulceration (Murray et al., 1996, Reiber et al., 1999, Sage et al., 2001, Nishide et al., 2009). In turn, the presence of foot ulcers, particularly non-healing ulcers, in patients with rheumatoid arthritis and diabetes contributes to a decreased quality of life that can be attributed to decreased mobility, which affects social function and leisure activities (Ribu et al., 2008, Firth et al., 2011). While the above studies show that pain from calluses may affect mobility, there is a lack of qualitative literature on patient perspectives of living with

callus and how these lesions affect their quality of life. These types of studies would be useful and may help to increase the volume of research conducted in the area.

A.3 Overview of interventions for callus and corns

The routine treatment for callus and corns is sharp or scalpel debridement. The profession that usually carries out this skilled procedure is that of podiatry. Aside from possible effects on plantar peak pressures (discussed in depth later), treatment studies have shown that debridement of callus may significantly reduce pain (Redmond et al., 1999, Woodburn et al., 2000, Balanowski and Flynn, 2005) and improve walking function (Balanowski and Flynn, 2005) in elderly adults and patients with rheumatoid arthritis. Only two randomised controlled trials have been conducted (Davys et al., 2005, Landorf et al., 2013) using real and sham treatments. While both the debridement and sham groups in each study showed improvements in pain scores, no significant improvement in pain reduction between treatment groups was found. Furthermore, Davys et al. (2005) found no significant difference in walking function between the two groups. Interestingly they found a non-significant reduction in pressure in the debridement group and an increase in pressure in the sham group compared to baseline immediately post-intervention. This could potentially be due to placebo effect. Studies assessing the placebo effect have reported that suggestion that an active treatment has been given can produce a placebo effect (reviewed by Price et al., 2008). The 9% increase in pressure found in the sham group could have been due to an expectation of improvement due to treatment, possibly resulting in increased loading during gait as a result of being less tentative with weight bearing over a painful callus. The small (< 5%) increase in contact area in the sham group may illustrate this. There was also an increase in walking speed in both groups (9% and 12.3% for scalpel debridement and sham groups respectively) which could have led to higher peak pressure values.

Siddle et al. (2013) conducted a randomised controlled trial that compared the efficacy of using scalpel debridement alongside a combined therapeutic approach to a control group receiving just a combined therapeutic approach over an 18 month period. They found that while pain was significantly reduced in both groups, there was no significant difference between the groups which suggests that scalpel debridement offers no benefit over combined therapy alone in individuals with rheumatoid arthritis. Again, there was no significant improvement in walking function. The authors suggest that

debridement should only be used in emergency cases where tissue integrity is at a serious risk (Siddle et al., 2013).

Aside from scalpel debridement, there are alternative approaches to callus and corn treatment. Salicylic acid, another widely used treatment, has been involved in research in corn treatment on healthy subjects (n = 198). The authors (Lang et al., 1994) found salicylic acid to be significantly more effective at removing corn tissue than placebo treatments with 62.4% of corns removed compared with 17.9% ($p < 0.001$). This may be due to the fact that salicylic acid activates plasmin which causes desquamation, and increases levels of plasminogen activators while decreasing inhibitors (Heda and Roberts, 2008). However, far more work focussing on corns is needed to better understand their physiology.

Other less common approaches have also been studied. A study by Colagiuri et al. (1995) on patients with diabetes (n = 9) presenting with plantar callus assessed the effect of a yearlong treatment using a custom made thermal pliable plastic insole worn seven hours per day. Clinical assessment of photographs taken before and after the study, by consensus of the three researchers who were blinded to patient and treatment, suggested that there was a significant improvement in callus grade ($p < 0.02$) compared with patients treated by scalpel debridement (n = 11) where no significant reduction was found. The orthotic patients were checked after 1-3 weeks and then at three monthly intervals as with the debridement group but no details on how the integrity of the orthotic insoles was assessed and whether or not they were routinely replaced are reported. The actual pressure reduction caused by the orthotic was also not measured. The poor level of callus reduction in the debridement group may have highlighted a need for more regular debridement which might have given better results. The limited qualitative measures employed in this study for assessing the skin and the interventions are a major pitfall. The results would have been more credible had quantitative measures of pressure changes and changes in callus lesions been performed. However, despite its drawbacks, this study is useful because it indicates the potential efficacy of orthoses as a treatment modality, but also highlights the need for much better quality research to understand the pathophysiology of callus.

A subsequent study looking at the effects of pressure relieving measures in adolescents with diabetes presenting with plantar callus has been conducted (Duffin et al., 2003). The authors recruited 211 participants for plantar pressure measures. They also assessed pressure reducing qualities in a small subgroup of individuals with callus (n = 17) and without callus (n = 17). They found over both groups that cushioning, orthoses without

cushioning and combined cushioning and orthoses significantly reduced peak plantar pressures ($p = 0.001$, 0.05 and < 0.001 respectively). Out of their sample, they recruited 23 subjects who used custom made orthotic insoles and 67 control subjects who did not use insoles and reassessed the calluses after a year. A significant reduction in in-shoe pressure was found ($p = 0.0003$) in the orthoses group when walking with no intervention but no significant difference was found in the control group. Out of the group that wore insoles over one year, six had callus at the beginning of the trial and in two the callus fully resolved. In the subjects who did not wear insoles, seven had callus at the beginning of the trial, and all seven still had callus after one year. However, no detail is given as to whether individuals were permitted to use foot treatments and no quantitative evaluation of the plantar skin as a whole is presented. Furthermore, no information is given as to whether the insoles were still delivering effective pressure reduction at the follow-up appointment. This brings into question whether the results were due to pressure reduction or other factors such as occlusion of the skin caused by the insole material, or use of topical treatments. While flawed, this study does indicate a possible link between pressure reduction and callus regression.

Khan et al. (1996) conducted a blinded randomised controlled trial of thirty patients comparing marigold tegetes erecta treatment to a placebo and found a significant reduction in callus dimensions and pain in the treatment group ($p < 0.001$). Furthermore, the authors found that applying a protective pad over the marigold preparation was significantly more effective than withholding the protective pad for both pain and callus dimensions ($p < 0.001$).

Akdemir et al. (2011) assessed the efficacy of a topical treatment containing cantharidin, salicylic acid and podophylin (Canthacur CS), which is applied after debridement. The sample included 65 plantar and 7 palmar calluses and corns. The patients were followed up over the period of a year with a maximum of four treatments over this period if necessary. Clinical assessment and patient satisfaction data was collected at each appointment. The authors reported a 100% success rate for all participants over a year with 79.2% of lesions resolved after one treatment, and 91.7%, 98.6% and 100% of calluses resolved after the second, third and fourth treatments respectively. However, no quantitative measures of the lesions were taken, no control group was used, and no randomisation or blinding was employed which makes the credibility of the trial questionable. Barnes and Brocklesby (2011) assessed the effect of using a silicone gel sheet (Cica-Care) on four patients with plantar callus and two with plantar scar tissue.

They reported a reduction in lesion dimensions and pain score in all cases but no quantitative data was presented in the paper to support this. As this employed a case study research design, the authors did not randomise participants into groups or blind participants.

The literature surrounding the treatment of callus is somewhat limited with the most rigorous studies concentrated to scalpel debridement treatment. While other studies have reported callus reduction with less common methods of treatment (Colagiuri et al., 1995, Khan et al., 1996, Akdemir et al., 2011, Barnes and Brocklesby, 2011) only one of these (Khan et al., 1996) provided quantitative measures, namely the dimensions of the callus. All others have relied upon subjective clinical examination which may be insensitive to the true physiological changes (or lack of) created by a treatment. This highlights the need for quantitative measures of callus morphology in order to accurately characterise lesions and thereafter measurement of treatment efficacy. However, aside from measuring dimensions of the lesions, there is a lack of other quantitative measurements of callus properties, which may offer more sensitive measures of changes in callus lesions, but also indicate the factors affecting creation and progression of callus.

A.4 Overview of skin structure

The epidermis, the most superficial of the two skin layers, is composed primarily of keratinocytes which account for around 95% of its cells (Mackie, 2003). Keratinocytes are in a constant state of self-renewal with new cells being produced in deep layers to replace those shed superficially. As the cells migrate superficially, they change from living cells to dead sheets of keratin (Wigley et al., 2008). The anatomy of the epidermis, the dermal-epidermal junction and the features of plantar skin are shown in Tables A.1 – A.3 and Figure A.3.

Table A.1 – Epidermal structure (in the order of deep to superficial)

Layer	Features
Stratum basale	One-cell-thick wall of dividing keratinocytes of a columnar/cuboidal shape which replace those lost superficially (Wigley et al., 2008).
Stratum spinosum	Composed of keratinocytes with a characteristic 'prickly' appearance due to the presence of desmosomes, important structural filaments which aid in cell cohesion, maintaining structure (Young et al., 2006, Wigley et al., 2008).
Stratum granulosum	The layer where keratinisation begins. Within this layer, lamellar granules appear and merge with the cell membrane, and these release glycophospholipids into the intercellular space forming the main constituent of the water permeability barrier (Smith et al., 1982, Potts and Francoeur, 1990, Imokawa et al., 1991, Wigley et al., 2008).
Stratum lucidum	In plantar (and palmar) skin, there is thought to be an additional thin layer of incompletely keratinised cells (Wigley et al., 2008). However, this might also be an artefact of histology specimen processing (Mackie, 2003).
Stratum corneum	Accounts for much of the epidermal thickness (the precise thickness of this layer varies with skin site), and consists of keratin sheets left by dead keratinocytes. The stratum corneum is replaced in around 16 days (Thomas et al., 1985, Wigley et al., 2008).

Table A.2 – Dermal-Epidermal junction and dermis

Structure	Role	
Dermal-epidermal junction (DEJ)	It is composed of types IV, V and VII collagen and functions to anchor the epidermis to the dermis, providing mechanical support and forming a permeable barrier between the two skin layers (Briggaman and Wheeler, 1975, Briggaman, 1982).	
Dermis	The deeper of the two skin layers, the dermis, is composed of collagen and elastic networks and contains nerves, vessels and lymphatics and provides mechanical strength (Wigley et al., 2008).	
	Papilliary layer	Contains finer layers of (type III) collagen and is responsible for providing mechanical support to the epidermis. It connects to the basal epidermal layer through the DEJ via keratin filaments and collagen fibrils. Finger-like rete ridges containing types I and III collagen interdigitate with rete pegs of the epidermis to provide epidermal anchorage (Wigley et al., 2008).
	Reticular layer	Contains thicker type I and III collagen fibres and elastic fibres and gives the skin much of its strength (Wigley et al., 2008).

Table A.3 – Plantar skin characteristics

Feature	Role
Thick epidermis (due to stratum corneum)	Protection of dermis and cubcutaneous tissues (Palastanga and Soames, 2012).
Epidermis and dermis tightly bound together	Well-developed rete ridges prevent horizontal skin displacement, allowing for improved grip (Young et al., 2006).

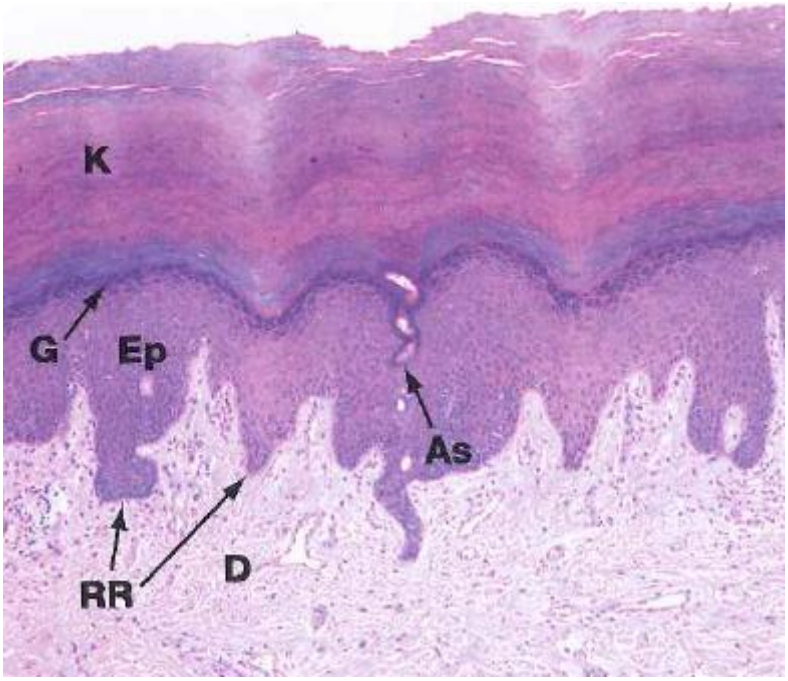


Figure A.3 – Plantar skin histology specimen showing the two layers of skin, the epidermis (Ep) and dermis (D). Within the epidermis, the stratum corneum (K), stratum granulosum (G), sweat ducts (As) and rete ridges (RR) are all visible (Young et al., 2006 p.184).

A.5 Histology and biochemistry of callus development

Plantar skin is known to be anatomically different from skin on other sites of the body (Table A.3; Figure A.3), and has been found to have a particular prominence of the keratin filaments K6, K7, K9, K16 and K17 which relate to the mechanical demands place upon it (Swensson et al., 1998). Histologically, callused skin has been shown to be markedly different from normal plantar skin. Thomas et al. (1985) observed callus to be 2-3 times thicker ($p < 0.001$) than normal plantar stratum corneum (normal stratum corneum = 123 ± 12 cell layers; callused stratum corneum = 349 ± 67 cell layers). The callus corneocytes had a similar surface area to normal plantar corneocytes. However, their volume was increased and their density was decreased. The greater number of cell layers present in callused stratum corneum indicates increased proliferation and the decreased density of the cells and indicates that the cells are not as well differentiated as normal plantar corneocytes in this layer (Thomas et al., 1985). It could be suggested that the increased rate of cell production in callus is a factor in the poorer cell differentiation as they are not given sufficient time to fully mature.

Biochemically, it has long been suspected that increased corneocyte cohesion plays a role in hyperkeratosis (Rubin, 1949). Kim et al. (2010) confirmed this through

immunohistochemistry and found several agents to be increased in anterior heel skin (which displays callus-like properties). Keratin genes K9 and K14 are expressed in greater quantities, as are the proteins involcurin, filaggrin, caspase 14, and CaSR, while PAR2 was found to be decreased (Kim et al., 2010). Levels of specific adhesion proteins (CDSN, DSG1 and DSC1) were also elevated. Corns have been linked to increased plasminogen activator inhibitor levels which decreases the rate of differentiation and desquamation (shedding of the stratum corneum) (Heda and Roberts, 2008). Collectively these increased levels of chemical triggers would likely cause an increased rate of proliferation and increased cell cohesion (Table A.4, Figure A.4) which would directly affect the mechanical properties of the skin and also the skin's barrier function.

Table A.4 – Biochemical triggers in callus and corn development

Feature	Role
Keratin K9	Activated by the gene Wnt5a and is expressed primarily in plantar keratinocytes of epidermal ridges above the stratum basale, and is seen during terminal differentiation (programmed cell death) (Knapp et al., 1986, Swensson et al., 1998, Rinn et al., 2008). It has been suggested that this specific keratin aids in reinforcing plantar skin making it more resistant to mechanical stresses (Swensson et al., 1998).
Keratin K14	Has an integral role in the skin barrier and is mutated in various skin diseases (Engelke et al., 1997, Jensen et al., 2000, Santos et al., 2002).
Protease activated receptor 2 (PAR2)	Responsible for increasing the intracellular concentration of calcium ions and has thus been shown to inhibit lamellar body secretion of lipids, delay epidermal barrier recovery and increase terminal differentiation (Demerjian et al., 2008, Jeong et al., 2008).
Calcium sensing receptor (CaSR)	Important in modulating calcium physiology. In the stratum basale, induces a pathway which causes differentiation resulting in an increased rate of epidermal permeability barrier formation and cell cohesion (by activating E-Cadherin) (Turksen and Troy, 2003, Tu et al., 2008).
Caspase 14	Increases the rate of corneocyte differentiation and terminal differentiation as a response to skin barrier damage (Raymond et al., 2007, Demerjian et al., 2008). It also has a crucial role in degradation of filaggrin and thus the generation of natural moisturising factors (NMFs) (Hoste et al., 2011).
Filaggrin	Profilaggrin is the precursor to filaggrin and is formed in the stratum granulosum as part of the keratohyalin granules but later breaks down into filaggrin towards the stratum corneum. It is a structural protein involved in cohesion of keratin filaments and thus plays a part in the skin barrier. It also contributes to the formation of cornified envelopes (Simon et al., 1996, McGrath and Uitto, 2008, Sandilands et al., 2009, Hoste et al., 2011). It is degraded in the stratum corneum by Caspase 14 into amino acids (Hoste et al., 2011).
Involucrin	Expressed in the suprabasal layers of the epidermis and acts as scaffolding in the wall of the cornified cell envelope (Yaffe et al., 1992, Steinert and Marekov, 1997).
Plasminogen activators	Expressed by keratinocytes at different stages of differentiation and converts plasminogen into plasmin. Plasminogen activators and their inhibitors have a role in terminal differentiation changes in cell morphology, formation of the cornified cell envelope and are distributed in the same regions as involucrin (Chen et al., 1993). They are also involved in regulation of desquamation (Lyons-Giordano and Lazarus, 1995).

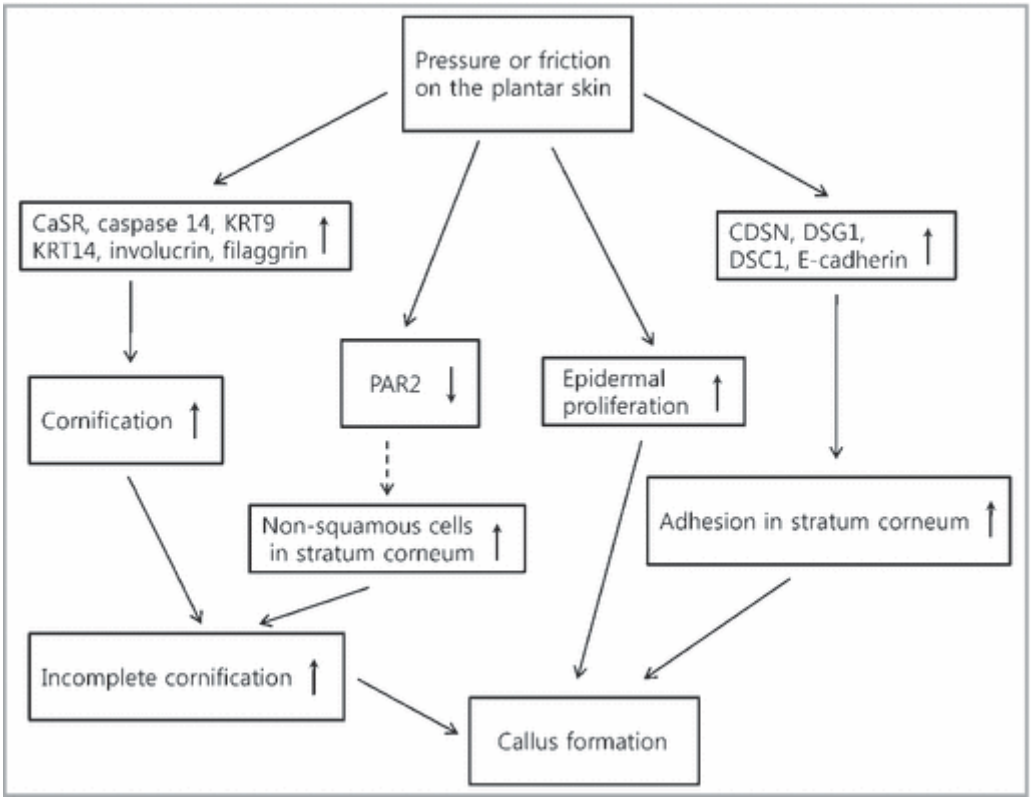


Figure A.4 – Molecular model for callus development (Kim et al., 2010 p.500). CaSR (calcium sensing receptor); KRT (keratin); CDSN, DSG1, DSC1 (adhesion proteins); PAR2 (protease-activated receptor 2).

The above studies can be used to hypothesise what might happen to the barrier properties of callused skin. The skin barrier is provided by lipids which help to prevent water loss from the skin and protect it from external chemical insults (Madison, 2003), thus the skin's barrier function is dependent on its lipid profile. The lipids are expressed during maturation of corneocytes and have several roles. They are situated in the intercellular spaces and regulate the permeation of water to prevent desiccation through their multi-lamellar organisation (which contributes to the skin's water holding and barrier function); as well as assisting in corneocyte cohesion in the stratum corneum (Elias and Friend, 1975, Elias et al., 1981, Smith et al., 1982, Wertz and van den Bergh, 1998, Imokawa et al., 1991, Baroni et al., 2012). The decreased differentiation of callused corneoocytes observed by Thomas et al. (1985), which could in part be due to a decrease in PAR2 (Kim et al., 2010), may alter the lipid profile of the skin. In cases where cells of the stratum corneum hyperproliferate, such as in plantar callus, the cells might not have adequate time to fully differentiate which reflects what was observed in callus by Thomas et al. (1985). Because the cells have not been given time to differentiate properly, this could lead to fragile cornified cell envelopes, which has been observed in soap-induced

dry skin (Harding et al., 2003). In biochemical experiments, these fragile cell envelopes stain positive for involucrin in the superficial aspect of the stratum corneum or in areas where the skin barrier is disrupted (Hirao et al., 2001); this was one of the chemical triggers found in increased quantities in callus-type skin by Kim et al. (2010). Thomas et al. (1985) noted that, at the most superficial aspect of the stratum corneum, there was a higher desquamation rate which might reflect scaling as seen in dry skin conditions as a result of fragile cornified envelopes. The fragile cornified envelopes will have a negative impact on the skin's barrier function (Wickett and Visscher, 2006). This will lead to an increased trans-epidermal water loss (TEWL) and decreased skin hydration as a result (Baroni et al., 2012). Decreased barrier function (and resulting decreased hydration) can also directly result from mechanical insults to the skin (Baroni et al., 2012). If there is a link between callus and external loading, the delivery of the loads to the callused site may directly affect the skin barrier prior to causing alterations in chemical triggers.

There is also evidence to suggest that hydration affects the mechanical properties of the skin. Studies have observed that increased moisture content of the stratum corneum leads to degradation of corneodesmosomes and intercellular lipids which probably leads to decreased cohesion resulting in increased desquamation (Warner et al., 1999, Bouwstra et al., 2003, Wu et al., 2006). It would be expected that in cases such as callused skin, skin stiffness is likely to accelerate with decreased hydration as cohesion is increased between keratinocytes.

The impact of the structural and biochemical changes in plantar skin has never been tested. As hypothesised above, an increased TEWL and decreased stratum corneum hydration is likely to be a feature of callused skin due to altered lipid profile while the increased cell production and cohesion of the corneocytes will increase the thickness of the stratum corneum. This is likely to increase the stiffness of the skin. Measures of these features could be useful therefore in characterising the properties of calluses and corns and changes in these due to interventions. However, to date there are few reports on these characteristics in plantar skin.

A.6 Plantar pressure

A.6.1 Application of loads to the plantar surface

The main ground reaction forces that occur during gait can be described as follows: the heel strikes the ground producing a vertical peak force approximately 1.2 times body

weight, coinciding with a posterior shear force of 0.2 times bodyweight (17% of vertical component). As the centre of mass raises to its highest point and the body is directly above the limb, a trough in vertical force is seen as load is transferred from the heel to the forefoot. At this point the vertical force is 0.7 times body weight and the horizontal force becomes 0. The heel then lifts and the bodyweight is transferred onto the forefoot (which is already loaded at this point) which then begins to propel the body forwards, causing a second vertical force peak of 1.2 times bodyweight which coincides with an anterior shear force of 0.2 times bodyweight (17% of vertical component) that passes through the forefoot. The vertical and horizontal forces then reduce as bodyweight is transferred to the opposite foot during the toe-off movement (Richards, 2008). This process produces a graph with two peaks for vertical loading force and a posterior and anterior peak for horizontal shear force (Figure A.5). The medio-lateral force magnitudes are relatively small compared to the antero-posterior shear forces during gait, being a maximum of 0.1 times bodyweight i.e. 50% of antero-posterior forces (Richards, 2008).

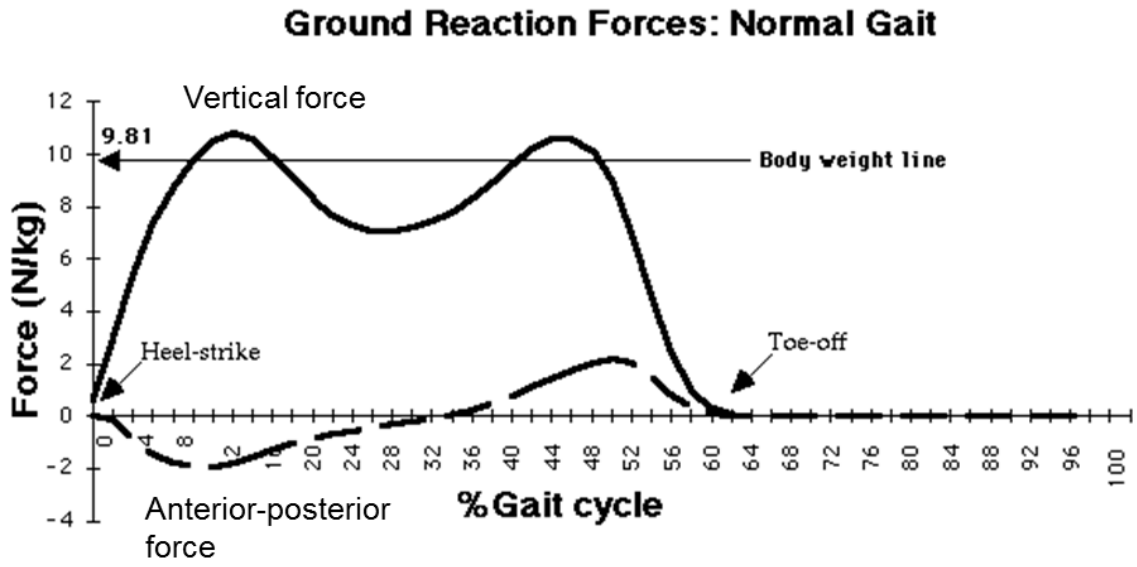


Figure A.5 – Vertical (compressive) and antero-posterior (shear) forces under the foot during gait (adapted from Kirtley, 2014). The second vertical peak and posterior to anterior peak represents the forces acting under the forefoot.

Pressure is the measure of force applied to an area (measured in kPa = 1,000 N/m^2), and is often used in clinical biomechanics because tissue damage relates to, not how much force is applied, but to how much tissue is involved in transmitting the force, its area (Richards, 2008). A large magnitude of force may not be damaging over a large area,

i.e. 1000N through the foot and ankle, but if this were to occur beneath a small area, e.g. a metatarsal head, this could then become damaging. Knowledge of how force is distributed beneath specific sites of the foot – how much pressure is applied – is imperative in understanding when skin may be at risk of developing ulcers (in the diabetes literature), or in the context of this thesis, how pressures relate to callus.

There is an underlying assumption that callus is formed as a result of the skin reacting to increased external pressure placed upon it and there is some evidence to suggest this is the case (Bevans and Bowker, 1999). Figure A.6 shows the factors which have been linked to callus development. Extrinsic factors influencing callus development are thought to include (1) footwear and (2) activity levels (Singh, 1996, Freeman, 2002, Grouios, 2004) which might both lead to increased plantar pressures during each step taken and increase the overall load experienced by the skin over a cumulative period. Intrinsic factors include foot deformities causing (3) bony prominences, and (4) those which may be secondary to altered physiology and disease (e.g. diabetes). The intrinsic factors may relate to age, sex and race (Singh, 1996, Freeman, 2002, Grouios, 2004). Below is a summary of how each of these factors is linked to pressure changes beneath the forefoot.

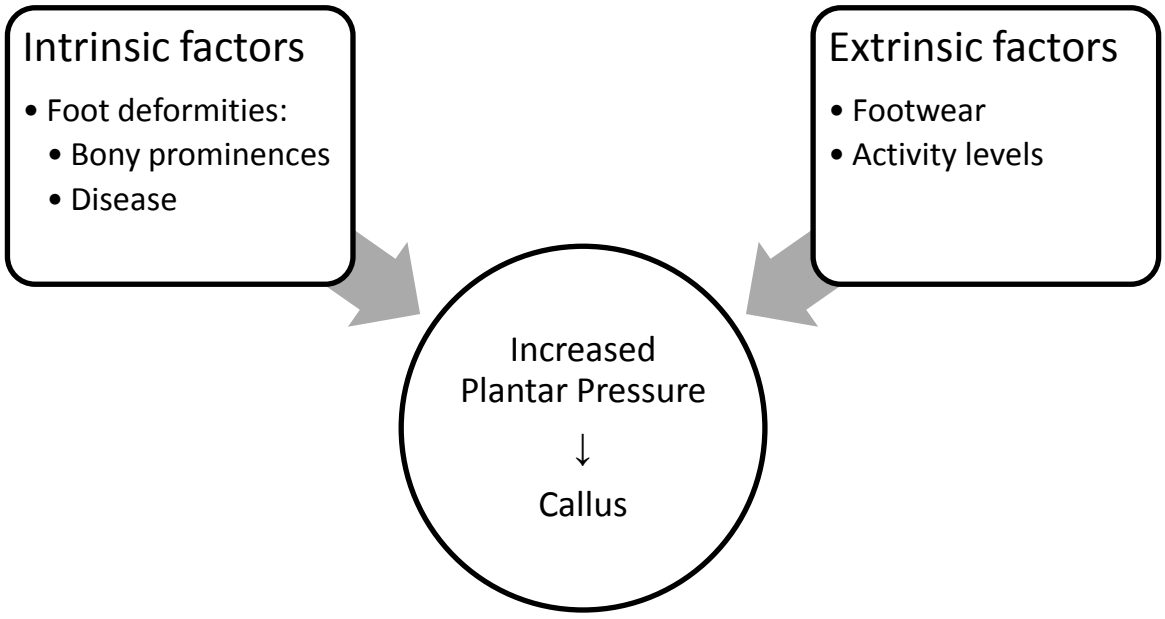


Figure A.6 – Causes of plantar callus

A.6.2 Footwear

Footwear factors that are thought to contribute to callus production include poorly fitting footwear such as wearing shoes that are too small or an incorrect shape for the foot;

irregularities within the footwear such as seams; wearing shoes that are not designed for the purpose for which they are being used; and not wearing footwear (Singh, 1996, Grouios, 2004). As far as the plantar pressure literature is concerned, there is an abundance of papers suggesting that high heeled shoes contribute to increased plantar forefoot pressures. With increasing heel height, the heel is offloaded and the forefoot pressure not only increases but also shifts from lateral to medial (Mandato and Nester, 1999, Speksnijder et al., 2005, Yung-Hui and Wei-Hsien, 2005, Ko et al., 2009, Cong et al., 2011) with in-shoe pressure increases reaching up to 71% ($p < 0.05$) beneath the first metatarsal with the just the introduction of a 2cm heel compared with a flat sole (Mandato and Nester, 1999). These studies suggest that increased heel height pronates the foot (Cong et al., 2011) and shifts the centre of mass anteriorly and medially (Ko et al., 2009). Interestingly, wearing high heels also significantly increases posterior shear force (Cong et al., 2011) which could increase the risk of skin problems as animal models have reported skin breakdown to occur faster with the introduction of shear force (Goldstein and Sanders, 1998).

Other footwear factors may also contribute to increased pressures. Branthwaite et al. (2013) found that toe box shape can influence pressures on the toes and over the metatarsal heads. The authors found significant differences between round, square and pointed toe boxes beneath all but the second metatarsal head. The percentage differences between conditions ranged from 3.3% (between square and round toe boxes at the third metatarsal head. $P = 0.005$), to 67.6% (between pointed and square toe boxes at the fifth metatarsal head. $P = 0.000$). Changes in pressure were attributed to toe box shape not conforming to foot anatomy (Branthwaite et al., 2013).

The presence of studs in football boots contribute to significantly increased pressures over the first, fourth, and fifth metatarsal heads when compared with running shoes (Carl et al., 2014). Percentage differences here ranged from 35.5% (at the fourth and fifth metatarsal head of the non-preferred foot. $P < 0.001$) to 42.6% (at the first metatarsal head of the preferred foot $p = 0.002$). Choice of running shoe can also affect peak forces with flat, racing style running shoes increasing peak forces under the lateral forefoot by 16.2% (p value not available) compared with normal cushioned training shoes (Queen et al., 2010). The hardness of the sole can contribute to pressures experienced under the forefoot. Medium and hard shoe soles have been found to lead to significantly increased pressures under the forefoot compared with soft soles (11% and 15.9% differences respectively. $P < 0.001$ and 0.009 respectively). Differences between medium and hard

soles were noted to be non-significant (4.3% difference. P = 0.114). This cannot be accounted for by contact area, as there was only a 1% decrease in hard compared to soft-soled shoes in this area, and a significant increase (10%. P = 0.001) increase in contract area in the midfoot between these conditions (Lane et al., 2014).

A.6.3 Activity levels

It has also been suggested that increased intensity of exercise, overtraining and excessive loads can increase callus risk (Grouios, 2004). Current evidence suggests that with an increase in activity levels comes an increase in the number of steps taken per day. A recent study with a large sample (n = 1,136) of US citizens has shown significant relationships between the number of steps taken per day with a pedometer and self-reported physical activity. The number of steps per day was shown to increase linearly with the number of days of strenuous exercise per week ($p < 0.001$). There was also a linear increase in number of steps per day with those who reported themselves to be physically active ($p < 0.001$) (Bassett et al., 2010). This introduces the point that with exercise comes increased accumulated loads over time which may have an impact on plantar skin.

In addition to increasing the number of steps taken per day, there is also evidence that exercise intensity can increase plantar pressure values. The largest volume of literature available on exercise intensity focusses on walking and running. Burnfield et al. (2007) reported that walking and running generate significantly higher pressures ($p < 0.001$) under the forefoot than stair climbing (48.6% and 48.2% respectively) and recumbent biking (83.8% and 83.7% respectively) exercises. For both walking and running, speed has been shown to have a significant effect. Burnfield et al. (2004) reported that with increased walking speed from slow (3.42 km/h) to medium (4.8 km/h) and fast (5.82 km/h), peak pressures significantly increased ($p < 0.006$) in the central and medial forefoot. An increase in speed from slow to medium (40.4% increase) brought an increase in peak pressure of 8.7% and 22.2% in the central and medial metatarsal heads respectively. Increasing the speed from medium to fast (9.1% increase) increased pressures at these areas by 8% and 9.1% respectively. Between slow and fast speeds (70.2% increase), there was an increase in pressure by 17.4% and 33.3% respectively.

Similarly to walking, an increase in running speed from 11.2 km/h to 17.8 km/h (58.9% increase) has been reported to result in a significantly increased peak pressure beneath the whole foot of 15.1% ($p < 0.01$) in a small population of 11 adolescent runners (Fourchet et al., 2012). This relationship between increased running speed and plantar

pressures has also been observed in 20 female runners in a Taiwanese study (Ho et al., 2010). Increasing jogging speeds from 1.5 m/s to 2.0 m/s to 2.5 m/s resulted in significant increases in peak pressures ($p < 0.05$) in the lateral, central and medial forefoot (medial forefoot was significant only between 1.5 and 2.5 m/s). From 1.5 m/s to 2.0 m/s (33.3% speed increase), pressures at the lateral, central and medial forefoot increased by 9.6%, 9.3% and 6.2% respectively; from 2.0 m/s to 2.5 m/s (25% speed increase), the percentage increases were 7.9%, 9.0% and 4.7% respectively; and from 1.5 m/s to 2.5 m/s (66.7% speed increase), the percentage increases were 18.3%, 19.1% and 11.2% respectively (Ho et al., 2010).

Other notable observations include a reported increase in peak plantar pressures beneath the forefoot after long-distance running. Bisiaux and Moretto (2008) observed a 10% increase ($p < 0.05$) beneath the second to fourth metatarsal heads using in-shoe pressure sensors after a 30 minute, intensive run. Nagel et al. (2008) also reported a significant increase in barefoot pressures beneath the second (12.6%), and third to fifth metatarsal heads (16.1%) ($p < 0.001$). Both studies reported a non-significant decrease in plantar pressure beneath the first metatarsal head and a significant decrease beneath the hallux ($p < 0.05$ and $p < 0.001$ respectively). Nagel et al. (2008) also reported a significant decrease in pressure ($p < 0.001$) beneath the lesser toes which suggests that muscle fatigue resulted in an offloading of pressure from the toes to the metatarsal heads which explains the increased peak pressures in this region. A subsequent study which measured peak forces beneath shod feet on pressure plates, before and after a 20km running race, reported very similar results, noting lateral force distribution during the push-off phase (Willems et al., 2012).

Overall, the studies in this section clearly highlight the relationship between type and intensity of exercise and magnitude of peak plantar pressures. Furthermore, these increased pressures coupled with an increased number of steps per day, will lead to an accumulation of load which may have a profound impact on plantar skin.

A.6.4 Bony prominences

There are various foot deformities which may affect pressure beneath the forefoot. One such deformity is hallux valgus. Plank (1995) and Galica et al. (2013) found that pressure beneath the lateral metatarsal heads were significantly lower in hallux valgus subjects than normal subjects with the latter study reporting a decrease of 4.4% ($p < 0.05$) compared with controls. Galica et al. (2013) also found a significant increase (4.1%. $p < 0.05$) in

pressure beneath the lesser toes while a decrease of 3.0% ($p < 0.05$) beneath the hallux was noted. Bryant et al. (1999), however, found that hallux valgus produced significantly higher peak pressures beneath the first, second and third metatarsal heads than control subjects with percentage differences of 43.8%, 57.6% and 53.4% respectively ($p = 0.000$ at each site). They also observed a significant increase beneath the lesser toes (35.3%. $p = 0.008$) and a non-significant increase beneath the hallux. Increased mean pressure beneath the first metatarsal head was observed by Martinez-Nova et al. (2010) who observed an increase of 7.9% ($p = 0.019$). These authors also noted a significant increase in pressure beneath the hallux of 124.2% ($p = 0.001$). The differences in pressure beneath the hallux observed by Martinez-Nova et al. (2010) and Galica et al. (2013) could be due to the fact that the former authors only included mild cases of hallux valgus whereas the latter authors also included severe cases which may have resulted in offloading as a result of discomfort (Galica et al., 2013). Differing stiffness properties of the joints in the first metatarsal and hallux, and deviation of the hallux could also possibly have contributed to differing pressure profiles. Collectively, these studies show higher forefoot loading medially than laterally and highlight how hallux valgus can change the pattern of pressure distribution in the foot.

While hallux valgus is well publicised, other anatomical conditions are known to affect plantar pressures. Some of these include hallux limitus, which significantly increases pressures beneath the hallux and lesser toes due to increased stiffness at the interphalangeal and metatarsophalangeal joint (Bryant et al., 1999, Zammit et al., 2008); Pes cavus, which significantly increases pressure beneath the metatarsal heads due to medial arch elevation, resulting in reduction in contact area (Burns et al., 2005, Gravante et al., 2005, Fernandez-Seguin et al., 2014); and acute Charcot arthropathy (Armstrong and Lavery, 1998) which is associated with increased pressures beneath the whole forefoot, and is possibly a precursor to destruction of the midfoot bones and joints.

A.6.5 Disease

There are certain diseases which are associated with increased plantar pressures, but by far the most publicised included diabetes and rheumatoid arthritis. Diabetic peripheral neuropathy has been linked with increased forefoot plantar pressures (Fernando et al., 2013) and in turn, high plantar pressures are associated with ulceration (Boulton et al., 1983, Frykberg et al., 1998) making them an important area of discussion.

In the diabetes literature, the two major factors affecting pressures includes tissue thickness and stiffness; and a reduction in joint mobility. Mueller et al. (2003) found that the greatest predictor of increased pressure beneath the forefoot in individuals with diabetes was hammertoe deformity, but they also reported soft tissue thickness to be important. Hammer toe deformities have been associated with increased peak plantar pressures in diabetes, with peak pressures reported to be 71.1% ($p < 0.001$) higher in the forefoot (Bus et al., 2005). The significance of the pressure increase also relates to the level of deformity with a significant negative correlation between toe angle and peak pressure ($r = -0.74$) (Bus et al., 2005). The elevated pressures are likely to be due to displacement and thinning of the fat pad beneath the metatarsal heads due to the hyperextension of the metatarsophalangeal joint (Bus et al., 2004a). Plantar soft tissue thickness beneath the metatarsal heads has been found to have an inverse relationship with peak plantar pressures (r ranges from 0.430 to 0.605 for second to fifth metatarsal heads. $P < 0.0001$) meaning that in cases where the tissues are thinner, the peak pressures tend to be higher (Young et al., 1995, Abouaesha et al., 2001). This may be due to thinner tissues which stiffen faster upon loading, causing the peak pressures to increase.

One other area relating to tissue stiffness, independent from the plantar fat pad and soft tissues, is Achilles tendinopathy, a common problem in diabetes resulting in a shortened, stiffened Achilles tendon, caused by dense packing of collagen fibrils with altered morphology in its tissue (Grant et al., 1997). This results in ankle equinus, a severe reduction in dosiflexion of the ankle joint which has been reported to significantly increase pressures by 7.6% ($p = 0.007$) in the forefoot, probably due to the foot being pulled into plantar flexion (Lavery et al., 2002). Additionally supporting this theory, Achilles tendon lengthening surgery has been shown to promote healing in forefoot ulceration (Holstein et al., 2004) due to a reduction in peak pressures at the forefoot by 26.7% ($p < 0.001$) (Armstrong et al., 1999).

Limited joint mobility, particularly of the subtalar and first metatarsophalangeal joints are also associated with high plantar pressure and ulceration in diabetes. Increases in peak pressure of up to 121.1% ($p < 0.001$) between individuals with no forefoot problems and those with limited subtalar joint mobility have been reported, and this may further increase up to 152.6% ($p < 0.001$) with the presence of neuropathy (Fernando et al., 1991). A more recent study conducted by Viswanathan et al. (2003) found individuals with neuropathy had significantly impaired joint mobility at the subtalar and first metatarsophalangeal joint ($p < 0.001$), resulting in increases in forefoot peak plantar

pressures compared with those without neuropathy (30.8%. $p < 0.001$). The reduction in joint mobility was further reduced ($p < 0.001$) and peak pressures dramatically increased by 58.0% in those with a history of ulcers, compared to individuals without neuropathy (Viswanathan et al., 2003). The pressure increases are probably due to the increased stiffness of the joint which results in less of the load being dissipated (Mueller et al., 1989). Increases in thickness of the plantar fascia beneath the whole foot has been shown to be inversely correlated to metatarsophalangeal joint mobility ($r = -0.53$) and directly related to increased vertical forces beneath the metatarsal heads ($r = 0.52$), probably due to making the foot more rigid (D'Ambrogi et al., 2003). Stiffness of plantar soft tissues in the forefoot has been found to be significantly increased in individuals with diabetic peripheral neuropathy which could lead to increased pressures (Klaesner et al., 2002, Sun et al., 2011).

The volume of literature surrounding rheumatoid arthritis and forefoot pressures is less than that of diabetes. The major foot deformities associated with rheumatoid arthritis include hallux valgus, metatarsal head depression, hammer or claw toes, tendocalcaneal bursitis or subplantar spur formation (Dimonte and Light, 1983) plus reduced range of motion of the ankle and subtalar joints (Locke et al., 1986). Peak forces under the hallux, lateral three toes and first metatarsal head in severe rheumatoid arthritis, cases with heel valgus and reduced joint motion, are significantly decreased ($p < 0.05$) with reductions ranging from 28.3% (first metatarsal) to 100% (second toe), compared to a normal population (Sharma et al., 1979). A subsequent study found similar results with 37.2% force reduction beneath the toes and 16.1% reduction beneath the first and second metatarsals (Simkin, 1981). Both studies found no significant difference in the loading of the lateral metatarsal heads, indeed the former study reported an 18.6% increase. The authors of both studies suggest discomfort and changes in the midtarsal joints results in loads being shifted laterally (Sharma et al., 1979, Simkin, 1981). Woodburn and Helliwell (1996), however, reported a significant decrease in pressure over the lateral metatarsal heads in rheumatoid patients with a valgus heel compared with normal rheumatoid and control groups (pressure reductions ranging from 28.1% to 52.1%. $p = 0.001$), probably as a result of medial distribution of pressures as a result of the valgus (everted) rearfoot. Tastekin et al. (2009) found no significant difference in dynamic pressure between heel valgus and normal rheumatoid patients, possibly because of pain interfering with gait.

In a study comparing rheumatoid arthritis patients with a healthy population, Otter et al. (2004) found that there were no significant differences in forefoot pressures, but the

rheumatoid group did show a significant increase in pressure time integral and decrease in force-time integral compared with the control group. The authors were unsure of the mechanism for this but suggested that patients may have had a slower gait cycle than the controls, possibly due to avoiding pain (Otter et al., 2004). There is also evidence of pressures being related to damage to the metatarsal joints. One study observed that joint damage had a linear relationship with peak plantar pressures (van der Leeden et al., 2006).

This section has shown that diseases, particularly diabetes and rheumatoid arthritis may have a profound effect on plantar pressures. These diseases have multi-tissue effects: pathological changes may result in tendons and soft tissues becoming stiffer (Grant et al., 1997), stiff tendons may cause joint deformity, causing hyperextension and as a result thinning plantar soft tissues by stretching them (Bus et al., 2004a). Thickened plantar fascia, which is related to altered joint function, may affect how load is distributed beneath the foot (D'Ambrogi et al., 2003). As a result of deformities and associated pain, individuals may reduce walking speed and load different sites of the foot, which may in turn also cause problems (Sharma et al., 1979). These factors all have a significant influence on pressures beneath the foot.

A.6.6 Plantar pressure and callus

There are few studies in the literature which demonstrate the hypothesised link between foot biomechanics and callus, but three of particular relevance do exist (Bevans and Bowker, 1999, Abouaesha et al., 2001, Menz et al., 2007). Bevans and Bowker (1999) compared lower limb biomechanical characteristics of three groups of individuals. The first (n = 22) contained subjects with diabetes presenting with forefoot callus; the second (n = 20) contained subjects with diabetes without plantar callus, and group 3 (n = 17) contained subjects free from diabetes presenting with forefoot callus. They found that both groups with callus (including individuals with diabetes, and individuals without) presented with a significantly higher total of structural anomalies than the group without callus (96 and 81 versus 65 for groups 1 and 3 versus group 2 respectively). The anomalies more prevalent in the callus groups included forefoot invertus, forefoot evertus, and equinus. These are static structural deformities that reflect differences in the alignment of the forefoot to the rearfoot, and limited ankle dorsiflexion. In the group without callus, the mean position of ankle dorsiflexion with the knee extended and the mean relaxed and maximally dorsiflexed hallux positions were significantly greater, up to 3.0% ($p = 0.03$), 16.5% ($p = 0.013$) and 12.8% ($p = 0.015$) for each parameter respectively; and the mean

eversion of the subtalar joint was significantly less than the two callus groups with angle reductions reaching 57.9% ($p = 0.001$). The callus group without diabetes had a significantly greater angle of rearfoot varus than the two groups (callus and non-callus) with diabetes. The angle of ankle dorsiflexion with the knee bent at 90^{o}, the range of motion of the first metatarsal joint and the angle of the subtalar joint in the relaxed stance position were similar in all three groups. From a discriminant function analysis, they found that the strongest predictor of callus development was subtalar eversion (or pronation) ($p = < 0.001$), but the decreased dorsiflexion with knee extension was also a predictor. No information on the position of calluses on the forefoot was given, so it is unclear whether certain types of biomechanical problems related to formation of calluses in certain areas, such as whether eversion might result in medial forefoot callus.

The significantly limited range of ankle dorsiflexion in the callus groups is likely to lead to increased plantar pressure due to compensatory changes in gait. The significantly limited range of hallux dorsiflexion in relaxed and maximally dorsiflexed positions in the callus groups may increase loading beneath the interphalangeal joint (Bevans and Bowker, 1999) and perhaps affect the way load is dissipated during gait, as with individuals with limited joint motion in diabetes (Mueller et al., 1989). The presence of diabetes seems not to be a factor because there were no significant differences in these parameters between the diabetes group with callus and the non-diabetes group with callus, while differences were shown between the callus and non-callused group (who had diabetes). Furthermore, the diabetes group with callus did not have a significant difference in blood levels of glycated haemoglobin compared to the diabetes group without callus which suggests that the differences in joint angle data is biomechanical, not biochemical in cause (Bevans and Bowker, 1999). Linking these results to the previous subsections, increased pressure could result from limited mobility of the subtalar and ankle joints (Mueller et al., 1989, Fernando et al., 1991, Viswanathan et al., 2003) particularly in individuals with diabetes where insensitivity caused by neuropathy is present.

Abouaesha et al. (2001) assessed plantar pressure, skin thickness and callus in individuals with diabetes ($n = 157$). They found that subjects with forefoot callus had a significantly increased pressure under each metatarsal head with pressure increases ranging from 21.7% (left foot, fourth metatarsal) to 51.8% (right foot, third metatarsal) compared to control subjects ($p < 0.05$). They also found these subjects had significantly reduced plantar tissue thicknesses under the second to fifth metatarsal heads ($p < 0.05$). This study and others discussed previously have also highlighted the inverse relationship

between tissue thickness and plantar pressures, particularly in cases where the metatarsophalangeal joint is hyperextended, causing displacement and thinning of plantar soft tissues (Young et al., 1995, Bus et al., 2004a). These may stiffen faster upon loading, causing increased pressure.

Menz et al. (2007) recruited 292 participants from a retirement home and screened them for callosities, which affected 52% of their sample (n = 151). Using a MatScan® pressure mat, they assessed barefoot walking and found that significantly higher plantar pressures existed under calluses overlying the hallux (13.8% increase; $p = 0.007$), second metatarsal head (10.4% increase; $p = 0.001$) and third to fifth metatarsal heads (14% increase; $p = 0.009$), but no significant differences beneath the first metatarsal head or lesser toes. From foot deformity assessments, they found that calluses were most likely to occur in females with moderate to severe hallux valgus, toe deformities and foot pain; with hallux valgus and toe deformities being significantly associated with callus ($p < 0.001$ and 0.016 respectively). This data relates to the previous discussed studies in hallux valgus and hammer toe deformities which have reported significantly increased pressures beneath the metatarsals (Bryant et al., 1999, Bus et al., 2005, Martinez-Nova et al., 2010). This study is very important in assessing the link between bony deformities, which have been shown to be associated with increased plantar pressures, and the presence of callus. It could be argued that callus builds in areas where there are increased pressures as a result of bony deformities, but this is not clear cut in the literature. The studies by Bevans and Bowker (1999), Abouaesha et al. (2001) and Menz et al. (2007) present compelling evidence that suggests structural abnormalities such as toe deformities, which may result in stretched, thinner soft tissues (Bus et al., 2004a, Abouaesha et al., 2001), or compensatory changes in gait (Bevans and Bowker, 1999), lead to increased peak plantar pressures, a hypothesised precursor to callus development. However, evidence supporting the contrary – callus being a cause, and not a result, of increased pressures, has also been presented in the literature.

Table A.5, shows several other studies have reported significantly higher ($p < 0.05$) peak plantar pressures under feet with callus compared with control groups (Potter and Potter, 2000b, Pataky et al., 2002, Duffin et al., 2003). However, it is unclear from the literature whether increased pressure beneath callus is a predisposing factor or a result of changes in skin thickness and characteristics. There appears to be conflicting evidence in the literature assessing the effect of callus debridement on pressure (Table A.6). For example, Pitei et al. (1999) and Pataky et al. (2002), both found that removal of callus in patients with diabetes significantly reduced plantar pressure ($p < 0.014$ and 0.001

respectively) which might indicate that callus causes elevated pressures. However, Potter and Potter (2000b) (pressures not shown in paper) and Woodburn et al. (2000) found no significant difference after removal of callus which might suggest that increased plantar pressure was not the outcome of the callus but rather a feature of the callus site prior to its development. In contrast to the studies investigating callus debridement in diabetes, Woodburn et al. (2000) reported a 17% mean increase ($p > 0.05$) in peak pressure after callus debridement. This was explained by the fact that there was a decrease in contact area over the metatarsal heads after callus removal (thus increasing pressures) and a statistically significant reduction in pain ($p = 0.01$) might have increased walking speed and pressures (based on evidence of decreased contact time post debridement). In a later study on rheumatoid arthritis patients (n = 18), Davys et al. (2005) found a non-significant decrease in pressure after callus debridement which would again suggest that callus debridement in this group of patients, while possibly beneficial for pain relief, does not impact on pressure reduction and that callus is not the cause of elevated plantar pressures, rather increased pressure is a pre-existing feature of the site.

Table A.5 Other studies reporting significant pressure increases beneath calluses

	Potter and Potter (2000b)	**Pataky et al. (2002)**	**Duffin et al. (2003)**	
	Healthy	With diabetes	With diabetes	Healthy
Callus	472.9 kPa	314 kPa	43 N/cm^2	46.5 N/cm^2
Control	355.1 kPa	128 kPa	39 N/cm^2	39 N/cm^2
% increase	33.17	145.31	10.26	19.23

Pressure changes calculated by ((diabetes group – control group) / control group) x 100.

Table A.6. Peak pressure changes following callus debridement (kPa)

	Pitei et al. (1999)			**Woodburn et al. (2000)**	**Pataky et al. (2002)**	**Davys et al. (2005)**
	Group 0	Group A	Group B			
Before debridement	374.8	351.7	241	241	340	828
After debridement	251*	240.5*	176.2*	285	141*	817
% change	-33.0	-31.6	-26.9	18.26	-58.53	-1.33

* = Significant change in pressure. Pressure changes calculated by ((post treatment – pre treatment) / pre treatment) x 100. Pitei et al. (1999): Group 0 - patients presenting with callus for first time, with no history of ulceration; Group A – history of ulceration, requiring callus debridement every 6 – 8 weeks; Group B – history of ulceration, requiring callus debridement every 3 – 4 weeks.

One possible factor causing the different outcomes in these studies might be differences in inclusion criteria. Pitei et al. (1999) treated patients with diabetes with neuropathy and a history of ulcers (n = 24), Pataky et al. (2002) treated 13 patients with diabetes without neuropathy, any skin problems or bony prominences (n = 13), Potter and Potter (2000b) recruited only healthy individuals without the presence of disease (n = 15; total of 36 calluses) who had callus removed every two to three months. They also reported not including subjects with bony abnormalities, those who had never had callus removed previously, or had regular callus removals frequently (every three to four weeks). Woodburn et al. (2000) recruited rheumatoid arthritis patients (n = 14). The results presented by Pitei et al. (1999) are particularly interesting because individuals with neuropathy and a history of ulceration are significantly more prone to biomechanical problems than those without a history of ulceration (Fernando et al., 1991, Viswanathan et al., 2003). The fact that callus removal resulted in comparable reductions in pressures in their two groups with a history of ulceration and the group with no history of ulceration, suggests that if biomechanical problems and increased pressures were a factor in their ulceration, they were not a factor in their callus development. The pressures beneath the calluses would remain relatively unchanged. In these four studies, it could be argued that the presence of diabetes is a factor contributing to the results, but neuropathy and structural deformities associated with the condition are not factors due to the fact that Pataky et al. (2002) did not recruit subjects with these characteristics.

The differing pressure measurement systems may have also played some part. Pitei et al. (1999) used an F-Scan insole and Pataky et al. (2002) used Force Sensing Resistors placed under each metatarsal head and these could have been more sensitive to changes in pressure after callus removal compared with the barefoot pressure mats employed by Potter and Potter (2000b), Woodburn et al. (2000). Davys et al. (2005) also used a pressure mat in their study. The variances in the participants of each study in relation to underlying disease pathology make it difficult to pool the results. As discussed previously, diabetes and rheumatoid arthritis have different features when compared to feet without underlying disease. Alterations in gait due to neuropathy and the anatomical changes discussed previously may have played a part in these different results.

Overall, the literature seems to point towards anatomical variations contributing to increased pressures leading to callus development, but this is not absolute because there are studies which have found that debriding callus also reduces the pressures (Pitei et al., 1999, Pataky et al., 2002) which would suggest that the presence of callus increases

pressure too. The only way to determine whether pressure is a cause of callus would be to conduct studies on normal plantar skin to determine whether pressure causes these types of skin changes. Either way, pressure studies are limited in the fact that the pressure measures only the compression element of the pressure which acts perpendicular to the foot – sensor interface. This does not provide any information about horizontal shear which may also have an impact on callus development.

A.7 Plantar shear pressure

Plantar pressure data in the literature, refers to forces applied perpendicular to the load sensor surface, and this ignores forces applied in medio-lateral and antero-posterior shear directions. Compared with plantar pressure studies that report vertical pressures, the volume of literature addressing specifically plantar shear pressure is scant; therefore how the anterior and posterior ground reaction shear forces are distributed across the forefoot as shear pressure is not clear. It follows that the role of shear in callus development, or increases in shear forces with callus, are poorly understood. However, there are some helpful papers which provide some insight into this area. In a sample of healthy males (n = 10) walking in conventional leather shoes, Pollard et al. (1983) found, using individual sensors placed under the metatarsal heads, hallux and heel, that the highest plantar shear force (values not available) occurred at the metatarsal heads; an anterior force occurred over the central and medial heads and a posterior force occurred over the lateral heads. In barefoot walking, the force was increased at all sites except the hallux. During propulsion, the gross forces applied to the forefoot are in the same direction so it is an interesting observation that shear occurred in two directions. They also found in a study of patients with diabetic neuropathy (n = 6), using the same instrumentation, that foot ulceration occurs at the site of maximum shear, the same site as maximum pressure (values not available) and this occurred with both barefoot and shod feet (Pollard and Le Quesne, 1983). Subsequently, the same research group (Tappin and Robertson, 1991) found in a sample of healthy individuals (n = 20) that the peak compression and peak shear forces in the first, fourth and fifth metatarsal heads occurred at the same time, which may have an effect of occluding skin perfusion. These authors used a semiconductor field coil with a centre tap placed on a 16mm diameter plate and a magnet placed on a second plate. The two plates were connected via silicon rubber allowing displacement to be measured (Pollard et al., 1983). However, the system only allowed unidirectional measurement in the

orientation of the grooves within the plates, so this could have led to some discrepancies in force magnitudes observed. It may have caused some medio-lateral shear to be recorded as antero-posterior shear as the mechanics of the sensor could have forced movement in an antero-posterior direction. The force tracings in these studies are graphically represented (in kg) but no numerical data is presented so the compression/shear ratio cannot be calculated.

Hosein and Lord (2000), using a similar system to Pollard et al. (1983) found in healthy individuals (n = 8) that peak plantar shear pressures occurred more laterally, under the third and fourth metatarsal heads (86.5 kPa and 71 kPa respectively), than peak vertical pressure, which occurred under the second and third heads (both 228 kPa). The compression to shear ratios (in kPa) were 5.8, 7.4, 2.6, and 2.1 per one kPa of shear stress under the first four metatarsal heads respectively. This range of ratios, if true, would suggest that shear pressures are not easily predicted or inferred from vertical pressure data. The same authors subsequently found that in cases of diabetes (n = 6), the peak shear pressures occurred more medially (72.7 kPa under the first metatarsal head) and peak compression pressure more medial (273 kPa under the second head) than the healthy group they reported in the previous study (Hosein and Lord, 2000). The medial metatarsal heads were found to be the most common area for ulceration corresponding to the site of peak shear pressure (Lord and Hosein, 2000). Like the previous authors, Perry et al. (2002) found in 12 individuals with diabetic neuropathy that peak compression pressure occurred in the medial metatarsal heads (189 kPa) while peak shear occurred in the lateral metatarsal heads (33 kPa). Furthermore, when the authors analysed the combined effects of compression and shear, they found that these forces occurred at the same site in 50% of individuals, but occurred at different times. Peak compression occurred at an average of 0.186s before shear in these individuals, but it is unclear what percentage of stance phase this was (Perry et al., 2002). Similarly, Yavuz et al. (2007) found that in 60% of their sample of patients with diabetic neuropathy (n = 10) that the sites of peak compression and shear pressures were different. Subsequently they observed that generally the magnitude of peak shear pressure was significantly greater at 83.3 kPa vs 62.3 kPa (33.7% difference; $p = 0.014$) in patients with diabetes (n = 15) than normal control subjects (n = 20) (Yavuz et al., 2008). The peak compression pressures were also higher in the diabetes group at 614.2 kPa vs 497.5 kPa (23.1% difference) but not significantly. However, neither study disclosed the specific locations of these shear forces in detail.

One study (Stucke et al., 2012) has specifically investigated whether the differences in the location of peak compression and shear pressures could be due to the movement of tissue during loading. The authors studied the gait of 11 healthy individuals, and from their data the forefoot peak pressure ratio was 1 kPa of shear for 14 kPa compression. They reported that compression and shear peak pressures occurred in different locations and at different times with peak compression occurring before peak antero-posterior shear in 57.6% of cases. They also observed, in 67% of cases, that the peak compression pressure was located between two peak shear pressure points. They concluded that 'since the peak pressure is located directly in between both of the peak shear values and the forces are moving in opposite directions about the peak pressure location radial ''spreading'' occurs' (Stucke et al., 2012 p.621). Perry et al. (2002) observed similar results in tissue activity where they found that skin was stretched to a greater degree than it was bunched in the forefoot.

One study has investigated shearing forces present under callused regions of the foot (Mori et al., 2012). These authors measured plantar shear force in patients with diabetes with (n = 9) and without (n = 41) forefoot callus. After normalising the data to bodyweight, they found a significant increase (31.6%; p = 0.03) in peak shear force in subjects with callus which, like the pressure studies presented in section A.6.6, may have preceded or succeeded callus development. The presence of callus may have increased the shear value as there is more tissue available to be deformed with the introduction of horizontal forces.

The varying results across the literature may be attributed to the fact that measurement of shear forces is technically challenging and each research group used different instrumentation and data collection techniques. Pollard et al. (1983) and Tappin and Robertson (1991) measured shear and compression forces in barefoot walking with separate compression and shear transducers. As their transducers were attached to the plantar skin and were 2.3mm thick, this could have caused increased loading and affected the loading at the foot/floor interface, and thus their measures of compression and shear could be directly influenced by the presence of the sensor. The device used by Lord and Hosein (2000) and Hosein and Lord (2000) was an in-shoe measurement device that could measure antero-posterior and medio-lateral shear pressures in separate gait cycles but they did not measure this at the same time as compression pressure (which was measured in a separate gait cycle using F-Scan insoles). Perry et al. (2002), Yavuz et al. (2007), Yavuz et al. (2008) and Stucke et al. (2012) used platforms designed to simultaneously collect shear

and compression data in barefoot walking. Perry et al. (2002) only analysed data collected from the initiation stage of gait and used the two-step method, whereas Mori et al. (2012) used an insole capable of measuring shear force and compression pressure at the same time. While all these studies are technically different, the results give a good insight into likely magnitudes, compression to shear ratio, and time of peak compression and shear forces.

In reality, peak shear pressure to compression pressure ratios vary greatly across the studies, with pressure ranging from 2.7 to 14 times greater than shear (mean across studies is 6.9 times greater). The far greater magnitude of compression than shear pressure concurs with ground reaction forces, which tend to be six times greater in the vertical direction than in the horizontal direction (Richards, 2008). Additionally, peak compression and shear pressures tend to occur at different sites. In the callus literature, calluses have been noted to occur at the areas of peak compression pressure in the forefoot (Pitei et al., 1999, Potter and Potter, 2000b, Pataky et al., 2002). Because callus and peak compression pressure are linked, it is pertinent that this area be explored in greater depth. However, in order to do so, one must understand how the skin reacts when forces are applied to it.

A.8 How the epidermis adapts to external forces

While the evidence linking external loading to plantar callus development is scant, some authors have investigated the effects of external insults on human skin structure in-vivo. Several have looked at the skin's response to frictional stimulus. The first of these, Rubin (1949), tested the effect of applying shear and compression by rubbing anterior thigh skin for 10 minutes with a tongue depressor and lubricant on 5 adults daily for 30 days. Biopsies showed that the thickness of the stratum corneum was increased by an average of 36% compared to the control site (the corresponding area on the opposite leg). Another study by Goldblum and Piper (1954) used a scratching machine to administer tolerable scratches (with 75 grams of weight) using a lucite 'fingernail' (1/8 inch diameter) for one hour a day on the backs of four male patients. Biopsies of the intervention skin showed evidence of hyperkeratosis which was not present in control skin samples (thickness not mentioned). However, each of these subjects had a history of inflammatory skin disorders which may have made them more prone to skin thickening.

While there is evidence of the skin's response to shear, even small load stimuli have been shown to elicit a response. Pinkus (1952) and Brophy and Lobitz (1959)

performed tape stripping experiments on volar skin on the forearm of a 45 year old male and skin on the back of a 34 year old male respectively. Pinkus (1952) tape stripped the skin repetitively until the stratum corneum was removed (the skin appeared red, shiny and dry). Brophy and Lobitz (1959) tape stripped 5 skin sites on the subject's back, and then repeated stripping in intervals of four, eight, 12, 24 and 48 hours later at each respective skin site. Biopsies were then taken at five minutes, four, eight, 11, 16, 25, 28, 32, 39, 49 and 73 hours after injury at each skin site. Pinkus (1952) found an increase in diameters of the most superficial, medium and deep layers of the epidermis peaked and plateaued between 48 and 72 hours where increases of 69.1%, 93.8% and 103.5% respectively, at 72 the hour timepoint. Brophy and Lobitz (1959) observed that cell mitotic activity increased after the first tape strip, but after subsequent strips at each site, the mitotic activity mimicked that of the skin samples taken from the first strip. This suggests that the skin showed a greater response to the first tape strip, and that repeated strips did not alter the skin's inflammatory response to the original strip (Brophy and Lobitz, 1959). These studies begin to show how mild skin irritation is enough to drive physiological changes.

In-vivo animal studies (Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998, Sanders et al., 2002) have further shown how skin adapts to friction, shear and compression. The main theme which has emerged is that under moderate levels of mechanical load, the skin mitotic levels increase and epidermal thickening occurs, while under a more severe mechanical stress, ulceration also occurs.

Mackenzie (1974a) and Mackenzie (1974b) studied the effect of friction on the epidermis of mouse ears. They administered friction using 10 circulations of a rotating brush, which rotated at a speed of 40 – 50rpm with a force of 8 – 9 grams (0.08 – 0.09 N), to three groups of mice ranging from one friction application to daily application for 35 days. A subsequent group received a greater number of circulations (x30) from the rotating brush between one and 10 days. It was found that mitotic activity initially decreased within the first 24 hours after a single friction application, but increased again to reach a peak at 48 hours, a similar finding to Pinkus (1952). In the mice who received daily friction application, it was found that mitotic levels remained high throughout the friction application period. For mice euthanized within 24 hours of the final daily friction application for seven, 14, 28 and 35 days of friction application, mitosis was 261.0%, 462.8%, 278.4% and 183.6% times higher than controls respectively (Mackenzie, 1974b). Furthermore, it was observed that the ear epidermis of mice that had received friction for seven days or longer contained between 49.0 – 82.3% more cells ($p < 0.05$) and the

stratum corneum was reportedly thicker than controls (measurements not available) (Figure A.7). There was no significant difference noted in thickness between the epidermis specimens that had received friction for seven days or longer which means that this hypertrophy must have occurred within the first seven days. In the group (n = 10) receiving 30 circulations, ulceration was noted and the skin thickening response was generally much greater than the group receiving less friction. In all cases, the stratum corneum was thickened and the strata basale and spinosum were increased in size, likely due to increased demand for cell production (Mackenzie, 1974a, Mackenzie, 1974b). The results of these studies suggest that 35 days is ample time for the skin to show a sustained response to a small mechanical stimulus at a force of 0.08 – 0.09 Newtons.

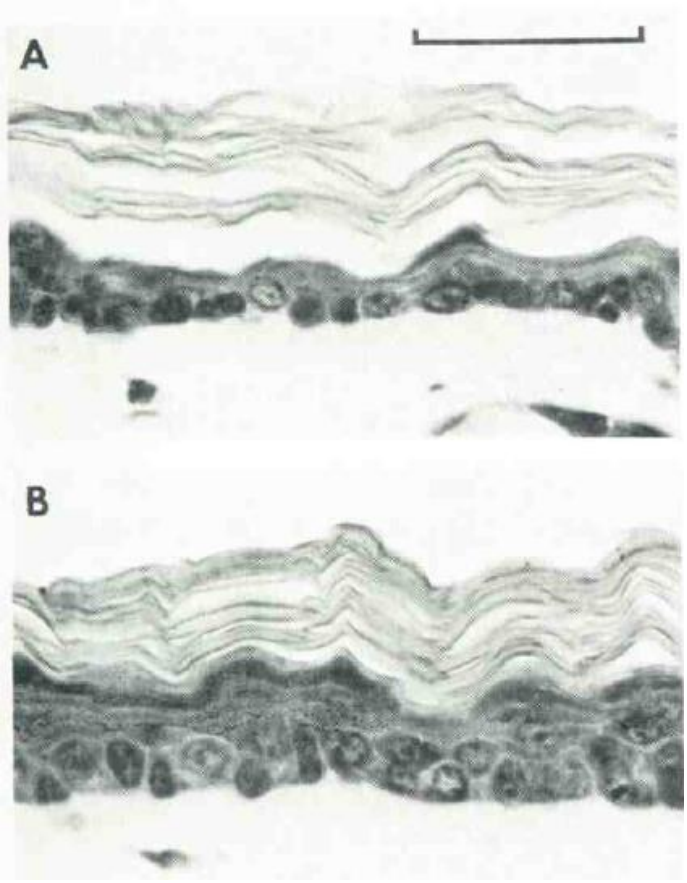


Figure A.7 – Specimens of mouse ear epidermis A = control; B = specimen after 7 days of daily friction application. Scale is 50 µm (Mackenzie, 1974a).

Goldstein and Sanders (1998), applied different combinations of compression and shear to the thigh skin of infant pigs (n = 8) using a load application device. The aims of the experiment were to test the effects of different combinations of compression and shear force application in an acute manner, whereby high loads were applied at 10 minute

intervals over 40 minutes on just one occasion (magnitudes not disclosed); and chronic manner, whereby the loads were applied for 40 minutes per day for three weeks. The 'chronic' load combinations included 5N compression with 2N shear, 6N compression with 1N shear and 3N compression with 1N shear; the magnitudes were lower than those used in the 'acute' experiments. Following the load application period the skin was histologically studied. The 'acute' load group showed that increased shear force magnitudes resulted in faster breakdown of the skin with abrasion and blister formation occurring. On skin where less shear force magnitude was applied, only skin redness was evident. For the chronic group, no significant changes were noted but there was some increase of epidermal volume and decrease in dermal volume which might suggest the beginning of a callus-like skin response. Again, this study demonstrates how skin may begin to change after several weeks of regular, moderate load application.

The dermis' resistance to mechanical stress is reportedly due to the morphology of collagen fibrils which has previously been linked to mechanical load bearing in evolutionary literature (reviewed by Sanders et al., 1995). Excessively high pressures over a prolonged period of time will lead to tissue breakdown and this is accelerated by increased age, smoking habits and moisture of the skin (Sanders et al., 1995). With regards to adaptation, collagen fibril diameters have been found to increase in tendon and skin as a result of increased compressive and shear forces. This in turn allows greater force tolerance (reviewed by Wang and Sanders, 2003). Thus external forces may lead to structural changes in the dermis as well as the epidermis.

In summary, there is a good body of evidence linking changes in skin structure to external forces placed upon it. The main theme which has emerged is that skin, in areas not designed for being loaded, adapts to mechanical loads up to a certain threshold of acute or accumulative load application, which if exceeded, is likely to cause skin breakdown and subsequent ulceration. Adaptation seems to occur under relatively small loads applied over a short period of time i.e. above seven days (Mackenzie, 1974b). It is a plausible but untested hypothesis that prolonged moderate loads under the metatarsal heads will alter chemical and physical properties of plantar skin and lead to hyperkeratosis. As the above studies have shown, increased loads may cause a skin response in as short a period as seven days. The separate contributions of compression and shear to any skin response are not known.

A.9 Gaps in the research into callus

Firstly, it is important to draw attention to the fact that corns have very seldom been studied, and barely feature in the literature. While callus and corns may often be considered together clinically, there is a lack of evidence to suggest they are similar in their biochemistry or in their aetiology. The vast majority of the literature published to date has focussed on callus; therefore callus will be the subject of the remainder of this thesis. Table A.7 shows the important gaps in the literature. Perhaps the most important omission from the literature is how calluses differ from normal plantar skin in terms of their form and functional properties. Whilst there is some understanding of how calluses develop and their prevalence, there are no studies investigating the biophysical characteristics of these lesions. In fact, there is little information which outlines the characteristics of normal plantar skin (such as profiles of hydration and mechanical properties) to provide a reference point against which abnormal skin can be measured. It is important to understand how normal and callused plantar skin differs because these differences could provide important insights into aetiology and treatments. Therefore, quantifying the characteristics of normal and callused plantar skin is an important first step.

Table A.7 - Gaps in the research into callus

Research gap	Importance
There are no studies which investigate the biophysical properties of callus	It is important to understand how normal and callused plantar skin differs because this could provide information into aetiology, inform treatment approaches and improve the investigation of treatment efficacy.
There are no studies which investigate the biophysical properties of normal plantar skin	
It is not known how normal and callused plantar skin differ in terms of biophysical properties	
The precise relationship between external loading and plantar callus development has never been scientifically investigated.	It is important to understand what mechanical factors cause hyperkeratosis in plantar skin because clinicians can then use this information to aid in callus prevention and treatment, particularly in at-risk groups such as rheumatoid arthritis and diabetes.

Characterising plantar callus first requires that suitable tools are available for characterising foot skin. Instruments for characterising skin are available but these have not previously been used on the foot. The thick epidermis of plantar skin and hyperkeratotic lesions which make the skin particularly hard may make measurement difficult as the devices available are primarily intended for use on soft skin, such as that on the face. Therefore, it is important to ensure that these devices will give reliable

measurements on plantar skin before attempting to use them for characterisation of normal and abnormal foot skin, such as callus.

There is also a possible link between callus development and external mechanical loads. It has been shown in the literature that high peak plantar pressures are associated with callused regions on the foot. Furthermore, it has been shown on other areas of the body and in animal studies that hyperkeratosis occurs as a result of compression and shear pressures which mean that it is highly likely that calluses develop as a result of these pressures. These studies highlight an area of importance; skin taken outside its physiological range of load tolerance, in terms of both magnitude and duration of load, causes it to change. Plantar pressure studies tend to only state the magnitude of pressure beneath the callused skin and do not express the accumulative load beneath this skin site. This is an important omission from the literature – increased duration of increased pressure will also have an accumulative effect, so to fully understand the relationship between pressure and callus, this needs to be taken into account. Further investigation into understanding the relationship between pressure and callus is essential for prevention and treatment of these lesions.

Part B. Skin measurement devices

B.1 Introduction to skin measurement parameters

In the context of the gaps in the literature, it is important to characterise the biophysical properties of plantar skin and how these properties are affected in cases of callus. The previous section highlighted that mechanical properties, trans-epidermal water loss (TEWL) and hydration are interrelated properties and may be useful measurement parameters to characterise plantar callus. The structural changes which occur in callus would most likely affect these properties and it is thus hypothesised that a relationship exists between callus development and regression, and skin properties.

However, there are other potentially useful parameters. The skin's surface topography could be useful as there is a noticeable change in appearance from normal to callused skin. The relationship between topography and biophysical properties would be useful in characterising the skin, especially in the context of clinical evaluations of skin, where observation may be the only tool available. Cross sectional anatomy and thickness measurement of callus would also be useful for similar reasons but would also potentially

allow analysis of how the lesions affect structures deep to the epidermis, such as the dermis and subcutaneous tissues. These measures may also assist the evaluation of intervention efficacy and also provide patients with a visual indication of improvement.

To investigate the biophysical properties of plantar skin, non-invasive approaches are preferred. Invasive biopsies have the benefit of being able to visualise and biochemically test the tissues but pose safety and ethical issues. They are far less practical when trying to characterise changing properties of lesions in-vivo, as repeated biopsies would need to be taken from the same skin sites over the test period. It is also important that techniques used will be able to characterise differences in both normal and callused skin, and therefore have sufficient sensitivity to changes in skin. It is important to note that while devices might prove reliable on some skin sites, because plantar skin is different in anatomy, it is likely to have different biophysical properties than non-plantar skin sites. Therefore reliability must be assessed separately on plantar skin to ensure that any devices used are fit for purpose in this area.

This section of the literature review will explore the different measurement modalities which can be used to measure the skin properties highlighted above including skin surface hydration, skin mechanical properties, skin imaging and skin surface analysis. Table B.1 shows details of the devices of interest and alternative modalities which will be covered in the following section.

Table B.1 – List of measurement devices and alternative measurement modalities

Device name	Skin property measured	Measurement principle (units)	Validated/ on plantar skin?	Alternative measurement modalities
Corneometer® **	Hydration.	Skin surface capacitance (aμ).	Yes / no	Conductance, NMR spectrometry, TTT.
Tewameter® *	Skin barrier function.	TEWL (g/h/m^2).	Yes / no	None.
Cutometer® *	Mechanical properties: viscoelastic and stiffness parameters under negative pressure.	Negative pressure application (mm).	Yes/ yes	Torsion ballistometry, indentation, ultrasound, digital image speckle correlation (direct measures), RRT (surrogate measures)
Reviscometer® **	Mechanical properties: direction and density of collagen and elastin fibres within the skin.	RRT (aμ).	Yes / no	Suction cup method, torsion ballistometry, indentation, ultrasound, digital image speckle correlation (all direct measures).
Diagnostic ultrasound (low frequency).	Cross sectional anatomy/ depth.	Reflection and scattering of ultrasonic waves (B-mode scan).	Yes / no	High frequency diagnostic ultrasound, OCT, MRI.
Dermatoscope	Skin surface topography: visual skin surface imaging.	Direct imaging of skin surface.	No / no	None.
Visioscan®	Skin surface topography: UV skin surface imaging with measures of texture parameters.	UV light emitting camera and specialised software analysing pixel and grey levels (aμ).	Yes / no	None.
Visioline®	Skin surface profilometry: negative replica analysis of wrinkles.	Shadow profilometry.	No / no	Quantimet (automated replica measurement system).

*Direct measure of skin property. **Surrogate measure of skin property (for non-imaging devices).
NMR – Nuclear magnetic resonance; TTT – Transient thermal transfer; TEWL – Trans-epidermal water loss; RRT – Resonance running time; OCT – Optical coherence tomography; MRI – Magnetic resonance imaging.

B.2 Skin surface hydration

The Corneometer® (Courage and Khazaka, Köln, Germany) (Table B.1) measures the stratum corneum hydration based on the principle of capacitance. The Corneometer® comprises two electrodes which are covered in a dielectric material and act as a capacitor. When a material is brought between the two electrodes and voltage is introduced, the amount of charge stored by the capacitor is the capacitance (displayed as an arbitrary unit). The capacitance is influenced by the hydration of the biological material placed in contact with the electrodes. An increase in hydration will lead to an increase in capacitance and vice versa. (Barel and Clarys, 2006, Courage-Khazaka, 2009a).

The Corneometer® has been used in many studies involving different skin sites on the body (Holm et al., 2006, Kim et al., 2002, Matsumoto et al., 2007, Sator et al., 2003, Eberlein-König et al., 2000) but in only two studies evaluating the efficacy of foot treatment (Garrigue et al., 2011, Papanas et al., 2011). The first of these by Garrigue et al. (2011) used Corneometry to compare hydration of the skin of 54 patients with diabetes after using Pedimed® moisturiser and a placebo treatment. The second, by Papanas et al. (2011), used the device to evaluate the efficacy of Neuropad Repair Foam® on the plantar skin of 20 patients with diabetes. Garrigue et al. (2011) only measured the dorsal aspect of the foot despite the fact that the treatment was designed to prevent ulcers on areas of hyperkeratosis and fissures which are generally found on the plantar aspect. Papanas et al. (2011) followed a similar protocol but measured plantar skin on an area free from hyperkeratosis, the rationale for this is unclear. Both studies found a significant increase in skin hydration post-treatment. Garrigue et al. (2011) observed a 48.9% and 57.3% increase in hydration after 14 and 28 days ($p = 0.0002$ and < 0.0001 respectively), and Papanas et al. (2011) observed an 8.9% and a 20.6% increase after seven and 14 days respectively ($p < 0.001$). These results show that the Corneometer® is sensitive to skin changes in the foot – changes which are shown to occur over a short period of time. However there is no data yet available for callused skin.

In terms of validation, there is sufficient evidence to support the accuracy and sensitivity of the Corneometer® for use in vitro (Barel and Clarys, 1997, Fluhr et al., 1999a). These studies report high correlations ($r > 0.8$) between the capacitance values and volume of water within filter paper, solutions with different dielectric constants and between Corneometer® and other hydration measurement instruments (Barel and Clarys, 1997, Fluhr et al., 1999a). Accuracy tests have also been conducted on forearm skin comparing Corneometer® measurements against the Skicon® which uses a conductance measurement method (and can therefore be considered a direct measurement of hydration) (Clarys et al., 1999). The two devices correlated highly ($r = 0.89$) and the intra-rater reliability of the Corneometer® was acceptable with a coefficient of variation (CV) ranging from 9-30% (Clarys et al., 1999). A similar comparative study by Fluhr et al (Fluhr et al., 1999b) found similar results in a population of 20 participants using seven skin sites. The Corneometer® and Skicon® (as well as the Nova DPM impedence and DermaLab capacitance devices) correlated highly and the CV of the Corneometer® ranged from 14-36.8% (Fluhr et al., 1999b). No validation work using the Corneometer® on the foot has yet been published and there has been no inter-rater or inter-day reliability data reported.

Aside from capacitance and conductance methods of measuring skin hydration, there are several other available systems (Table B.1). Nuclear magnetic resonance (NMR) spectrometry provides a direct measurement of epidermal hydration. The technique involves the use of a magnetic field to study the proton content of water (this parameter is termed 'resonance of hydrogen'). Transient thermal transfer (TTT) involves the measurement of heat transfer from the body by sending a thermal pulse through the epidermis (Girard et al., 2000). These two measurement techniques have been compared in a study with the Corneometer®. Girard et al. (2000) investigated different moisturising products on 12 subjects and found good intra-rater reliability with all three devices (Corneometer® CV < 7%; TTT CV < 4% and 2% on different depths; NMR CV < 0.3%). These results were echoed in tests over a three day period using the Corneometer® and TTT. The NMR and TTT methods are able to measure different layers of the epidermis and superficial dermis whereas the Corneometer® only measures the stratum corneum which could contribute to the higher variance as the moisture content of this layer fluctuates, particularly with application of topical agents (Girard et al., 2000).

While the NMR and TTT could be of particular value in foot dermatology research, the Corneometer® is more readily available, far cheaper and easy to use; and has been shown to provide reliable data in several studies. Combined with other instruments measuring different biophysical parameters, it could be of value in the characterisation of plantar skin.

B.3 Trans-epidermal water loss (TEWL)

The Tewameter® TM300 (Table B.1) belongs to a group of electronic instruments called evaporimeters which are used to quantify TEWL through the stratum corneum; a measure of skin barrier function (Elkeeb et al., 2010). The Tewameter® TM300 probe consists of a hollow, cylindrical head (dimensions = 1 x 2cm) which is open at both ends and is thus termed an open chamber evaporimeter. Within this cylindrical chamber lies one pair of sensors which measure moisture and temperature. The location of these sensors allows the moisture gradient between them to be measured. The unit of measurement is diffusion flow rate which is expressed in g/h/m² and derives from Fick's law of Diffusion (Courage-Khazaka, 2012).

In order to produce valid measurements, the evaporimeter method must be proven to be a valid measure of skin barrier function, and the different types of devices available

must correlate with each other. Elkeeb et al. (2010) used TEWL measurement on titrated skin in vitro using three evaporimeters (Tewameter®, Vapometer™ and AquaFlux). They found that TEWL values correlated well with thickness of skin samples (AquaFlux r^2 = -0.44, p = 0.01), two of the three device measurements correlated with the flux of titrated water (Tewameter r^2 = 0.5, p = 0.0. AquaFlux r^2 = 0.034, p0.04) and all the devices could measure skin barrier function over time. Steiner et al. (2011) found that the Tewameter® open and Tewameter® closed devices, and the Tewameter® open and Vapometer devices showed high levels of agreement (r = 0.98 and 0.7 respectively). The Tewameter® has been shown to be more sensitive in the low to ranges of TEWL between 15 – 40 g/h/m^2 and less sensitive above 50 g/h/m^2 (Steiner et al., 2011).

However, not all studies have shown positive results. Chilcott et al. (2002) investigated the relationship between skin permeability using titrated water and TEWL in vitro. They found no significant correlation existed between TEWL and human epidermis (p = 0.72) or porcine skin (p = 0.068) with titrated water, or tape stripping and needle punctures on porcine skin (p = 0.64 and 0.13 respectively) (Chilcott et al., 2002). It is important to note, however, that this study evaluated skin damage in vitro, which may not have provided conditions where skin barrier function could be adequately measured (Levin and Maibach, 2005).

Although measurements from closed and open chamber evaporimeters have been shown to correlate, it is worth explaining the merits of each. One study concluded that a closed chamber device generally does not need to be used in a controlled climate as the chamber shields the measurement site from any air turbulence and can be used in a variety of different orientations whereas the open chamber device shows greater differences during these tests (Tagami et al., 2002). These authors sought to analyse the difference between a closed and open chamber evaporimeter device (Nikkiso-YSI and DermaLab respectively). Like the studies mentioned above, there was a good correlation between the two devices (r^2 = 0.92, p = 0.0001).

Overall, the evaporimeter method of measuring TEWL has been shown to be a valid measure of barrier function. The Tewameter® correlates with other evaporimeter devices which indicate that it accurately performs its task of measuring TEWL. This device has potential in the characterisation of plantar skin and could be particularly valuable when comparing normal skin to callused skin which may have altered barrier function due to possible changes in intercellular lipid profile.

B.4 Measurement of the mechanical properties of the skin

The Cutometer® (Courage and Khazaka, Köln, Germany) (Table B.1) measures the viscoelastic properties of the skin under negative pressure application (Courage-Khazaka, 2010a, Barel et al., 2006). This deformation of the skin is measured (in mm) by an optical light system (Figure B.1) and the data is displayed as a strain-time or stress-strain curve from which many mechanical parameters can be calculated (Courage-Khazaka, 2010a, Barel et al., 2006). The Cutometer® has been used to investigate the effects of aging (Ryu et al., 2008, Sungyeon et al., 2007, Krueger et al., 2011), the effectiveness of different skin grafts (Nguyen et al., 2010, Rahmanian-Schwarz et al., 2011, Sín et al., 2010); and in conditions such as connective tissue disorders (Catala-Petavy et al., 2009), infection (Dobrev, 1998), psoriasis (Dobrev, 2000) and Raynaud's phenomenon (Dobrev, 2007). The Cutometer® has been tested for reliability on scar tissue by two studies (Fong et al., 1997, Draaijers et al., 2004). Fong et al (Fong et al., 1997) found from measurements taken on 12 scar sites that the intraclass correlation coefficient (ICC) value showed good reliability ($r = 0.776$) between three observers using the maximum distension parameter. Draaijers et al (Draaijers et al., 2004) found the reliability to be better on normal skin ($n = 20$) than scars ($n = 49$) with normal skin showing high ICC values for all parameters (> 0.75) and scars showing a range of low to high values ($0.35 – 0.93$) for different parameters with the lowest being obtained from the viscoelastic (UV) parameter. The intra-rater reliability was analysed using CV and this was found to be between 16.1-33.8% for normal skin, and 22.5-36.0% for scar tissue. The authors explained that the lower reliability readings for scars might be because the tissue is stiffer than normal skin, thus giving lower values for each parameter. These, in turn, will be lower compared to the resolution of the device (Draaijers et al., 2004).

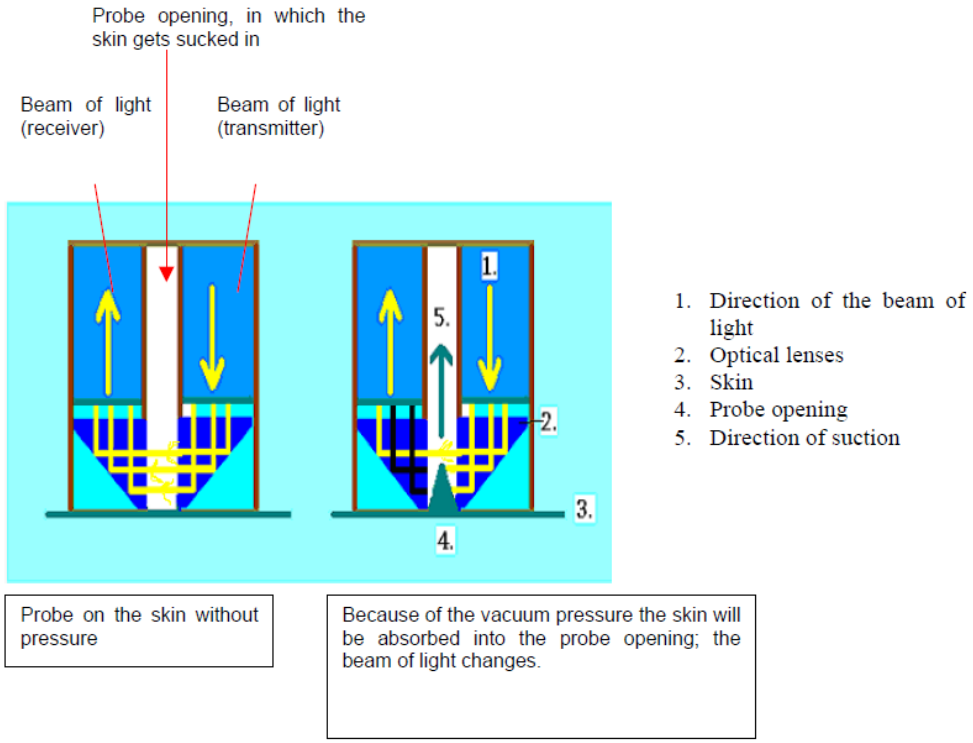


Figure B.1 - Measurement principle of the Cutometer (CK, 2010a p.14)

The Cutometer® has also been tested for use on foot skin by Hashmi and Malone-Lee (2007). The authors analysed pressure data for square wave formations and corrected the data to fit the square wave curves to assess device error on three sites of the foot in 20 people. The error was found to be between 1.3 and 3.3%. The stress-strain relationship was assessed by plotting skin displacement against pressure and the relationship between these was found to be linear ($r = 0.51 – 0.96$) in agreement with Hooke's law which states that stress applied to a material is proportional to the resulting strain (Vincent, 1982). The intra-rater reliability was found to be low ($CV = 0.3 – 0.6\%$). The second stage of the study analysed viscoelastic parameters of the skin on different sites of the foot of 87 people and found that plantar metatarsal skin exhibited the lowest series elastic element, viscoelasticity and plasticity compared to dorsal and medial arch skin (Hashmi and Malone-Lee, 2007). In a study by the same authors (Hashmi et al., 2006), when normal, healthy plantar skin ($n = 87$) was compared with the skin of individuals with diabetes ($n = 103$), the series elastic element on retraction and plasticity of the skin was found to be significantly greater in those with diabetes while the viscoelastic properties were not significantly different. These differences could potentially be due to increases in thickness and stiffness of soft tissues in the feet of individuals with diabetes (Duffin et al., 2002,

Chao et al., 2011). Tests on the inter-day reliability of the Cutometer® have not been carried out to date.

The duration of negative pressure application (*on-time*) and duration of skin relaxation after cessation of negative pressure (*off-time*) can be set by the researcher and influence the elastic (the length of time the skin takes to return to its original state) and plastic (the degree of deformation in shape that occurs) deformation of the skin (Sín et al., 2010, Courage-Khazaka, 2010a). However, the durations of on- and off-times could be a potential source of variation. Having repetitive measures on the same site cause skin creep, meaning that more deformation occurs and the way in which it retracts becomes altered due to being measured in a non-normal state (Dobrev, 2005). Similarly, it is likely that having a single long on-time will also increase skin creep. To obtain accurate information about mechanical properties, an optimal on-time and off-time needs to be set. For the foot, the skin is thicker and less-elastic (Hashmi and Malone-Lee, 2007) which means a greater on-time is required if skin mechanics are to be accurately measured. Hashmi and Malone-Lee (2007) used 60 seconds as on-times and off-times in their work on pedal skin, to achieve this accuracy. This would allow ample time for enough skin to be drawn into the device to minimise any reliability issues, such as were described on scar tissue by Draaijers et al. (2004)

Other, less widely reported mechanical property measuring modalities (Table B.1) include torsion ballistometry (Jemec et al, 2001), indentation (Delalleau et al, 2006; Pailler-Mattei et al, 2008; Zahouani et al, 2009), ultrasound (Diridollou et al, 1998), Frictiometry (Neto et al., 2013) and digital image speckle correlation (Staloff et al, 2008). Few of these techniques have been compared, but those studies which are available are now discussed

Murray and Wickett (1997) found in their study on the mechanical properties of human calf skin that Cutometer® data and Dermal Torque Meter® (another suction cup device) data did not correlate well with each other, or with the Dermal Phase Meter® (a skin capacitance device). This was deemed to be due to the different types of forces applied to the skin (Murray and Wickett, 1997). Jemec et al. (2001) compared a suction cup device (DermaFlex) with a ballistometer (Dia-Stron Torsion Ballistometer) on palmar, and volar and hairy forearm skin, and found a low to moderate correlation between results of the two methods ($r = 0.315 – 0.540$). Again this was most likely due to difference in measurement techniques: the DermaFlex draws skin through an aperture while the ballistometer analyses skin recovery after tapping it with a known force (Jemec et al.,

2001). A later study (Woo et al., 2014) compared Ballistometer parameters to those of Cutometer on forearm, cheek and forehead skin. They found good correlations ($r > 0.07$) between Ballistometer parameters and three of the Cutometer parameters based on distensibility on only cheek skin. Other sites did not show good correlations between devices, again due to differences in measurement techniques (Woo et al., 2014). Pedersen et al. (2003) compared two suction cup devices (DermaLab® and DermaFlex®) and found moderate correlations ($r = 0.383 - 0.437$) in data obtained from volar forearm skin. The authors considered design difference between devices to be the main issue as the devices use Young's Modulus (stress to strain ratio) in their measurements in slightly different ways. The DermaFlex® measures the distance of distension under stress while the DermaLab® relies on elasticity constant and material thickness values in addition to measuring deformation of the skin (Pedersen et al., 2003). Neto et al. (2013) compared the Reviscometer® to the Cutometer® and a friction based skin mechanics measurement device, the Frictiometer® on the abdomen of 34 female volunteers. There was no correlation between the Frictiometer® and Reviscometer®. Weak to moderate correlations were found between Frictiometer® and Cutometer® parameters, and Cutometer® parameters and Reviscometer®. Again, the authors cited difference in methodology as a reason for poor correlations. The literature suggests that these devices can clearly identify differences between skin sites, but the lack of agreement between some of the devices aiming to measure similar parameters is an issue. There is a lack of standardisation in the literature of a single 'best' technique, making it difficult to compare data.

The suction cup method has been the most extensively studied. The advantage of the suction cup based Cutometer® is that an 8mm diameter aperture probe is available which makes it ideal for determining the mechanical properties of the full skin thickness (including the dermis which influences the skin's mechanical properties), and more rigid callused skin, which might otherwise result in unreliable measures using a small aperture probe. Having a wider aperture will allow for greater vertical deformation of the skin (Barel and Clarys, 1995). Ballistometry involves tapping a very small area of skin to assess how it responds (Jemec et al., 2001) but as plantar skin is thick and quite stiff, it may not be able to create a suitable deflection of the skin. Indentation involves the application of a vertical force to the skin causing an indent which is a more accurate measure of softness and water content (Manny-Aframian and Dikstein, 1995) which again might not be suitable for assessing very rigid plantar skin.

The Reviscometer® (Courage and Khazaka, Köln, Germany) (Table B.1) is a relatively new device which purports to measure the elasticity of skin. It is based on the principle of acoustic shear wave propagation whereby an acoustic shockwave is sent between two sensors (approximately 2mm apart) through the skin (Figure B.2). The time which the wave takes to pass from one sensor to the other (resonance running time or RRT) is a surrogate measure of skin stiffness and is displayed as an arbitrary unit (Courage-Khazaka, 2005).

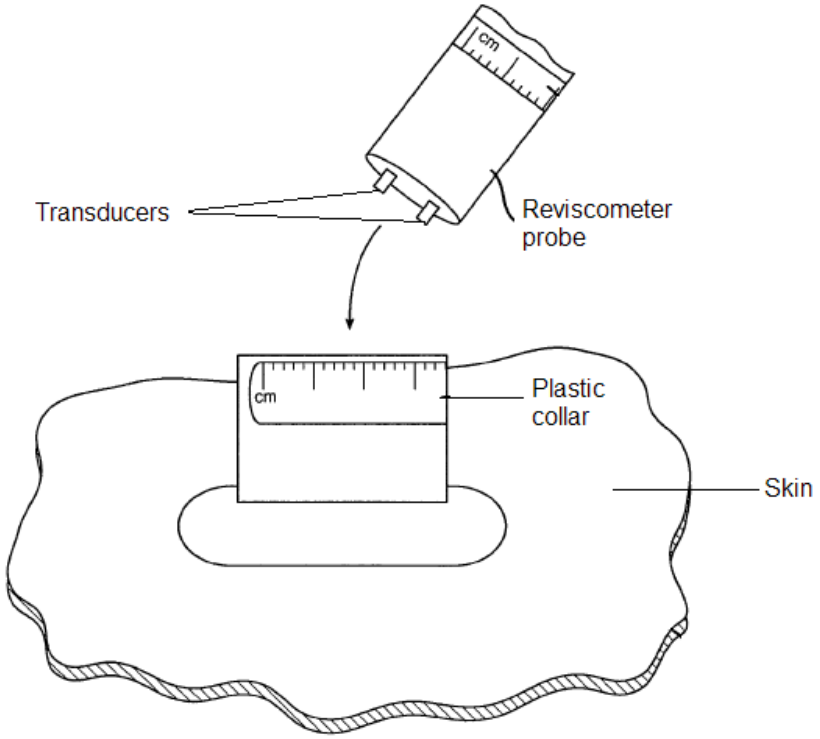


Figure B.2 – Diagram of the Reviscometer® probe (Courage-Khazaka, 2005).

The device has been used in many published studies including tests of skin treatments (Paye et al., 2007, Uhoda et al., 2002) and the assessment of skin variations with age, BMI, body site and gender (Ohshima et al., 2011, Xin et al., 2010, Hermanns-Lê et al., 2001). Two validation studies have been undertaken (Barel et al., 2005, Verhaegen et al., 2010). The first of these (Barel et al., 2005) showed an inverse linear relationship between RRT and polymer stiffness. The RRT showed a significant negative correlation with Young's Modulus from data collected from the polymers using a Cutometer®. The second study by Verhaegen et al. (2010) sought to assess the reliability of the instrument on normal skin (of the forearm, upper arm and abdomen) and scar tissue. Two investigators used the Reviscometer® to take multidirectional measurements from normal skin (n = 50) and scar tissue (n = 50). An intraclass correlation coefficient (ICC) analysis

revealed that the inter-rater reliability was >0.79 on normal skin and >0.86 on scar tissue for all outcome parameters (mean RRT, mean amplitude and mean ratio). The intra-rater reliability was >0.66 for normal skin and >0.75 for scar tissue. This relatively high reliability might be partly because the measurement probe was left in the same position for both investigators. The reliability may have been reduced had each investigator placed the measurement probe on the skin before taking their sets of measurements, as would happen in the clinical setting. No studies have been published detailing the use of the Reviscometer® on pedal skin or on inter-day reliability; however this initial work on reliability appears generally promising.

In areas with a thicker stratum corneum such as plantar skin, there could be an element of error as there is the possibility of the thickness acting as a barrier to shear waves. However, a study by Vexler et al. (1999) used a device similar to the Cutometer® on agar gels of varying thicknesses over silicon rubbers of varying stiffness and found that their standard deviations were less than 3%. However, the same study found that the thinner the agar, the more influence the silicon rubber below had on the measurements (Vexler et al., 1999) which might mean that measurements of the dermal elasticity might be more accurate when the epidermis is thinner or less dense. In an unpublished study (Van Engelen et al., 2008) the shear wave penetration depth by the Reviscometer® was found to be 0.7mm which means that there could be potential issues with measurement on skin sites with a very thick epidermis.

There is some disagreement in the literature about what the RRT (shear wave) measurement values actually mean. Some argue that RRT measures the density and stiffness of the skin (Hermanns-Lê et al., 2001, Koehler et al., 2009). Others argue that RRT may also indicate of degree of alignment - i.e. a lower mean RRT on one site compared to another may indicate that the fibres run more parallel (Verhaegen et al., 2010). However, fibre orientation may have no bearing on skin stiffness and therefore give false indications of differences in stiffness. Some argue that RRT is indicative of direction of skin tension (Uhoda et al., 2002, Quatresooz et al., 2006, Ohshima et al., 2011) while others argue that it reflects the direction of elastic fibres (Dang et al., 2005). All of the studies took multidirectional measurements by rotating the probe between repeated measures. The mean elasticity is equal to the mean value of all measurement orientations so is in effect a composite measure, reflecting variations in stiffness in various rotations. The direction can be identified by determining the maximum (tension) and minimum (laxity and firmness) RRT values (Hermanns-Lê et al., 2001). Anisotropy, the direction of

the skin's tensile strength, can be determined by calculating the mean amplitude or mean ratio (these, in effect, have been found to correspond to the same variable, but the mean amplitude has been shown to be a more reliable measure) (Verhaegen et al., 2010). It can also be calculated from the coefficient of variance (Hermanns-Lê et al., 2001, Uhoda et al., 2002).

It is surprising that many studies using the Reviscometer® have been conducted but none have explicitly investigated what the device actually measures. A future validity study would certainly be of benefit and clarify the device's true value. If the device measures what it claims to, it could have potential in assessing mechanical properties in the skin of patients with diseases such as rheumatoid arthritis and diabetes where the skin's integrity is compromised. In these cases, a device which applies external force such as the Cutometer® would be contraindicated and the Reviscometer® might provide a useful alternative.

There are many devices available which measure the mechanical properties of the skin directly and as a surrogate measure. The Cutometer® is the most common device occurring in the literature and has been validated for use on normal skin, scar tissue and pedal skin. The Reviscometer® has been validated on polymers, normal skin and scar tissue. Both of these devices show promise in the results of the available studies and would likely be of benefit for characterising pedal skin provided they can deliver reliable results. Because they have different measurement methods, they could be of significant worth in gaining a more robust mechanical profile of the skin

B.5 Imaging of the skin

Ultrasound (Table B.1) is the use of high frequency sound waves to visualise anatomical structures within the body. The principle is reflection and scattering of the waves off anatomical structures of variable densities, and different tissues of variable densities within a structure. The transducer creates a pulse and the echoes from the reflection and scattering of the sound waves are received by the transducer. These are then processed by the device and an image is created based on the strength of the echoes (Kremkau, 2006). The cross-sectional image, characteristic of diagnostic ultrasound, is seen with B-mode scanning whereby many A-mode scan pulses (the display which represents the amplitudes of a single line of ultrasound echoes) are processed into a greyscale image (Serup et al., 1995).

The use of diagnostic ultrasound in dermatology is not a new concept. Generally in skin research, high frequency ultrasound (above 20MHz) is used as it gives a high level of clarity of both the epidermis and dermis (general use of ultrasound in dermatology is reviewed by Fornage (1995), Schmid-Wendtner and Dill-Muller (2008), Jasaitiene et al. (2011) and Kleinerman et al. (2012)).

There have been many studies that have validated ultrasound for skin thickness measurements by comparing malignant skin lesion images with histological specimens (Fornage et al., 1993, Lassau et al., 1999, Bessoud et al., 2003, Pellacani and Seidenari, 2003, Lassau et al., 2006, Bobadilla et al., 2008, Machet et al., 2009, Vilana et al., 2009). For low frequency ultrasound between 7 and 15 MHz, the correlation between images and specimens is above 0.9 (Lassau et al., 2006, Bobadilla et al., 2008, Vilana et al., 2009), and for high frequency ultrasound at 20MHz, the correlation is above 0.89 (Fornage et al., 1993, Lassau et al., 1999, Bessoud et al., 2003, Pellacani and Seidenari, 2003, Machet et al., 2009). These studies are evidence of the potential accuracy of both low and high frequency ultrasound in skin thickness measurement. Particular advantages of this modality are that it is readily available and poses no risk to patients.

Plantar skin ultrasound studies are scarce and have focussed on the epidermal thickness of patients with diabetes. Duffin et al. (2002) found no significant difference in plantar skin thickness between healthy controls and individuals with type 1 diabetes. However, this study used a 7.5MHz transducer which may not have been sensitive enough to detect minute differences. Hashmi et al. (2006), using 20MHz, found plantar skin to be significantly thicker in individuals with type 2 diabetes than the healthy control group ($p = 0.017$). Using a 55MHz transducer, Chao et al. (2011) found plantar skin on average to be 6% thicker in patients with diabetes compared to controls.

Despite the good quality images that high frequency ultrasound can deliver, the challenge increases with the presence of hyperkeratosis. Low frequency ultrasound can be used to detect structures deep within the dermis (Jasaitiene et al., 2011) and has been used to assess the mechanical properties of deeper plantar soft tissues (Bygrave and Betts, 1992, Cavanagh, 1999, Hsu et al., 2005). Nishide et al. (2009) used low frequency ultrasound (10MHz) to assess inflammation caused by callused tissue as a predictor of ulcer development in patients with diabetes (Nishide et al., 2009). However, this study did not assess the lesion itself, only the oedema present in the subcutaneous tissue. Potter and Potter (2000a) used A-mode ultrasound to study the speed of callus re-growth. From their pilot work, they determined that A-mode scanning gave reliable results (CV < 4%) for

measuring callus thickness. However, this mode of scanning is wrought with potential error. Because there is no image production in A-mode scanning, repeatable positioning of the transducer is very difficult, the sonographs have to be carefully interpreted, and accompanying anatomical features that may affect data may go completely unnoticed (Potter and Potter, 2000a).

In the context for the research conducted for this thesis, high frequency ultrasound would likely be little value in measuring calluses because thickness and density of the stratum corneum would obscure the image. Keratotic material can cause shadowing and strong reflection obscuring an image (Serup et al., 1995, Schmid-Wendtner and Dill-Muller, 2008). A-mode ultrasound scanning would not be practical due to the lack of an image and difficulty with repeatable probe positioning. An example of a low 7.5MHz frequency image of the plantar metatarsal area (PMA) is shown in Figure B.3.

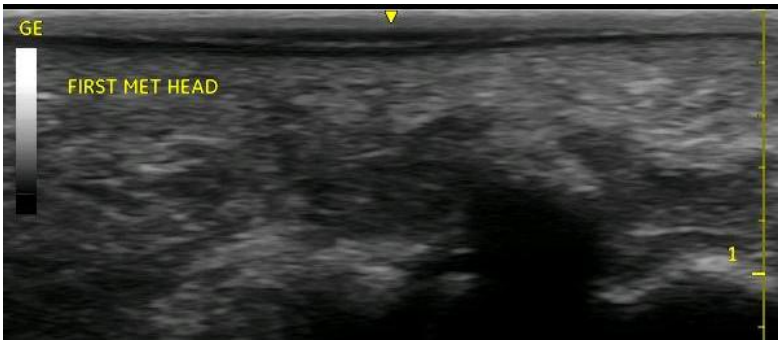


Figure B.3 – Low frequency ultrasound image of tissue overlying the first metatarsal head. The black stripes near the top of the image reflect the type of obscurity that results from measuring plantar skin. The ultrasound is unable to produce a clear image of the skin due to the amount of keratotic material present in plantar skin.

Other methods for measuring the thickness of skin are available (Table B.1). The most recent innovation is optical coherence tomography (OCT), which uses optical light reflections to produce a high resolution cross-sectional image (Gambichler et al., 2005). Several studies have used the device in the assessment of skin thickness. The OCT measurement technique provides epidermal thickness data which correlates to that obtained by histology, although due to the removal and processing of histological specimens, the explicit values are different (Gambichler et al., 2006, Gambichler et al., 2007, Silver et al., 2012). However, identification of the dermal-epidermal junction (DEJ) is necessary to provide accurate measurements (Gambichler et al., 2006, Josse et al.,

2011). Although no studies measuring plantar skin thickness with this device have been published, Michelson Diagnostics (2012) have provided the author with images of plantar skin which clearly show the layers of the epidermis and DEJ. Figure B.4 shows an OCT image of plantar heel skin. This method of imaging shows promise in dermatology and would be a useful commodity for this research project. However due to the very high cost, it is not readily available.

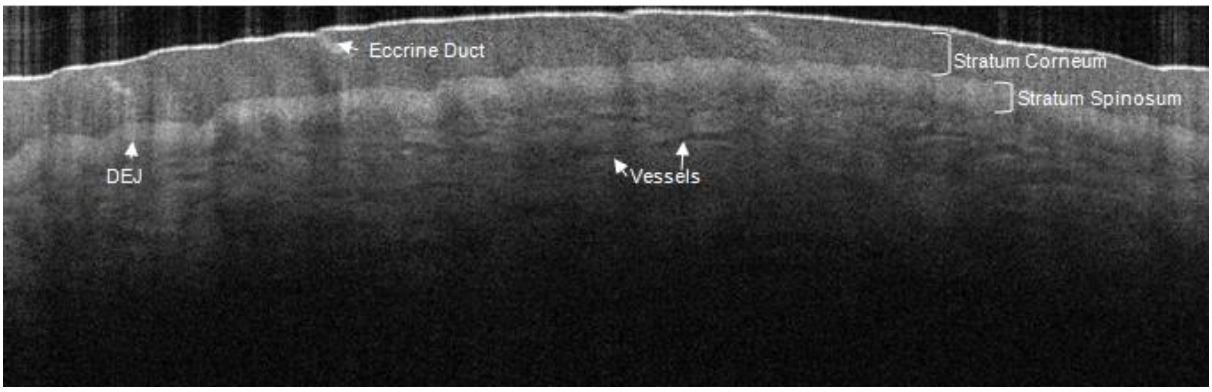


Figure B.4 – OCT image of plantar heel skin. Here, various layers and landmarks within the skin can be clearly observed.

Magnetic resonance imaging (MRI) is another option for the study of skin thickness. This technique has been deemed to be acceptable in measurement of skin thickness in healthy individuals (Sans et al., 2011). It can also detect changes in the behaviour of water within the skin layers (Richard et al., 1991, Richard et al., 1993, Mirrashed and Sharp, 2004a) and can be useful in assessing states of altered hydration such as in lymphodema (Idy-Peretti et al., 1998). Research into the hydration of skin using moisturisers has also been undertaken and has found MRI to be a useful modality (Mirrashed and Sharp, 2004b).

MRI could potentially be a very effective method in the assessment of plantar skin in the proposed research. However it is expensive, largely inaccessible to researchers outside of radiology, requires specialist training to use, is impractical for recruitment purposes and is also potentially hazardous due to its powerful magnetic field which would pose a danger to subjects or researchers with metal implants or jewellery.

Overall, there are several useful modalities that could be useful for imaging of the skin. MRI and OCT would certainly provide excellent quality images but are unfortunately very expensive and not readily available. Diagnostic ultrasound has been widely used in the characterisation of skin carcinomas and has been validated inr this area. Low frequency

ultrasound is likely to be the best choice for imaging callused skin but may lack image clarity.

B.6 Measurement of skin topography

The measurement of skin topographical properties would be a useful adjunct alongside other measurements, such as hydration and mechanical properties, as there is a noticeable visible change from normal skin to callused skin which likely reflects changes in the microstructural and mechanical properties occurring. There are several instruments available which measure the skin's surface topography. As topography is a wide subject area, the devices available measure different parameters of the skin's surface.

Dermoscopy (Table B.1) is a technique where direct imaging of the skin's surface is achieved (Figure B.5). A wealth of research has been conducted using this modality in the field of melanoma diagnosis (reviewed by Braun et al. (2009)). Several papers have reported the use of dermoscopy in the assessment of pedal skin lesions. Bae et al. (2009) highlighted the usefulness of dermoscopy in differentiating between plantar warts, calluses and corns by assessing the topographical characteristics under magnification. Saida et al. (2004), Miyazaki et al. (2005), and Altamura et al. (2006) have used dermoscopy as a tool for differentiating between benign and malignant melanocytic lesions on pedal skin through analysing the specific topographical patterns. The value of using dermoscopy to assess skin topography is clear from the research papers, and this could have potential value in assessing the skin surface characteristics of plantar callus.

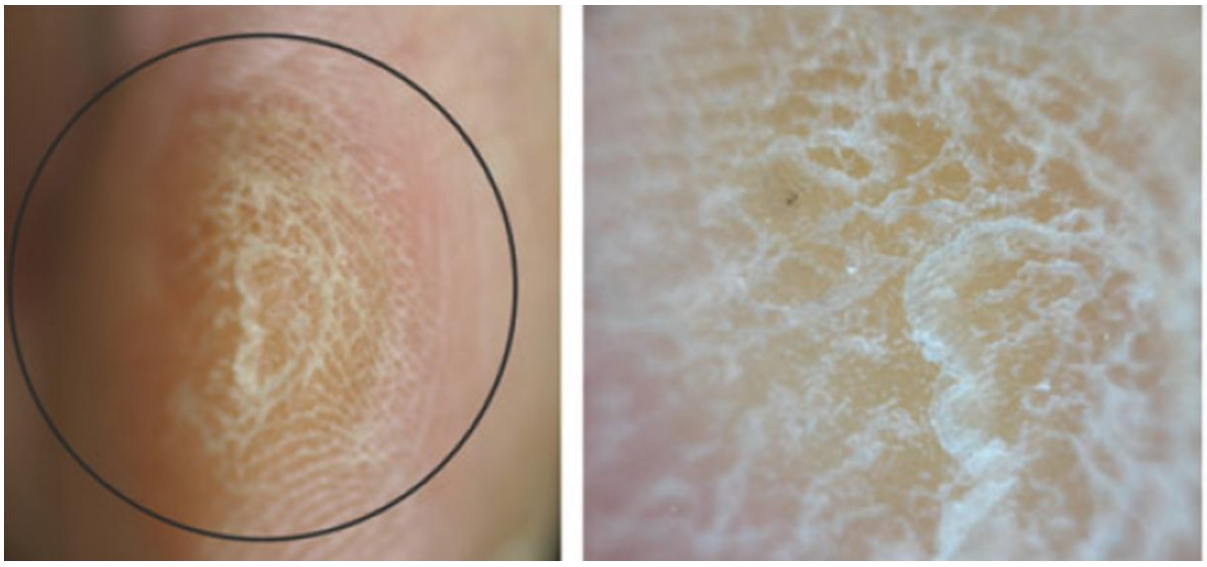


Figure B.5 - Callus photograph (A) and corresponding dermatoscope image (B) (Bae et al., 2009, p.222).

The Visioscan® (Courage and Khazaka, Köln, Germany) (Table B.1) is a device which uses a UV light emitting camera to capture images of the surface of the skin producing a high definition image of the stratum corneum surface (Figure B.6). The images are then processed using specialised software (SELS software) based on chosen parameters of skin surface measurement (Courage-Khazaka, 2009b, Tronnier, 1999). The device has been used in various studies analysing skin treatments (Pena Ferreira et al., 2010, Berardesca et al., 2006, Choi et al., 2012), evaluation of the scalp (Pierard et al., 2012, Pierard-Franchimont et al., 2011, Xhauflaire-Uhoda et al., 2010), and the effects of photoaging (Petit et al., 2003, Quatresooz et al., 2011). One study has assessed the reliability of the device (Kottner et al., 2013). Three measures were taken from four volar forearm sites on 12 participants. The ICC was calculated to assess intra-site reliability and it was found that the Visioscan® SELS and roughness parameters all gave highly reliable results (ICC > 0.95). However, these favourable results may have been due to the fact that each set of images was taken by a single investigator (it is not stated whether investigators were changed between participants), all of which were taken without removing the device away from the skin. Also forearm skin is generally homogenous whereas studies of callus involve normal and callused areas. No research with this device has been undertaken on the foot and there are no studies which have investigated the device's inter-rater or inter-day reliability.

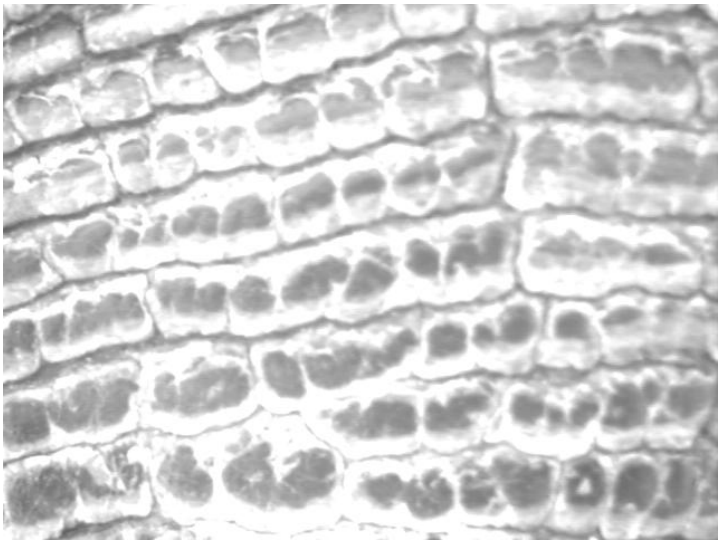


Figure B.6 - Visioscan® image of callused skin.

The Visioline® (Courage and Khazaka, Köln, Germany) (Table B.1) is a device which is designed specifically for shadow profilometry of wrinkles, undulations in the skin's surface. A silicone negative replica is taken of the skin surface and then placed under an oblique light source. A shadow is formed by the replica where the skin surface shows depressions and wrinkles allowing for the difference in height between high and low areas to be measured (Courage-Khazaka, 2010b). Shadow profilometry is not a new concept, and studies have been undertaken exploring skin furrows multidirectionally on silicone skin replicas using automated measurement systems (Corcuff et al., 1983, Corcuff et al., 1984, Corcuff et al., 1987, Corcuff et al., 1991). So far however, the Visioline®, a system whereby replicas are oriented and measured manually, has been the topic of very few published research papers and its relationship to other measures of topography have not been reported. Two papers identified by the author include mouse model studies: one assessing the effects of ingestion of collagen tripeptide on photo aging and skin barrier function (Pyun et al., 2012) and the other assessing the effects of cultured fibroblasts on wrinkled murine skin (Jeong et al., 2015). In both cases the device was used for skin replica analysis. The proposed advantages over Visioscan® might include the fact that an image of a high quality replica can give exceptional detail of the skin surface contours (Figure B.7) including those caused by striations and sweat ducts.

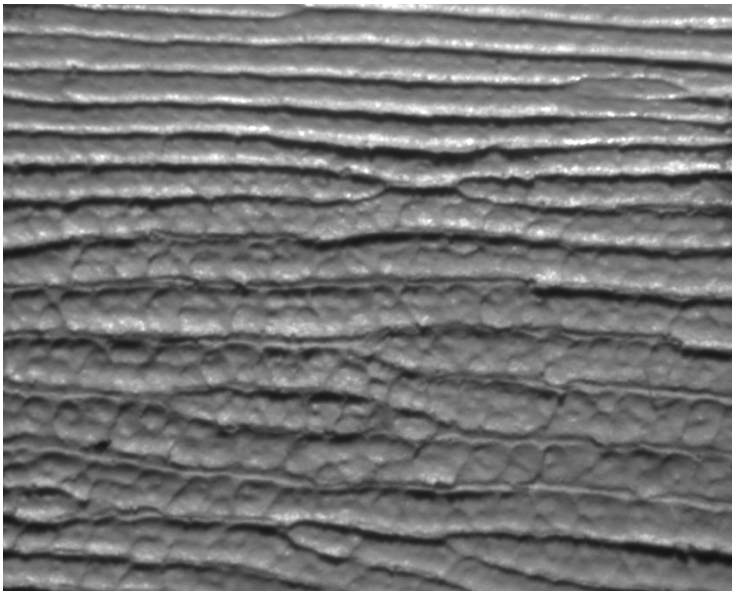


Figure B.7 - Visioline® replica of a callus strip.

Unlike other devices measuring biophysical properties of the skin discussed previously, the validation literature surrounding devices measuring skin surface topography is scant. Dermoscopy has been shown to be useful in visually characterising malignant lesions while the Visioscan® has been validated on forearm skin. While the Visioline® has no supporting literature available yet, it may also be of use for visual analysis of skin topographical characteristics. Because it uses shadow profilometry, it may be useful for assessing alternative skin characteristics to the dermoscopy such as skin striation or sweat ducts which might be affected in callused skin.

B.7 Gaps in skin measurement research

This section has highlighted several key areas which need to be addressed. (1) With the exception of the Cutometer®, few measures of skin's biophysical properties have been used and validated on the foot (Corneometer® has been used in efficacy studies involving pedal skin (Garrigue et al., 2011, Papanas et al., 2011) but has not been tested for reliability in this area). (2) There is also an overall lack of literature which supports the reliability of these devices in general. While some studies have looked at inter- and intra-rater reliability within a single session using CV and ICC, there has not yet been a study published which has looked at inter-day reliability. (3) The relationship between skin hydration, TEWL, mechanical properties and topography has never previously been explored in plantar skin research.

Conclusion

This review has enabled a substantial acquisition of knowledge in the area of plantar skin physiology, biochemical and biomechanical skin properties, and devices available to measure plantar skin properties. This has enabled the main research problems related to understanding of plantar callus to be identified.

One main conclusion is that there is a lack of research characterising normal and hyperkeratotic plantar foot skin. There is also a lack of literature linking external mechanical factors (compressive and shear pressures) to plantar callus development. An improved scientific knowledge of normal, callused skin and the pathway to callus development would be useful in the evaluation and design of interventions to prevent and treat these common, troublesome skin lesions, and could be fundamental to clinical practice.

In order to characterise the skin, skin hydration, TEWL, mechanical properties, thickness and topography should be considered. These are expected to be interdependent properties. For each skin property, a number of measurement techniques are available. This critical review supports the use of the following instruments: the Corneometer®, the Cutometer®, the Reviscometer®, diagnostic ultrasound, dermoscopy, the Visioline® and the Visioscan® in that what they measure has face validity as a minimum and some show good reliability for non-foot applications. However, before being used to characterise plantar skin, they need to be validated for use in this area of the body.

Therefore, the purpose of the first study in this thesis will be to assess the inter-rater and inter-day reliability of these devices which will inform whether these devices are appropriate for plantar skin research (Chapter 3). This will then inform the instruments which are used to characterise normal and callused plantar skin in the second study of this thesis (Chapter 4). Once a robust knowledge of normal and callus skin properties has been established, further work will seek to investigate whether it is feasible to induce callus-like skin changes to normal skin through mechanical loading (Chapters 5 and 6), and changes in callused skin as a result of pressure reduction (Chapter 7).

Chapter 3: Inter-rater and inter-day reliability of non-invasive instruments measuring the biophysical properties of foot skin

3.1 Introduction

In Chapter 2, the author highlighted the need to assess the reliability of available biophysical skin measurement tools for use on the foot. If the devices are deemed to provide reliable data, they can then be used in further research to investigate the biophysical characteristics of normal and callused plantar skin which will be useful in the clinical and research settings. Reliability must be evident between days for one investigator using a measurement device (inter-day) and also between two or more investigators using the same device in the same session (inter-rater). These factors must be investigated because there is a lack of literature that outlines the reliability of devices required to characterise normal and callused foot skin. Also, because plantar skin is weight bearing, it is thicker and stiffer than normal skin, and may have completely different biophysical properties as a result, so its measurement using devices that are designed for other areas of the body might be difficult. Therefore, fitness for purpose and proof of reliability is essential if these devices are to be used in plantar skin research.

In this study, three specific biophysical properties of the skin were selected for measurement which would provide comprehensive information of plantar skin. These properties included skin surface hydration, skin stiffness, and skin surface profilometry. The instruments of choice were: Corneometer® (skin surface hydration), Cutometer® (distensibility) and Visioscan® (skin surface roughness). Aside from the Cutometer®, none of these devices have been validated for use on pedal skin.

Five additional devices that were reviewed in the previous chapter were excluded from the study. Diagnostic ultrasound was excluded as preliminary tests with the device revealed that images of callus lesions were not clear enough to measure. Dermoscopy was excluded on the grounds that high quality quantitative data could be collected with Visioscan®. The Tewameter® was excluded as in-vivo and in-vitro preliminary tests showed the device to be too sensitive to movement to use. It was also impractical for use on the foot as the device is designed to be held horizontally at a perpendicular angle to the skin, which would make foot positioning difficult and many surfaces on the foot are not flat. The Reviscometer® was excluded because it was found that the data collected on hard, callused skin was invalid and produced outlier data. As the Reviscometer® provides only a

surrogate measure of skin mechanical properties, the Cutometer® was preferred due to its direct measurement of distensibility. The Visioline® was excluded due to the fact that while striation and sweat duct topography might be interesting skin measures, they are time consuming and difficult to measure, prone to human error and are as not important for understanding the properties of callus as the other measures.

3.2 Aims and objectives

The aims of this study were to test the inter-rater and inter-day reliability of the Corneometer®, Cutometer® and Visioscan® to assess whether they could be of use in subsequent research studies. The objectives included collecting biophysical data from each device on normal and callused skin on a small sample of participants with two investigators over two days, and analysing agreement between raters and between days.

3.3 Materials and methods

Ethical approval for this study was granted by the University of Salford's College Research Ethics Panel (application number HSCR12/09).

3.3.1 Subjects

Participants were recruited via a poster campaign on the University campus including the Podiatry Clinic and an automated email was sent to staff and students. Participants who had a plantar forefoot callus were recruited provided they did not meet any of the following exclusion criteria which could potentially affect the skin's properties: compromised cardiovascular or neurological status, connective tissue disorders (such as lupus erythematosus), diabetes, autoimmune disorders (such as rheumatoid arthritis), peripheral vascular disease, or wounds/ ulcers of the legs and feet, and eczema, psoriasis or other dry skin disorders affecting the plantar skin.

The foot was assessed by the author of this thesis and confirmed by a HCPC registered podiatrist. Pulses from the posterior tibial and dorsalis pedis arteries were assessed by palpation. Presence of neuropathy was assessed by vibration perception using a 128MHz tuning fork and 10g monofilament test on the hallux, first and fifth metatarsal heads. If these tests, skin assessment and medical history were satisfied, the volunteers were recruited to participate in the study.

3.3.2 Data collection

Data was collected on two consecutive days. At each session, the foot was allowed to acclimatise for approximately 15 minutes before measurements were taken. Temperature and humidity during each session was monitored but not controlled. The participant lay on a plinth with the plantar surface of the foot facing the investigators.

The skin sites measured included: centre of callus, edge of callus, adjacent skin (the distance of the lesion's diameter medial or lateral from the centre of the lesion), and two normal weight bearing skin sites including (1) non-callused skin overlying the plantar metatarsal area (PMA), and (2) adjacent skin overlying the base of the fifth metatarsal (figure 3.1). The callus centre was chosen because of its location at the centre of the lesion, and could thus be considered the most 'callused' area. The callus edge was chosen because of its potential importance in later longitudinal studies; a change in callus properties post-treatment may potentially become apparent faster at an area of 'less callused' skin, such as the boundary of the lesion itself. Adjacent skin located close to the callus was chosen because of the potential for skin that is normal in appearance to still display callus-like properties due to its close proximity to the lesion, which could thus aid to evaluation of interventions. Normal weight-bearing (PMA) and semi weight-bearing (fifth metatarsal base) skin sites were chosen to compare with callused skin and to give a good profile of normal skin properties.

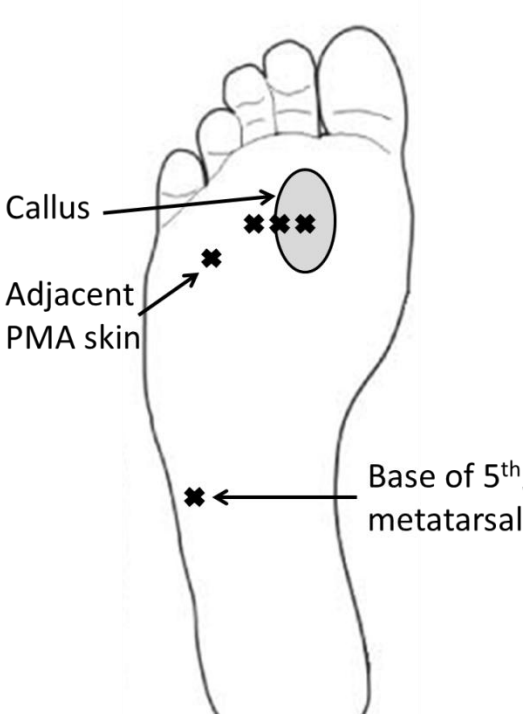


Figure 3.1 - The measurement sites on the callus include the centre, edge, and skin directly adjacent to the callus; and skin overlying the plantar metatarsal area (PMA) and base of the 5th metatarsal (adapted from Hashmi and Malone-Lee, 2007 p.253).

On day one, the skin sites to be measured were marked with a water soluble ball point pen. The centre and edge of the callus were identified and the width was measured using a ruler. Generally there is a difference in skin texture and hardness between callused and non-callused skin so the boundary between these areas, the callus edge, can be visualised and palpated. The adjacent skin was positioned 200% of the radius length away from the callus centre. The fourth metatarsal was used as a control site, unless the callus lay over this site, in which case the first metatarsal head was used. The fifth metatarsal base of the same foot was used as a second control site. Both control sites were identified through palpation and marked. The distance of the radii of the measurement probes were marked over the centre of each skin measurement site to allow accurate probe placement.

For each skin site and device, the first investigator would take measurements followed by the second investigator. Each investigator was blinded to their own measurements during testing to eliminate measurement bias. On the second day, the investigator order was reversed. Measurements were taken based on manufacturers' instructions but adjusted for the needs of plantar skin testing as follows. For each investigator, 10 measurements were taken per skin site using the Corneometer® and one image was taken per site for the Visioscan®. For the Cutometer®, 500mbar of negative

pressure was applied. The on-time and off-time were set to 30 seconds each. One cycle of on-time and off-time was used on each skin site by both investigators.

Single data values for hydration are produced by the Corneometer® and a mean was taken from the 10 measurement values obtained from each skin site. The Cutometer® produces a range of different values based on stiffness, elasticity and plasticity. The Uf parameter, which measures maximum skin distensibility (a surrogate measure for stiffness), was chosen based on the hypothesis that callused skin is stiffer than normal plantar skin. Two variables, *variance* and *homogeneity*, were obtained from the Visioscan® images. These were chosen based on the hypothesis that callused skin is rougher than normal skin and therefore more variable and less homogenous in appearance. The homogeneity parameter is a measure of how homogenous the skin is and measures the combinations of grey levels in the image. The more frequent the grey level combination appears in the image, the lower the homogeneity and the rougher the skin (Courage-Khazaka, 2009b). The variance parameter is a measure of how variable the grey level pixel values are within the image and is higher the rougher the skin is (Courage-Khazaka, 2009b).

3.3.3 Statistical analysis

To compare inter-rater and inter-day reliability, the ability of an instrument to distinguish between individuals (de Vet et al., 2006), the intraclass correlation coefficient test was used (Shrout and Fleiss, 1979). This test calculates reliability by dividing the within subject variance (σ_p^2) by the within subject variance plus measurement error: the systematic variance between raters (σ_{pt}^2) and the variance not explained by test subjects or raters ($\sigma_{Residual}^2$) (de Vet et al., 2006). Systematic variance between raters were included in the measurement error because these are important to take into account when devices are intended to be used by different investigators (de Vet et al., 2006).

Intraclass correlation coefficient

$$ICC = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_{pt}^2 + \sigma_{Residual}^2}$$

(de Vet et al., 2006, p.1036)

This test was chosen over Kappa because it is designed for continuous data whereas Kappa is designed for categorical data (Landis and Koch, 1977). Based on reliability statistic criteria for the Kappa equation, agreement ratings were assessed according to the following: slight (r = 0.0 – 0.2), fair (r = 0.21 – 0.4), moderate (r = 0.41 – 0.6), substantial (r = 0.61 – 0.8), and high (r = 0.81 – 1.0) (Landis and Koch, 1977, p.165).

To assess the inter-rater and inter-day agreement, the standard error of measurement (SEM) and smallest detectable change (SDC, also known as smallest detectable difference, minimum detectable change or minimum important difference) (de Vet et al., 2006) were calculated. The SEM gives the measurement error by calculating the square root of the error variance. This is done by dividing the standard deviations of the mean differences by the square root of two. The square root of two is used because change is calculated by the difference between two measurement values (de Vet et al., 2006).

Standard error of measurement

$$SEM = \frac{SD_{Differences}}{\sqrt{2}}$$

(de Vet et al., 2006, p.1037)

The SEM is useful alone when there is a clear understanding of the data, and of what magnitude of difference is important. However, when this is unclear, the SDC can be calculated and conceptualises the SEM value. The SDC gives the value which a change must exceed in order to be considered real and not due to error - it gives a value which can be considered a guide for detecting clinically relevant changes in data (de Vet et al., 2006). It is calculated by multiplying the SEM by the square root of two and 1.96, and produces the same value at Bland and Altman's Limits of Agreement.

Smallest detectable change

$$SDC = 1.96 \times \sqrt{2} \times SEM$$

(de Vet et al., 2006, p.1038)

Descriptive data including means, 95% confident intervals (CI) and percentage differences between datasets were also calculated. Percentage difference was calculated by

subtracting value 2 from value 1, dividing by the value 1 and multiplying by 100. In this study, Day 2 and Investigator 2's device readings were taken as value 2 for inter-day and inter-rater differences.

Percentage difference

$$\%\, difference = \frac{Value\ 2 - Value\ 1}{Value\ 1} \times 100$$

Statistical analysis was undertaken on SPSS 20.0 and Microsoft Office Excel 2010.

3.4 Results

The following section outlines the result of the study. 'Inter-rater' reliability refers to reliability assessed between the two investigators on day 1, then again on day 2. 'Inter-day' reliability refers to reliability for each investigator between the two days. Inter-day reliability may also be referred to as intra-rater reliability because it assesses two sets of results of one rater, but the term 'inter-day' was deemed more appropriate because measurement sessions were carried out one day apart. Eight healthy adults (with a total of eight calluses) gave informed consent to take part in the study. Of the eight calluses, three were consistent with Merriman's grade 1 'no specific callus plaque, but diffuse or pinch callus, or present in narrow bands' and five were consistent with grade 2 'circumscribed, punctuate oval or circular, well-defined thickening of keratinized tissue' (Springett and Merriman, 1995, p.207).

3.4.1 Corneometer®

The inter-rater and inter-day reliability for the Corneometer® data is shown in table 3.1 – 3.2. ICC values were high across most skin sites (> 0.8) for both inter-rater and inter-day reliability with a few exceptions. For the callus centre, the inter-day reliability was moderate (r = 0.44) for Investigator 1; and fair (r = 0.25) for Investigator 2. The SEM and SDC values ranged from 0.73 – 2.28au and 1.9 – 6.32au respectively between investigators. Between days, the SEM and SDC values ranged from 1.36 – 3.69au and 3.76 – 10.23au respectively. The percentage differences between investigators were variable. The highest percentage differences on Day 1 were found at the callus edge and skin overlying the fifth metatarsal base (14.9% and 7.8% respectively). On Day 2, the highest

differences were at the callus edge and normal PMA skin sites (13.9% and 11.2% respectively). The largest percentage differences for Investigator 1 were at the callus centre and adjacent skin sites (28.5% and 13.6% respectively). For Investigator 2, the largest differences were at the callus centre and normal skin overlying the PMA (21.9% and 10.2% respectively).

Table 3.1 – Corneometer® inter-rater reliability

Skin site	Mean (95% CI) Investigator 1	Mean (95% CI) Investigator 2	% difference	ICC	SEM	SDC
Day 1						
Callus centre	4.39 (1.48 - 7.31)	4.53 (1.39 - 7.67)	3.1	0.8	1.56	4.32
Callus edge	7.92 (1.18 - 14.67)	6.74 (1.77 - 11.71)	14.9	0.87	2.28	6.32
Skin adjacent to callus	11.99 (2.69 - 21.29)	11.39 (2.65 - 20.13)	5.0	0.98	1.36	3.77
Normal PMA skin	13.57 (5.72 - 21.42)	14.05 (5.67 - 22.43)	3.6	1.0	0.89	2.47
Skin overlying 5th metatarsal base	15.33 (9.34 - 21.32)	14.14 (9.49 - 18.79)	7.8	0.93	1.36	3.77
Day 2						
Callus centre	5.65 (3.12 - 8.17)	5.52 (3.25 - 7.80)	2.2	0.94	0.68	1.9
Callus edge	7.10 (2.53 - 11.66)	6.11 (2.31 - 9.90)	13.9	0.92	1.21	3.36
Skin adjacent to callus	10.36 (3.84 - 16.89)	10.61 (5.25 - 15.97)	2.4	0.93	1.82	5.04
Normal PMA skin	13.92 (6.95 - 20.89)	15.49 (6.53 - 24.44)	11.2	0.94	1.98	5.49
Skin overlying 5th metatarsal base	14.66 (9.33 - 19.98)	15.35 (9.24 - 21.46)	4.7	0.98	0.73	2.02

Mean, 95% CI, SEM and SDC represent hydration values in arbitrary units.

Table 3.2 – Corneometer® inter-day reliability

Skin site	Mean (95% CI) Day 1	Mean (95% CI) Day 2	% difference	ICC	SEM	SDC
Investigator 1						
Callus centre	4.39 (1.48 - 7.31)	5.65 (3.12 - 8.17)	28.5	0.44	2.2	6.09
Callus edge	7.92 (1.18 - 14.67)	7.10 (2.53 - 11.66)	10.4	0.85	2.51	6.97
Skin adjacent to callus	11.99 (2.69 - 21.29)	10.36 (3.84 - 16.89)	13.6	0.9	2.63	7.28
Normal PMA skin	13.57 (5.72 - 21.42)	13.92 (6.95 - 20.89)	2.6	0.95	1.87	5.18
Skin overlying 5th metatarsal base	15.33 (9.34 - 21.32)	14.66 (9.33 - 19.98)	4.4	0.94	1.53	4.25
Investigator 2						
Callus centre	4.53 (1.39 - 7.67)	5.52 (3.25 - 7.80)	21.9	0.25	2.59	7.18
Callus edge	6.74 (1.77 - 11.71)	6.11 (2.31 - 9.90)	9.4	0.87	1.76	4.88
Skin adjacent to callus	11.39 (2.65 - 20.13)	10.61 (5.25 - 15.97)	6.8	0.8	3.69	10.23
Normal PMA skin	14.05 (5.67 - 22.43)	15.49 (6.53 - 24.44)	10.2	0.97	1.36	3.76
Skin overlying 5th metatarsal base	14.14 (9.49 - 18.79)	15.35 (9.24 - 21.46)	8.6	0.89	1.86	5.15

Mean, 95% CI, SEM and SDC represent hydration values in arbitrary units.

3.4.2 Cutometer®

The inter-rater and inter-day reliability for the Cutometer® data is shown in Tables 3.3 – 3.4. The ICC values on callused skin were high (r = 0.68 – 1.0) for both inter-rater and inter-day reliability, aside from inter-rater reliability for adjacent skin on Day 2 which was moderate (r = 0.42). For both inter-rater and inter-day reliability tests, the ICC values ranged from fair to moderate for PMA skin (r = 0.27 – 0.54) and slight to substantial for the 5th metatarsal base (r = 0.1 – 0.7). The SEM and SDC values ranged from 0.01 – 0.26mm and 0.04 – 0.71mm respectively between investigators. Between days, the SEM and SDC values ranged from 0.05 – 0.28mm and 0.14 – 0.78mm respectively. The percentage differences between investigators were varied. On Day 1, the callus centre had the greatest percentage difference (37.8%) followed by the normal PMA and skin overlying the 5th metatarsal base which were also large (15.1% and 16.6% respectively). For Day 2, the largest differences included callus edge and skin overlying the 5th metatarsal base (20.8%, and 20.9% respectively). Between days, for Investigator 1 the

largest percentage differences included callus centre and edge (36.6% and 18.7% respectively). For Investigator 2, the largest percentage differences were at the skin adjacent to callus and the skin overlying the base of the fifth metatarsal (6.2% and 8.5% respectively).

Table 3.3 – Cutometer® inter-rater reliability

Skin site	Mean (95% CI) Investigator 1	Mean (95% CI) Investigator 2	% difference	ICC	SEM	SDC
Day 1						
Callus centre	0.83 (0.30 - 1.36)	0.52 (-0.01 - 1.04)	37.8	0.71	0.26	0.71
Callus edge	0.65 (0.10 - 1.21)	0.65 (0.17 - 1.13)	0.3	0.91	0.18	0.51
Skin adjacent to callus	1.00 (0.66 - 1.34)	1.03 (0.84 - 1.23)	3.1	0.77	0.15	0.43
Normal PMA skin	1.24 (1.08 - 1.39)	1.05 (0.90 - 1.20)	15.1	0.53	0.06	0.17
Skin overlying 5th metatarsal base	1.03 (0.81 - 1.26)	0.86 (0.74 - 0.98)	16.6	0.51	0.11	0.31
Day 2						
Callus centre	0.52 (0.11 - 0.94)	0.51 (0.09 - 0.93)	3.1	1.0	0.01	0.04
Callus edge	0.77 (0.42 - 1.13)	0.61 (0.19 - 1.03)	20.8	0.87	0.12	0.52
Skin adjacent to callus	1.03 (0.76 - 1.30)	0.97 (0.81 - 1.12)	6.1	0.42	0.19	0.52
Normal PMA skin	1.16 (1.01 - 1.31)	1.05 (0.96 - 1.14)	9.6	0.54	0.07	0.21
Skin overlying 5th metatarsal base	1.00 (0.89 - 1.10)	0.79 (0.74 - 0.83)	20.9	0.1	0.07	0.2

Mean, 95% CI, SEM and SDC represent maximum distension values in mm.

Table 3.4 – Cutometer® inter-day reliability

Skin site	Mean (95% CI) Day 1	Mean (95% CI) Day 2	% difference	ICC	SEM	SDC
Investigator 1						
Callus centre	0.83 (0.30 - 1.36)	0.52 (0.11 - 0.94)	36.6	0.63	0.28	0.78
Callus edge	0.65 (0.10 - 1.21)	0.77 (0.42 - 1.13)	18.7	0.74	0.26	0.73
Skin adjacent to callus	1.00 (0.66 - 1.34)	1.03 (0.76 - 1.30)	2.9	0.87	0.13	0.36
Normal PMA skin	1.24 (1.08 - 1.39)	1.16 (1.01 - 1.31)	6.2	0.27	0.14	0.39
Skin overlying 5th metatarsal base	1.03 (0.81 - 1.26)	1.00 (0.89 - 1.10)	3.5	0.7	0.11	0.3
Investigator 2						
Callus centre	0.52 (-0.01 - 1.04)	0.51 (0.09 - 0.93)	1.4	0.95	0.12	0.34
Callus edge	0.65 (0.17 - 1.13)	0.61 (0.19 - 1.03)	5.8	0.99	0.05	0.14
Skin adjacent to callus	1.03 (0.84 - 1.23)	0.97 (0.81 - 1.12)	6.2	0.68	0.11	0.3
Normal PMA skin	1.05 (0.90 - 1.20)	1.05 (0.96 - 1.14)	0.2	0.37	0.11	0.31
Skin overlying 5th metatarsal base	0.86 (0.74 - 0.98)	0.79 (0.74 - 0.83)	8.5	0.26	0.08	0.23

Mean, 95% CI, SEM and SDC represent maximum distension values in mm.

3.4.3 Visioscan®

The inter-rater and inter-day reliability for the Visioscan® data is shown in Tables 3.5 – 3.6 (homogeneity) and 3.7 – 3.8 (variance). Across both parameters, the inter-rater and inter-day reliability ranged from moderate to high (r = 0.52 – 0.91). For the homogeneity parameter, the SEM and SDC values ranged from 0.02 – 0.06au and 0.06 – 0.16au respectively between investigators and 0.04 – 0.08au and 0.1 –0.23au respectively between days. For the variance parameter, the SEM and SDC values ranged from 0.4 – 0.93au and 1.11 – 2.58au respectively between investigators and 0.49 – 0.81au and 1.37 – 2.24au respectively between days. For homogeneity, the largest percentage differences between investigators was at skin overlying the base of the fifth metatarsal on Day 1 (3.1% difference) and over the PMA on Day 2 (1.5% difference). Between days, the highest percentage difference for both Investigator 1 and Investigator 2 was at the normal PMA skin site (1.3% and 2.9% respectively). For variance data, the largest difference between investigators was for normal PMA skin on Day 1 (12.2%) and on Day 2, the callus centre (4.1%). The largest inter-day percentage difference for Investigator 1 was at the normal PMA skin site (3.6%) and for Investigator 2, the callus centre (12.6%).

Table 3.5 – Visioscan® homogeneity inter-rater reliability

Skin site	Mean (95% CI) Investigator 1	Mean (95% CI) Investigator 2	% difference	ICC	SEM	SDC
Day 1						
Callus centre	1.36 (1.23 - 1.49)	1.39 (1.29 - 1.49)	1.9	0.83	0.05	0.14
Normal PMA skin	1.45 (1.34 - 1.56)	1.49 (1.39 - 1.59)	2.8	0.91	0.02	0.06
Skin overlying 5th metatarsal base	1.42 (1.34 - 1.49)	1.46 (1.39 - 1.53)	3.1	0.54	0.05	0.14
Day 2						
Callus centre	1.36 (1.26 - 1.45)	1.34 (1.25 - 1.44)	1.0	0.75	0.05	0.15
Normal PMA skin	1.47 (1.38 - 1.56)	1.45 (1.33 - 1.57)	1.5	0.76	0.06	0.16
Skin overlying 5th metatarsal base	1.40 (1.33 - 1.48)	1.42 (1.34 - 1.51)	1.3	0.87	0.03	0.09

Mean, 95% CI, SEM and SDC represent homogeneity values in au.

Table 3.6 – Visioscan® homogeneity inter-day reliability

Skin site	Mean (95% CI) Day 1	Mean (95% CI) Day 2	% difference	ICC	SEM	SDC
Investigator 1						
Callus centre	1.36 (1.23 - 1.49)	1.36 (1.26 - 1.45)	0.5	0.87	0.05	0.13
Normal PMA skin	1.45 (1.34 - 1.56)	1.47 (1.38 - 1.56)	1.3	0.68	0.06	0.17
Skin overlying 5th metatarsal base	1.42 (1.34 - 1.49)	1.40 (1.33 - 1.48)	0.8	0.7	0.05	0.13
Investigator 2						
Callus centre	1.39 (1.29 - 1.49)	1.34 (1.25 - 1.44)	3.2	0.82	0.04	0.1
Normal PMA skin	1.49 (1.39 - 1.59)	1.45 (1.33 - 1.57)	2.9	0.54	0.08	0.23
Skin overlying 5th metatarsal base	1.46 (1.39 - 1.53)	1.42 (1.34 - 1.51)	2.5	0.52	0.06	0.16

Mean, 95% CI, SEM and SDC represent homogeneity values in au.

Table 3.7 – Visioscan® variance inter-rater reliability

Skin site	Mean (95% CI) Investigator 1	Mean (95% CI) Investigator 2	% difference	ICC	SEM	SDC
Day 1						
Callus centre	6.43 (4.41 - 8.45)	5.97 (4.56 - 7.38)	7.2	0.76	0.93	2.58
Normal PMA skin	5.10 (3.44 - 6.76)	4.48 (3.11 - 5.84)	12.2	0.88	0.41	1.14
Skin overlying 5th metatarsal base	5.68 (4.47 - 6.89)	5.15 (4.09 - 6.22)	9.3	0.66	0.69	1.91
Day 2						
Callus centre	6.46 (5.02 - 7.90)	6.72 (5.29 - 8.14)	4.1	0.8	0.72	1.99
Normal PMA skin	4.91 (3.45 - 6.37)	4.96 (3.49 - 6.42)	0.9	0.99	0.4	1.11
Skin overlying 5th metatarsal base	5.84 (4.68 - 7.00)	5.68 (4.31 - 7.04)	2.8	0.91	0.41	1.14

Mean, 95% CI, SEM and SDC represent variance values in au.

Table 3.8 – Visioscan® variance inter-day reliability

Skin site	Mean (95% CI) Day 1	Mean (95% CI) Day 2	% difference	ICC	SEM	SDC
Investigator 1						
Callus centre	6.43 (4.41 - 8.45)	6.46 (5.02 - 7.90)	0.4	0.86	0.76	2.12
Normal PMA skin	5.10 (3.44 - 6.76)	4.91 (3.45 - 6.37)	3.6	0.86	0.67	1.85
Skin overlying 5th metatarsal base	5.68 (4.47 - 6.89)	5.84 (4.68 - 7.00)	2.7	0.86	0.49	1.37
Investigator 2						
Callus centre	5.97 (4.56 - 7.38)	6.72 (5.29 - 8.14)	12.6	0.79	0.55	1.52
Normal PMA skin	4.48 (3.11 - 5.84)	4.96 (3.49 - 6.42)	10.8	0.79	0.68	1.87
Skin overlying 5th metatarsal base	5.15 (4.09 - 6.22)	5.68 (4.31 - 7.04)	10.1	0.61	0.81	2.24

Mean, 95% CI, SEM and SDC represent variance values in au.

3.4.4 Order of measurement observations

On observation of the mean values (Tables 3.1 – 3.8), no patterns were identified in terms of the effect of the order of measurement (Investigator 1 vs Investigator 2) except for the Cutometer® where Investigator 1 consistently obtained higher values in general over both days. Investigator 1 collected data first on day 1 and second on day 2.

3.5 Discussion

Generally, the inter-rater and inter-day reliability has been shown to be good, though variable, across the different instruments. The values concur with the available reliability literature (Fong et al., 1997, Draaijers et al., 2004, Kottner et al., 2013). For example Fong et al. (1997) reported Cutometer® inter-rater reliability scores (r = 0.78) on scar tissue and these are similar to ICC values found on callused skin sites in this study (generally high r > 0.7 in 83.3% of cases).

For the Corneometer®, the inter-rater reliability was good while the inter-day reliability was low for the centre of callus. There are several possible reasons why this might be the case. Firstly, because callused skin is particularly hard and protrudes from the foot, this could potentially affect the amount of pressure applied by the probe. Hard, protruding calluses may have caused greater deformation of the springs in the probe head leading to higher measurement values. Indeed, Clarys et al. (2011) found that in very dry skin, there was an increase of over 40% in capacitance reading when the operator applied a higher manual pressure, which would deform the springs in the probe head more than a lighter pressure. At the driest skin (12.6 au) there was an increase of 42% when applying high pressure. Preliminary work done by the author of this thesis found a mean increase of 24.4% when deliberately applying high pressure over different plantar skin sites on a single subject. Thus, variations in the pressure applied by the operators may have had some influence on measurements and thus affecting the reliability. These factors might explain the low inter-day reliability, but it does not explain why the inter-rater reliability was still high. Perhaps callused skin is more prone to hydration changes between days that the other sites – hydration is more likely to vary between days than in a single session which might explain why inter-rater reliability was higher than inter-day reliability. The low measurement values compared to the other skin sites are at the lower end of the device range which might also affect results. Inter-day variation in skin hydration could also have contributed to poor inter-day reliability, whereas hydration is less likely to consistently vary in a single session and inter-rater reliability was higher. Given this fact, it might be that having each investigator measure twice on the same day would have given higher reliability scores, instead of having measures between days. However, since the instruments are intended to be used longitudinally, it was appropriate to test reliability between days, despite the fact that it could have given intermittently lower reliability scores.

The percentage differences between investigators and between days were variable for the Corneometer® with the highest inter-rater difference being observed for the callus edge on Day 1 (14.9%) and the highest inter-day difference observed on callus centre for Investigator 1 (28.5%). However, on Day 1, the inter-rater differences between PMA and skin overlying the fifth metatarsal base were 3.6% and 7.8% respectively, and for Investigator 1, 2.6% and 4.4% respectively. Across the entire dataset, 90% of the differences were less than 15%. The mean percentage difference between callus and control sites across both investigators and days (65.5%) was larger than the differences between investigators and days by a considerable margin.

The Cutometer's® inter-rater and inter-day reliability for callused skin was generally moderate to good and ICC values on callused skin sites reflect the literature on normal and scar tissue (Fong et al., 1997, Draaijers et al., 2004). However, inter-rater and inter-day reliability was more variable for the adjacent and control skin sites. For non-callused PMA skin, there was moderate inter-rater agreement but poor inter-day agreement. This again might suggest natural skin variation over a day-to-day period. The fifth metatarsal base was even more variable with poorer inter-rater agreement on the second day, and poorer inter-day agreement for Investigator 2. For these skin sites, Investigator 1 had slightly higher readings than Investigator 2 which could be a result of differences in probe application pressure. One paper, published after the study in this chapter was completed, has highlighted that applying a heavy load to the probe can significantly affect the measurements ($P < 0.005$) compared to applying a light or no load (Bonaparte et al., 2013). The authors recommended applying minimal force to the probe when holding it to the skin. However, it must be noted that due to the nature of the skin sites in this study and the fact that the probe is positioned perpendicular to the foot and parallel to the ground, the application of some pressure is necessary to keep a tight seal between the probe and skin, and to prevent movement of the probe. Holding the probe so the spring loaded head was in line with the outer rim, but without pressing excessively hard, was observed to give a smoother distension/ time curve than applying too little pressure – where fluctuations in skin distension readings were evident during a pressure application cycle due to minute movements. The spring loaded head is intended to ensure constant probe pressure (Courage-Khazaka, 2010a) and given that both investigators in the study had experience of handling the probe and applying constant pressure between measurements, any effect of probe pressure was likely to be minimal.

The high reliability values on callused compared to normal skin sites with the 8mm diameter Cutometer® probe suggests it might be better suited to stiffer skin than normal skin, such as that overlying the PMA and base of the fifth metatarsal. The fact that skin overlying the PMA and fifth metatarsal base is more convex in nature compared to callused skin might also have played a part in the initial position of the skin inside the probe which could have led to discrepancies in reliability. Methodologically, there are unlikely to be any aspects of the protocol that could be modified to counteract this effect. Using a smaller aperture measurement probe might have given more reliable results on control site skin, but at the expense of less accurate data obtained from callused skin (assuming a smaller aperture becomes challenging on stiffer skin). The positioning of the probe was as accurate as possible and the probe was repositioned between each measurement which has been shown to be more reliable than leaving the probe attached to the skin between measurements, which could lead to other factors, such as distortion of the skin, or skin occlusion, which will affect the measurements (Bonaparte and Chung, 2014).

The percentage differences between investigators and between days were variable for the Cutometer® with the highest inter-rater and inter-day difference being observed on the callus centre for Day 1 (37.8%) and Investigator 1 (36.6%) respectively. However, the percentage differences between days for the PMA and base of fifth metatarsal were 15.1% and 16.6% respectively, and for Investigator 1, 6.5% and 3.2% respectively. Across the entire dataset, 80% of differences between investigators and between days were below 20%. The mean difference between callus and control sites across both investigators and sessions was 41.6%, which is much larger than most of the inter-rater and inter-day differences.

The reliability of the Visioscan® is generally moderate to good for callused skin sites. While the ICC values were lower than those reported by Kottner et al. (2013), the probe was manually positioned by each investigator before imaging and was not fixed in position. The device is very sensitive and any slight change in probe position can affect the values. While a guidance mark was placed at each skin site, the probe would still have to be aligned by each investigator before taking the measurement, and between days. This phenomenon and variations in other skin properties, which may have affected the skin's surface profile, together could account for the moderate agreement. For the homogeneity parameter, the percentage differences between investigators and between days were very small ranging from 0.5% to 3.2%. These differences are smaller than the mean percentage difference between callus and control sites across investigators and days which was 5.8%.

For the variance parameter, the largest percentage difference between investigators was seen for normal PMA skin on Day 1 (12.2%). The largest percentage difference between days was Investigator 2's callus centre data (12.6%). On day 1, the differences were 9.3% for the fifth metatarsal base, and for Investigator 2, 10.1%. However, across the entire dataset for this device, 66.67% of differences were below 10%; and 50% of differences were below 5%. Compared to the mean percentage difference between callus and control sites across investigators and days (23%), the inter-rater and inter-day differences are very small.

Across all the devices, the SEM and SDC values were variable with callused sites generally showing less agreement than normal sites. The SDC, which is calculated from the SEM, suggests in some cases, particularly skin hydration and stiffness at callus sites, that quite large changes in data might be necessary to identify a change that can be deemed as 'real' and not as a result of measurement error. However, this should not be an issue so long as control sites, which showed marked differences from callused skin in this study are used as a comparison (on average the control sites were 65.6%, 41.6%, 5.6% and 24.2% different from callused skin for hydration, distensibility, homogeneity and variance respectively). Thus, variation between days in the measurements for Investigator 1 and 2 are lower than the differences one might expect to see between callus and non-callused sites. The level of agreement in absolute units of measurement may appear quite low in some cases, but this must be taken into context with the other results. The agreement results may be a product of the fact that plantar skin properties can deviate in a short space of time (i.e. Day 1 to Day 2) and the devices are sensitive to these skin changes as well as the variation in the investigators' operating skills. This suggests use of control sites is important in future research designs and that change in skin properties compared to changes at a control site is a key approach to data analysis. Given that reliability and explicit differences in mean scores are acceptable and show that differences between skin sites that are greater than differences between investigators and days, the SDC scores are not concerning in that, for the intended studies of foot skin, the measurement approaches appear fit for purpose.

This study was undertaken as part of a larger project investigating the reliability of biophysical measurement devices which was published in the Journal of Foot and Ankle Research (Hashmi et al., 2015b). The results from this study also relate to corn and heel fissure skin which was not included in this chapter. The main difference between this study and the data presented in this chapter are the inclusion of the Reviscometer® probe. The

author wrote this chapter after the study had concluded and it was clear from the Reviscometer® data that it was greatly affected by hardness of the skin, and regularly showed error values. Because this phenomenon was evident from preliminary tests, before the study had commenced, the decision was made to omit the results. A further difference between the results of this chapter and the published study are that the Visioscan® parameters are different. The homogeneity and variance parameters were chosen by the author of this thesis because they reflected what the author felt were the most important aspects to measure, and anecdotally reflected what was observed during testing. These parameters were also consistently reliable, which is of importance for future testing.

3.6 Conclusions

This chapter has demonstrated development of a greater understanding of research techniques in terms of design and methodology for skin measurement techniques, and in data handling and statistical analysis. Furthermore, it has allowed evaluation of the skin instruments thus enabling the author to make judgements on their limitations and potential use in further research.

The results of the study have shown good inter-rater and inter-day reliability of the Corneometer®, Cutometer® and Visioscan®. The ICC values were generally good and comparable to the literature and the percentage differences in mean values between investigators and days were smaller than differences between normal and callused skin sites. This means that variations between investigators and days should not mask the expected differences in skin properties between sites.

The SDC data suggests that control site data will be important when collecting data over a longitudinal time period as normal variations in skin properties over time could mask skin changes caused by a potential intervention or other independent variables (such as an increase or decrease in load applied to the foot site). The inter-rater reliability could have been affected by variation in pressure applied by each investigator and probe positioning, and skin measurement tools remain sensitive to operator judgement. The inter-day reliability across all the devices could have been affected by variations in the skin's properties and probe positioning, and thus reflect more than simply variation due to operator technique.

Chapter 4: The biophysical characteristics of normal and callused plantar skin

4.1 Introduction

Chapter 2 highlighted that there was a gap in the research literature regarding the biophysical properties of normal and callused plantar skin, and the relationship between pressure and callus development. Therefore, various skin properties were highlighted which would be useful to measure to characterise the skin including hydration, mechanical properties, TEWL, skin surface topography and cross sectional thickness. Chapter 3 investigated the inter-rater and inter-day reliability of the measurement devices that characterised the hydration, mechanical properties and topography of normal and callused plantar skin. The study reported on the extent to which reliable data collection was possible with the Corneometer® (hydration), Cutometer® (distensibility) and Visioscan® (skin surface topography parameters: homogeneity and variance), and thus the limits within which any study of differences between normal and callused skin sites would be possible.

Knowledge of the normal characteristics of plantar skin is essential if one is to quantify what an abnormal change in skin is and evaluate the effectiveness of a treatment to return skin to a normal state. Also, understanding how an abnormal skin site is different may point at treatment strategies more/less likely to work, because approaches that target a specific difference between sites (e.g. in hydration) could be prioritised.

In addition, to investigate what causes callus to develop, or regress, a researcher may seek to manipulate an independent variable (e.g. external load) and observe change in an area of skin, either away or towards normal values. Thus, quantifying normal and abnormal skin properties helps define the boundaries for studies such as this. Work of this sort is described in subsequent chapters. The study outlined in this chapter was concerned with collecting biophysical data from normal and callused plantar skin on the forefoot which could be used to characterise their biophysical properties. The hydration, mechanical and topographical properties of these two skin states can be characterised to develop a biophysical profile.

4.2 Aims and objectives

The aim of this study was to characterise normal and callused plantar skin. The objectives were to measure the hydration, stiffness and topographical properties of these skin sites in a large sample of individuals and use this data to create a biophysical profile.

4.3 Materials and methods

Ethical approval for this study was granted by the University of Salford's College Research Ethics Panel (application number HSCR12/55).

4.3.1 Subjects

Participants were recruited via a poster campaign on the University campus including the Podiatry Clinic and an advert in a local newspaper. Participants who had a plantar forefoot callus were recruited provided they did not display any of the following exclusion criteria which could potentially affect the skin's properties: compromised cardiovascular or neurological status, connective tissue disorders (such as lupus erythematosus), diabetes, autoimmune disorders (such as rheumatoid arthritis), peripheral vascular disease, or wounds/ ulcers of the legs and feet, and eczema, psoriasis or other dry skin disorders affecting the plantar skin.

The foot was assessed by a podiatrist registered with the HCPC. Pulses from the posterior tibial and dorsalis pedis arteries were assessed by palpation. Presence of neuropathy was assessed by vibration perception using a 128MHz tuning fork and 10g monofilament test on the hallux, first and fifth metatarsal heads. If these tests, skin assessment and medical history were satisfied, the volunteers were recruited to participate in the study.

4.3.2 Data collection

Data was collected in a single session. The foot was allowed to acclimatise for approximately 15 minutes before measurements were taken. Temperature and humidity were monitored but not controlled.

The skin sites measured included: centre of callus, edge of callus, adjacent skin (the distance of the lesion's diameter medial or lateral from the centre of the lesion), and two normal weight bearing skin sites including (1) non-callused skin overlying the plantar metatarsal area (PMA), and (2) adjacent skin overlying the base of the fifth metatarsal

(figure 4.1). The callus centre was chosen because of its location at the centre of the lesion, and could thus be considered the most 'callused' area. The callus edge was chosen because of its potential importance in later longitudinal studies; a change in callus properties post-treatment may potentially become apparent faster at an area of 'less callused' skin, such as the boundary of the lesion itself. Adjacent skin located close to the callus was chosen because of the potential for skin that is normal in appearance to still display callus-like properties due to its close proximity to the lesion, which could thus aid in evaluation of interventions. Normal weight-bearing (PMA) and semi weight-bearing (fifth metatarsal base) skin sites were chosen to compare with callused skin and to give a good profile of normal skin properties.

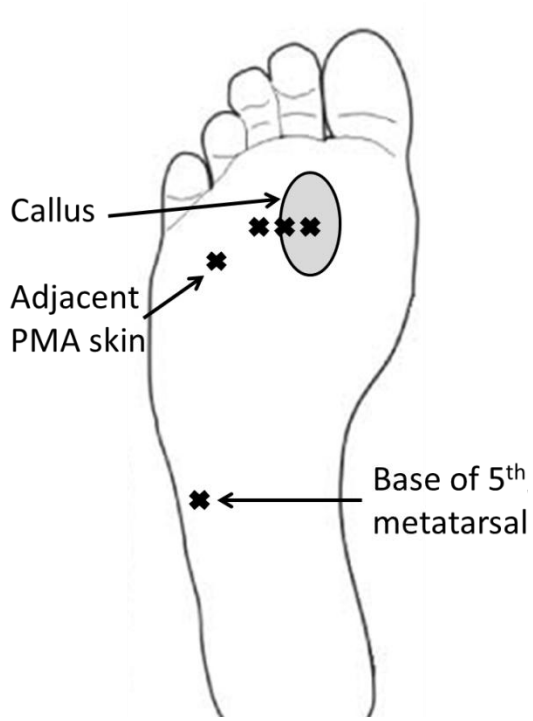


Figure 4.1 - The measurement sites on the callus include the centre, edge, and skin directly adjacent to the callus; and skin overlying the plantar metatarsal area (PMA) and base of the 5th metatarsal (adapted from Hashmi and Malone-Lee, 2007 p.253).

The skin sites to be measured were marked with a ball point pen. The centre and edge of the callus was marked and the width of the lesion recorded. Generally there is a difference in skin texture and hardness between callused and non-callused skin so the boundary between these areas, the callus edge, can be visualised and palpated. The adjacent skin was positioned 200% of the radius length away from the callus centre. The fourth metatarsal was used as a control site, unless the callus lay over this site, in which

case the first metatarsal head was used. The fifth metatarsal base of the same foot was used as a second control site. Both control sites were identified through palpation and marked. The distance of the radii of the measurement probes were marked over the centre of each skin measurement site to allow accurate probe placement.

Measurements were taken based on manufacturers' instructions but adjusted for the needs of plantar skin testing as follows. 10 measurements were taken per skin site using the Corneometer® and one image was taken per site for the Visioscan®. For the Cutometer®, 500mbar of negative pressure was applied. The on-time and off-time were set to 30 seconds each. One cycle of on-time and off-time was used on each skin site.

Single data values for hydration are produced by the Corneometer® and a mean was taken from the 10 measurement values obtained from each skin site. The Cutometer® produces a range of different values based on stiffness, elasticity and plasticity. Skin distensibility (a surrogate measure of stiffness) was chosen based on the hypothesis that callused skin is stiffer than normal plantar skin. Two variables, *variance* and *homogeneity*, were obtained from the Visioscan® images. These were chosen based on the hypothesis that callused skin is rougher than normal skin and is therefore more variable and less homogenous in appearance. The homogeneity parameter is a measure of how homogenous the skin is and measures the combinations of grey levels in the image. The more frequent the grey level combination appears in the image, the lower the homogeneity and the rougher the skin (Courage-Khazaka, 2009b). The variance parameter is a measure of how variable the grey level pixel values are within the image and is higher the rougher the skin is (Courage-Khazaka, 2009b).

4.3.3 Statistical analysis

Statistical analysis was undertaken on SPSS 20.0 and Microsoft Office Excel 2010. Means and 95% confidence intervals were calculated for each skin site. Percentage differences were also calculated to show explicit differences between skin sites.

Percentage difference

$$\%\ difference = \frac{Skin\ site\ 1 - Skin\ site\ 2}{Skin\ site\ 2} \times 100$$

To assess whether these differences were statistically significant, the repeated measures Analysis of Variance (ANOVA) with post-hoc Bonferroni adjustment, or Friedman's ANOVA with post-hoc Wilcoxon Signed-Ranks Test were used for parametric and non-parametric data distributions respectively. Differences were tested between all skin sites including callus centre, callus edge, skin adjacent to callus, normal PMA skin and skin overlying the fifth metatarsal base.

The repeated measures ANOVA relies on the F-statistic, a value used to assess significance of variance between means, which is calculated by dividing the residual mean of squares by the model mean of squares. To calculate the mean of squares, one must first calculate the within subject sum of squares (SS_W) by multiplying the degrees of freedom by the within subject variance, and the model sum of squares (SS_M) by calculating the difference between the mean of each group and the overall mean. These are then squared, multiplied by the number of subjects and then the values of each group are summed together. Once these have been calculated, the residual sum of squares (SS_R) can be found by subtracting the model from the within subject sum of squares. The mean squares are calculated by the sum of squares divided by the degrees of freedom (df). The model (the variation explained by the model) and the residual (the variation not explained by the model) mean of squares is found by dividing the sums of squares by the degrees of freedom. The F-ratio is found by dividing the model by the residual mean squares. The F-ratio is compared against a value based on its degrees of freedom, and the significance level is determined which highlights differences between the groups (Field, 2009).

Repeated measures ANOVA

$$SS_W = \sum_{i=1}^{n} (x_i - \bar{x}_i)^2$$

$$SS_M = \sum n_i \, (\bar{x}_i - \bar{x}_{grand})^2$$

$$SS_R = SS_W - SS_M$$

$$df_R = df_W - df_M$$

$$MS_M = \frac{SS_M}{df_M}$$

$$MS_R = \frac{SS_R}{df_R}$$

$$F = \frac{MS_M}{MS_R}$$

(Field, 2009, pp.465 - 468)

The Friedman's ANOVA is the non-parametric equivalent of the repeated measures ANOVA and is based on ranks as opposed to actual scores. The data for each condition for each participant is ranked. The ranks of each condition are then summated (R_i) and the Friedman statistic (F_r) is calculated as below. The degrees of freedom and the significance of the test statistic is then given (Field, 2009).

Friedman's ANOVA

$$F_r = \left[\frac{12}{Nk\,(k+1)}\sum_{i=1}^{k} R_i^2\right] - 3N\,(k+1)$$

(Field, 2009, p.574)

To assess the correlation between skin measurement parameters at each skin site, the Spearman correlation coefficient was used. This statistical test was chosen over Pearson's correlation coefficient because it allows for non-normally distributed data (Field, 2009) which was a feature with different datasets in this study. It is calculated by summing all of the squared differences in ranks of data, multiplied by six and then divided by the number of samples multiplied by the number of samples squared minus 1. This value is then subtracted from 1 and the resulting value is the correlation coefficient (or r value) (Lund and Lund, 2013).

Spearman's rank order correlation

$$r = 1 - \frac{6 \sum d_i^2}{n\,(n^2 - 1)}$$

(Lund and Lund, 2013)

4.4 Results

In total 51 healthy adults with a total of 61 calluses were enrolled onto the study. Out of the sample, 80% were female. The participants had a mean height of 164cm (± 13); weight of 74.9kg (± 15.9); and BMI of 27.9 (± 5.3). Of these calluses, 16 were consistent with Merriman's grade 1 'no specific callus plaque, but diffuse or pinch callus, or present in narrow bands' and 45 were consistent with grade 2 'circumscribed, punctuate oval or circular, well-defined thickening of keratinized tissue' (Springett and Merriman, 1995, p.207). The following sections detail the skin results by parameter measured.

4.4.1 Skin hydration

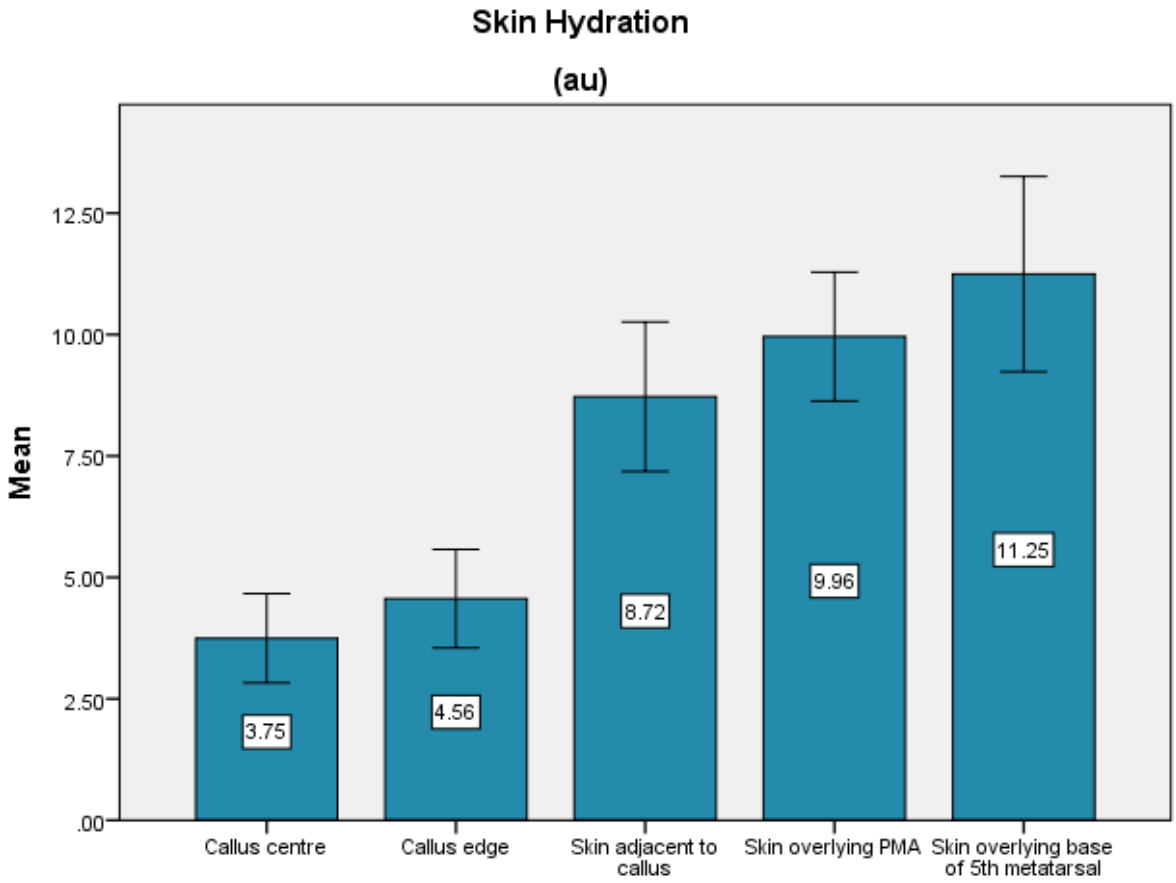


Figure 4.2 – Mean and 95% CI hydration data for each of the five measurement sites.

Skin at the centre of the callus was 17.8% less hydrated than skin of the callus edge (p = 0.02); 57.0% less hydrated than skin adjacent to callus (p = 0.000); 62.4% less hydrated than skin overlying the PMA (p = 0.000) and 66.7% less hydrated than skin overlying the fifth metatarsal base (p = 0.000). Skin of the callus edge was 47.7% less hydrated than skin adjacent to callus (p = 0.000); 54.2% less hydrated than skin overlying the PMA (p = 0.000) and 59.4% less hydrated than skin overlying the fifth metatarsal base (p = 0.000). Skin adjacent to callus was 12.4% less hydrated than skin overlying the PMA (p = 0.085) and 22.4% less hydrated than skin overlying the fifth metatarsal base (p = 0.062). Skin overlying the PMA was 11.5% less hydrated than skin overlying the fifth metatarsal base (p = 0.451).

4.4.2 – Skin distensibility

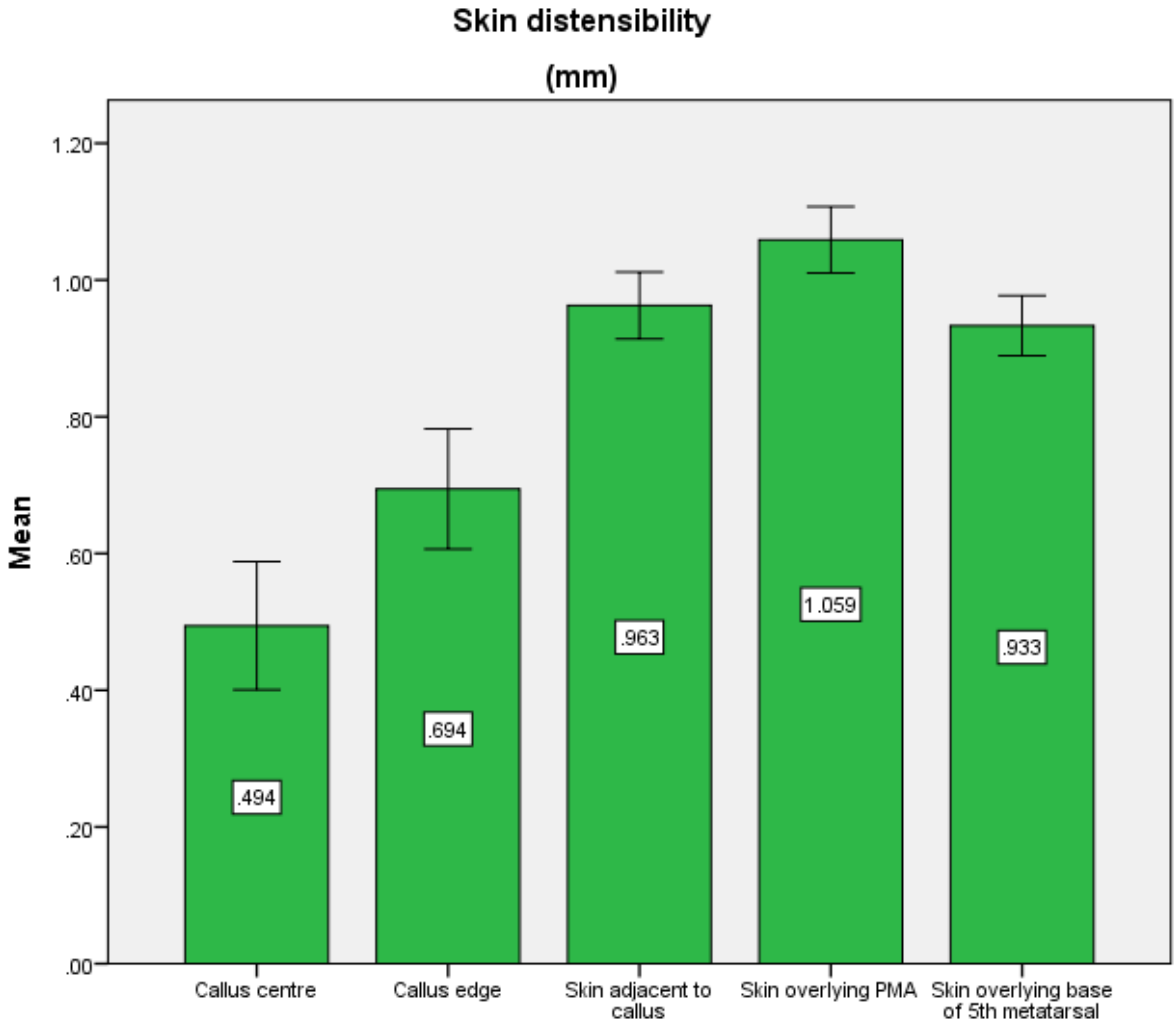


Figure 4.3 – Mean and 95% CI distensibility data for each of the five measurement sites.

Skin at the centre of the callus was 28.8% less distensible than skin of the callus edge (p = 0.000); 48.7% less distensible than skin adjacent to callus (p = 0.000); 53.3% less distensible than skin overlying the PMA (p = 0.000) and 47.0% less distensible than skin overlying the fifth metatarsal base (p = 0.000). Skin of the callus edge was 27.9% less distensible than skin adjacent to callus (p = 0.000); 34.4% less distensible than skin overlying the PMA (p = 0.000) and 25.6% less distensible than skin overlying the fifth metatarsal base (p = 0.000). Skin adjacent to callus was 9.1% less distensible than skin overlying the PMA (p = 0.006) and 3.2% more distensible than skin overlying the fifth metatarsal base (p = 1.0). Skin overlying the PMA was 13.4% more distensible than skin overlying the fifth metatarsal base (p = 0.000).

4.4.3 Skin Topography – homogeneity

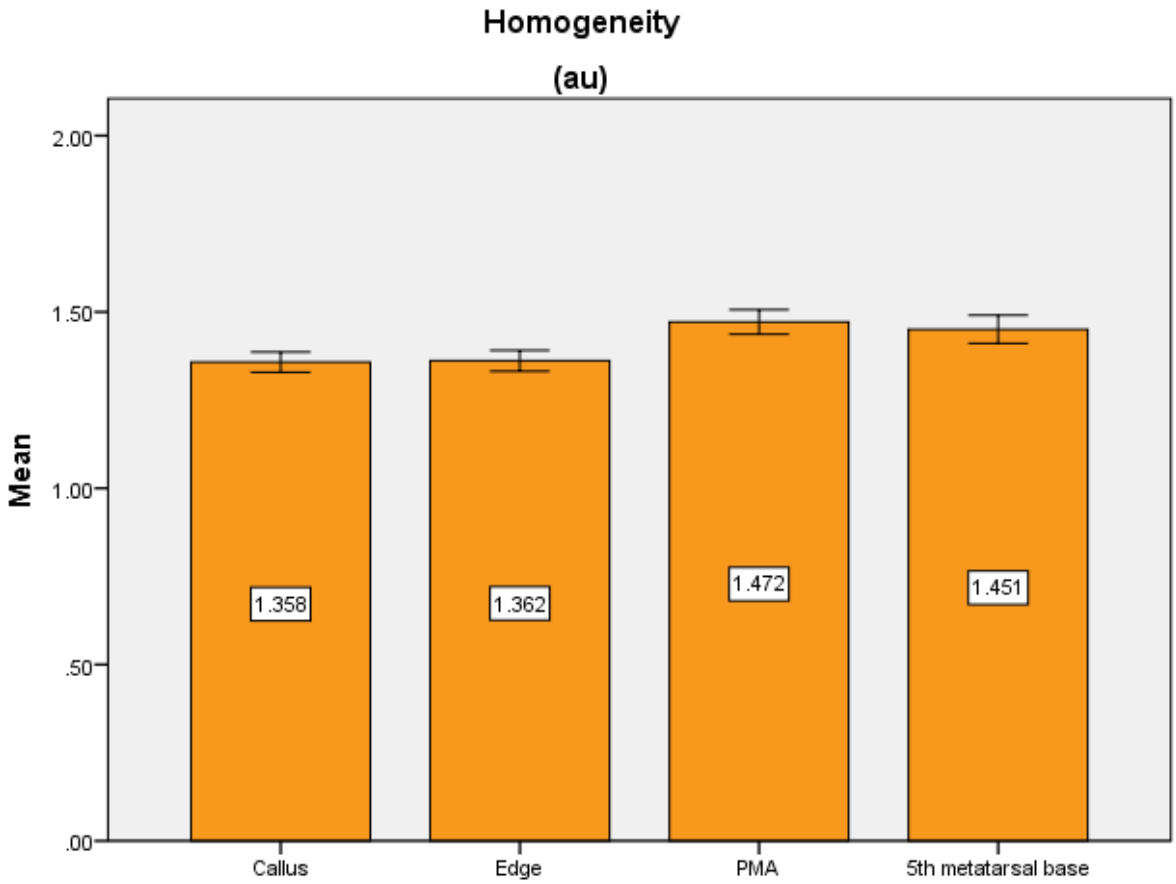


Figure 4.4 – Mean and 95% CI homogeneity data for each of the four measurement sites.

Skin at the centre of the callus was 0.4% less homogenous than skin of the callus edge (p = 1.0); 7.5% less homogenous than normal skin overlying the PMA (p = 0.000); and 6.3% less homogenous than skin overlying the base of the fifth metatarsal (p = 0.000). Skin at the callus edge was 7.1% less homogenous than normal skin overlying the PMA (p = 0.000) and 5.9% less homogenous than skin overlying the base of the fifth metatarsal (p = 0.001). Normal skin overlying the PMA was 4.9% more homogenous than skin overlying the base of the fifth metatarsal (p = 1.0).

4.4.4 Skin Topography – variance

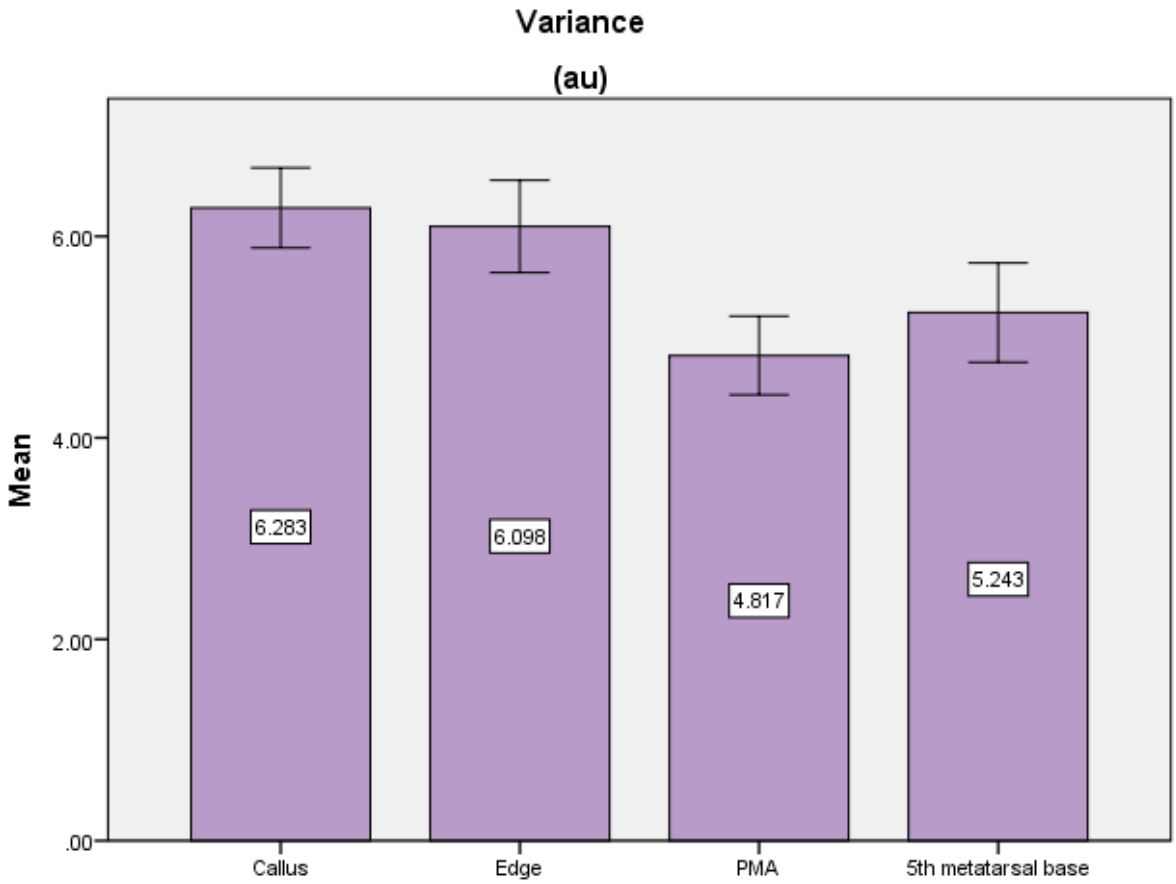


Figure 4.5 – Mean and 95% CI variance data for each of the four measurement sites.

Skin at the centre of the callus was 3.7% more variable than skin of the callus edge (p = 1.0); 29.6% more variable than normal skin overlying the PMA (p = 0.000); and 19.6% more variable than skin overlying the base of the fifth metatarsal (p = 0.001). Skin at the callus edge was 24.9% more variable than normal skin overlying the PMA (p = 0.000) and 15.3% more variable than skin overlying the base of the fifth metatarsal (p = 0.01). Normal skin overlying the PMA was 13.4% less variable than skin overlying the base of the fifth metatarsal (p = 0.323).

4.4.5 Correlations between variables

Table 4.1 shows the correlation coefficient for the biophysical parameters at each skin site measured. Between hydration and stiffness parameters, there was a moderate significant correlation at the callus centre (r = 0.56) and edge (r = 0.55), and a weak significant correlation on skin adjacent to callus (r = 0.33). Between hydration and homogeneity, there was a moderate significant correlation on PMA skin (r = 0.69) and skin overlying the fifth metatarsal base (r = 0.59). There was a moderate significant negative correlation at these

two sites between hydration and variance parameters (r = -0.68 and -0.66 at PMA and fifth metatarsal base respectively). There was also a weak significant negative correlation between stiffness and variance at PMA skin sites (r = -33). At each skin site there was a very strong and significant negative correlation between homogeneity and variance parameters (r ranged between -0.91 to -0.96).

Table 4.1 Correlation coefficient between biophysical parameters at each skin site

	Callus centre	Callus edge	Skin adjacent to callus	PMA	5th met. base
Hydration v stiffness	0.56**	0.55**	0.33*	0.25	0.2
Hydration v homogeneity	0.2	0.24		0.69**	0.587**
Hydration v variance	- 0.12	-0.25		-0.68**	- 0.66**
Stiffness v homogeneity	0.18	0.18		0.3*	0.17
Stiffness v variance	-0.14	-0.26		-0.33*	-0.14
Homogeneity v variance	-0.91**	-0.94**		-0.96**	-0.96**

Spearman's correlation: r value. * = significant correlation (p < 0.05); ** = significant correlation (p < 0.01).

4.4.6 Differences between callus grades

There was a significant difference in hydration (p = 0.045) at the callus centre between grades 1 and 2 callus (according to Merriman's classification system). There were no significant differences identified between grades for distensibility, homogeneity and variance parameters.

4.5 Discussion

The aim of this study was to define the characteristics of normal and callused plantar skin. The results showed that callus was significantly less hydrated, less distensible (stiffer), less homogenous in appearance, and more variable in appearance (rougher) than normal plantar skin sites. This is one of very few studies that have sought to characterise the biophysical properties of skin as a primary outcome. At the time of writing, there have been only two which tested differences between skin sites using similar measures. Marrakchi and Maibach (2007) studied differences in hydration between skin sites on the face, using the Corneometer® and observed that the skin of the neck had significantly higher hydration values than other skin sites. Ryu et al. (2008) measured many mechanical parameters of different skin sites using a Cutometer® and found significant differences, particularly in stiffness and elastic parameters, between the face and other skin sites. Other studies have also observed variations between skin sites, but did not test for significance as they were

not a primary outcome measure. Krueger et al. (2011) and Luebberding et al. (2014) observed large variations in mechanical properties between skin sites using the Cutometer®; and Firooz et al. (2012) observed variations in hydration and mechanical properties between skin sites, using the Corneometer® and Cutometer®.

The study described in this thesis chapter has added to the work of previous authors by specifically investigating the biophysical properties of foot skin, which no others have attempted to characterise, and in particular, the differences between callus and normal skin. As a measure of skin hydration, two previous studies have used the Corneometer® on foot skin (Papanas et al., 2011, Garrigue et al., 2011), and one study has evaluated mechanical properties on the foot using the Cutometer® (Hashmi and Malone-Lee, 2007). In this study, the skin hydration values for normal skin are much lower than those reported by Papanas et al. (2011). This study found a mean hydration on normal skin overlying the PMA and the base of the fifth metatarsal of 9.96au and 11.96au respectively which is much lower than 26.55au on non-callused plantar skin reported by Papanas et al. (2011). However they used participants who had diabetes and they measured non-callused plantar skin on the 'centre' of the plantar aspect, which might have meant the plantar arch, which is not fully weight bearing. The results in this chapter might also suggest that individuals who have callus display generally drier plantar skin than those with healthy plantar skin.

Hydration results cannot be compared to those reported by Garrigue et al. (2011) as they only included participants with diabetes who had xerotic skin. It is also not appropriate to compare skin stiffness results with those obtained by Hashmi and Malone-Lee (2007), since they used a 2mm diameter probe and analysed different aspects of the time displacement curve to this study. There are no available studies which have previously measured skin surface topography on plantar skin to which the results here can be compared.

The observations in this study, that callused skin is less hydrated, stiffer, and rougher in appearance than normal non-callused skin sites, were expected given findings of previous studies showing marked histological differences between callused and normal skin. Thomas et al. (1985) found callused stratum corneum was 2-3 times thicker ($p < 0.001$) than normal plantar stratum corneum (349 versus 123 cell layers thick) and now this study has shown that histological differences are also related to biophysical differences between callus and control sites.

There are several reasons that could explain why callus is significantly less hydrated and stiffer than normal plantar skin. Through immunohistochemistry tests, Kim et

al. (2010) attributed callus development to an increase in: Keratins K9 and K14; adhesion proteins CDSN, DSG1 and DSC1; the proteins involcurin, filaggrin, caspase 14, and CaSR. Collectively this would cause an increased rate of proliferation and increased cell cohesion which thus increases the rate of keratinisation (Kim et al., 2010). As a result of increased cell proliferation, the cells may not have enough time to fully differentiate (Thomas et al., 1985) leading to intercellular lipids becoming fragile (Harding et al., 2003). As a result, the skin's barrier function suffers (Wickett and Visscher, 2006) resulting in decreased hydration (and increased TEWL) (Baroni et al., 2012).

It is not clear whether the effect of altered lipid profiles directly affects the stiffness of the skin. In theory, lipids become more fragile due to lack of differentiation (Harding et al., 2003) which might cause weaker bonds between cells. However, as cell cohesion and proliferation is accelerated (Kim et al., 2010), this increased cell turnover might be enough to increase skin stiffness alone through the skin becoming thicker which might overrule any possible weakness caused by fragile lipids. It could be that fragile lipids may not have any effect on stiffness; they might only affect the epidermal barrier function and hydration. As the biochemical triggers involved in callus formation (Kim et al., 2010) probably increase lipid secretion and thus cohesion, this might be enough to increase stiffness even if they are fragile. Hydration may also have an effect on stiffness. The decreased hydration may have the effect of contributing to increased skin stiffness by possibly increasing cell cohesion through retention of corneodesmosomes (Warner et al., 1999, Bouwstra et al., 2003, Wu et al., 2006).

Increased skin roughness in callus, illustrated by decreased homogeneity and increased variance, are a visible effect of the processes above. The scaliness of the skin is likely to be a result of altered desquamation which has been observed by Thomas et al. (1985) who reported that in the most superficial aspect of callused stratum corneum, the rate of cell loss was increased suggesting less tightly bound keratinocytes. It could possibly be due to fragile intercellular lipids as discussed above. While it is not known for sure why this is the case, it does explain why the surface of callused skin can appear scaly in nature.

The devices used in this study (Corneometer®, Cutometer® and Visioscan®) have been shown to clearly identify differences between skin sites at a statistically significant level. They have effectively described the properties of callused and normal plantar skin. Correlation analysis has revealed that on callused skin, hydration and distensibility (stiffness) parameters moderately correlate with each other showing that there when skin

becomes less hydrated, it becomes stiffer. There were also moderate correlations between the roughness parameters and hydration at non-callused skin sites, but not at callused sites. This may suggest that when skin becomes callused, the roughness and hydration changes. While the changes are significant in both cases, they don't closely mimic each other in all individuals. The homogeneity and variance parameters showed a very strong negative correlation to one another which suggests that they are very closely related.

The results of this study could be far reaching in terms of providing targets for treatment evaluation. To the best of the author's knowledge, this has never been done before. But previous literature has used the same skin devices to evaluate treatments. Two studies have been published which have used hydration as a parameter for evaluating treatment. Garrigue et al. (2011) and Papanas et al. (2011) used the same skin hydration device (Corneometer®) to evaluate the efficacy of skin moisturising products in xerotic skin. The studies reported hydration increases of up to 57.3% post-treatment, showing that skin hydration changes to treatments can be detected on the foot using the device. There are no available studies which have evaluated foot treatments, using the 8mm (or other diameters) Cutometer® probe, but treatments on other areas of the body have been evaluated effectively using this method. One such example by Josse et al. (2009) used the Cutometer® to assess mechanical properties in arm skin treated with a topical corticosteroid cream. The device was able to detect a 30% pre- and post-treatment change in stiffness. Like the Cutometer® device, the Visioscan® has never been used to evaluate foot skin treatments, but it has been used on other sites. The best example involved the evaluation of different moisturising agents applied to the face. The device was able to detect statistically significant differences over the 28 day study period (mean values and % differences not available) in pre- and post-treatment roughness parameters (Pena Ferreira et al., 2010).

The results presented in this chapter can be used as a guide for what magnitude of skin change, as a result of treatment or intervention, can be expected or aimed for using the skin devices. For example, if one were to aim to improve callused skin hydration to the point where it were 'less callused', a target of 17.8%, which would improve the central callused skin to same hydration as the callus edge, might be appropriate as a short term goal. Likewise, an increase of 62.4% hydration, the increase required to equal the hydration of normal PMA skin, might be a reasonable long-term target. As with the skin hydration, the distensibility and skin roughness data might be used in the same way, with differences between skin sites acting as a guide. Using these devices together gives an all-

round profile of the skin and can therefore allow clinicians and researchers to draw inferences about the data. For instance, if callused skin were to show characteristics like normal PMA skin after an intervention, one could suggest that the intervention has had the effect of reversing some of the callus biochemical changes. For example the proliferation of cells has sufficiently slowed down, leading to less thickened skin, and allowing more time for keratinocyte differentiation, leading to improved lipid profile and an improved barrier function; thus greater hydration (Thomas et al., 1985, Kim et al., 2010, Baroni et al., 2012).

In reality, however, the situation is not as simple as this. Basic treatments such as moisturisers tend to treat the symptom – i.e. stiffness, dryness, roughness, which may lead to discomfort – as opposed to the cause of the callus. The superficial skin will be reduced using a scalpel, and perhaps softened and hydrated using a moisturiser. While there may be some temporary effect on the biochemical process of hyperkeratosis, the underlying mechanism is likely to remain. If the treatment stops, it is likely that callused skin will return, as previous research has documented (Potter, 2000). This mechanism is hypothesised to be plantar pressure and the evidence links to this (Bevans and Bowker, 1999, Abouaesha et al., 2001, Menz et al., 2007). The following chapters of this thesis will aim to use the new knowledge gained from the study in this chapter to try to change the properties of the skin biomechanically. The next study will aim to increase loads beneath normal plantar skin, and use the skin measurement devices to evaluate whether or not the normal skin becomes 'callus-like', i.e. whether it shows signs of reduced hydration, distensibility, and homogeneity, and increased variance, which would point to hyperkeratosis. The final study will use the devices to determine whether reducing pressure beneath existing calluses leads to skin properties similar to, or beginning to change in the direction of, normal PMA skin. If the intended skin changes in these two studies occur, it can be argued that alterations in the biomechanics of the skin have had an effect on the biochemical process of hyperkeratosis. Conversely, if no changes occur, the opposite can be argued.

The main limitation to this skin characterisation study is that means of visualising an actual cross-section of the skin were not attempted. Ideally, histological analysis of skin at each of the measurement sites could have been used to greater link the biophysical properties to the cellular differences between callused and normal skin. However, this would not have been well tolerated by participants because it would have involved skin biopsy. This could introduce pain, the risk of infection, and accidental injury to the

participant or investigator, and consequently result in a large reduction in participant numbers. Also, because this research is intended to be applied to the clinical and research settings in analysing the effects of skin treatments and interventions, performing biopsies at follow up appointments would be inappropriate. Pain and inflammation which might result from skin punctures, will almost certainly have an effect on gait (Mickle et al., 2010), and thus pressure distribution – an important area of investigation in the context of this thesis. Inflammation could also inadvertently affect the skin properties. Thus, while a limitation to this study, withholding the use of invasive measures was an appropriate decision.

One other limitation of this study is that the majority of the sample (80%) was female, which could potentially have affected the data. Previous, large sample studies have shown gender differences on non-plantar skin sites such as the face and forearm. Differences have been noted in TEWL and skin hydration between genders. Young men have been shown to have more hydrated skin than young women, and a lower TEWL. However, the TEWL of both genders becomes more similar in fifth decade of life, and men's skin loses hydration and women's skin remains similar or increases in hydration depending on skin site (Luebberding et al., 2013). Similar differences were found in mechanical properties, with women shown to have significantly stiffer but more elastic skin than men up until the fourth decade, where the differences begin to diminish with increased age (Luebberding et al., 2014). However, the agreement between studies is not consistent with some showing no gender-related differences in hydration or mechanical properties (Man et al., 2009, Firooz et al., 2012). Gender differences in other parameters such as sebum level and PH have also been reported but vary between studies (Man et al., 2009, Luebberding et al., 2013). Nevertheless, the available research suggests that gender differences do exist, so the possibility of having a population with 80% females might affect the results. Future plantar skin characterisation studies might benefit from gender-matching their participants to minimise gender bias affecting data.

A control group of individuals who are age- and gender-matched without callus may have been beneficial to include in this study and would have allowed comparisons of the general skin quality between individuals with and without callus. This could give valuable information about individuals who might be at risk of callus development, based on their skin properties, and would be an interesting area to research.

This study was undertaken as part of a larger portfolio of work investigating the biophysical properties of callus, corns and heel fissures, and the characterisation data was

thus used to evaluate treatments to these lesions. At the time of writing, the characterisation portion of the project has now been published in the Journal of Foot and Ankle Research (Hashmi et al., 2015a). Two other studies, one evaluating topical and podiatry treatments for callus (Hashmi et al., 2015, under review) and heel fissures (Hashmi et al., 2015, under review) have been submitted for publication and are both currently under review. These studies showed large increases in hydration (increases ranged from 46% - 152%) and distensibility (increases ranged from 15% - 100%) in callused skin sites between baseline and post-treatment were possible in podiatry and topical treatment groups. There were no significant changes in the control site across the study. Such a large difference suggests that the instruments used in this study are effective in tracking callused skin longitudinally through periods of intervention, and the techniques will be employed to track skin through biomechanical changes in the remaining studies in this thesis.

4.6 Conclusion

This chapter demonstrates development of new knowledge through an original research study in an area that has never been investigated – the characterisation of foot skin, and namely plantar callus. This is despite management of normal and callused skin sites being fundamental to long standing areas of podiatry practice across the world. The importance of the data presented here is therefore potentially very high. Using the same skin measurement techniques evaluated in the previous chapter, this chapter has, for the first time, reported the biophysical properties of callused skin in relation to normal plantar skin. Callused skin is less hydrated, stiffer and rougher in appearance than normal plantar skin and reflects the biochemical changes associated with callus. This information could potentially be useful in the clinical setting, where treatment approaches can be quantitatively evaluated and the ability to return callused skin to normal can be judged. It could also be useful in assessing skin which might be becoming ‘callused’ as a result of exogenous insults, such as a poorly fitting shoe or bony prominences. In the clinical setting and research, this information will be very valuable in evaluating skin treatments. In the context of this thesis, it is valuable as the effect of applying load to normal skin can now be quantitatively evaluated using the properties of callused skin as a target for effects of increasing/decreasing load.

Chapter 5: Development of a device to apply external loads to plantar skin

5.1 Introduction

The previous chapter (Chapter 4) has demonstrated that callus is significantly less hydrated, less distensible (stiffer), less homogenous and more variable (rougher) than normal plantar skin. Now that a biophysical profile of plantar skin has been constructed, the role of external load in the development of callus can now be investigated. Chapter 2 identified that there is a hypothesised association between callus and external loads applied to skin, but this link has never been explicitly studied systematically. The specific unresolved issue is whether increased plantar pressure causes callus; research on other skin sites suggests that increasing external load leads to callus creation. Despite the fact callus is more common on the foot than any other area of the body, all prior work has been on sites other than the foot. The purpose of the next two chapters is therefore to analyse the effect on plantar skin properties of increases in external load. To achieve this in controlled laboratory conditions, i.e. apply increased loads systematically and in a safe manner, a device was required to apply loads on living subjects. The outline idea was to apply in-vivo like pressures to a single site under the foot, in repetitive cycles over a period of several weeks. This chapter will describe the development of a device to apply loads to the plantar skin, focussing particularly on the target loads to be applied and the design and repeatability of the device. The subsequent chapter describes the application of this device in an experimental study.

5.2 Determining the forces and pressures to be applied by the loading device

A device was designed to apply external loads to plantar skin in a controlled way and mimic, as far as was possible, in-vivo and gait like loading conditions. As discussed in Chapter 2, during gait the foot is subject to compressive (vertical) loads and shear (horizontal) loads. The vertical loads reach a peak of around 1.2 times bodyweight while the shear loads generally reach 0.2 times bodyweight in both the posterior and anterior directions as a result of acceleration and deceleration (Richards, 2008). These loads are applied over the metatarsal heads resulting in the well reported plantar pressure patterns in the literature. Ideally the device for this study must be able to apply pressures similar to those experienced in gait in terms of three key parameters (1) magnitude of pressure, (2)

timing of pressure application, and (3) direction of pressure application. In order to do this, adequate force must be applied over a specific contact area at an appropriate rate to replicate in-vivo vertical and shear plantar pressures. The device would ideally apply both compression and shear pressure so that the relationship between skin properties and these forces can be investigated. As was discussed in Chapter 2, however, shear forces and pressures are thought to be much smaller than vertical compression forces and pressures. Also, there is far better data on vertical pressures upon which to design a device to apply additional loads.

To estimate how much force might be necessary to apply pressures that are realistic, two studies that reported normal pressure over each of the metatarsal heads using a pressure mat (Novell Emed®) were selected to provide target vertical plantar pressure data (Bryant et al., 1999, Putti et al., 2008). These studies were selected due to the fact that they are the only studies identified that reported pressures over each of the metatarsal heads separately and did so in large groups of healthy participants. Bryant et al. (1999) and Putti et al. (2008) both selected healthy subjects (n = 30 and 53 respectively) without the presence of any foot deformities, previous surgery, pain or any factors that might affect their gait so their data was as representative of 'normal' as possible. Also, and in the context of the research questions being addressed, these studies reported barefoot pressures, and were deemed to be more helpful than in-shoe pressure data (such as Pedar®) since they are greater and therefore would set higher target pressures.

However, since plantar pressures are person specific and plantar skin likely pre-conditioned to the pressure normally applied, then the target pressures applied by the device should be adjusted on a participant by participant basis. This would be necessary to ensure that the pressures applied were suitably towards the upper limits (or outside) those normally experienced by the foot and the skin of the foot, and thus more likely to stimulate some change in the skin. The assumption here was that, to investigate whether callus-like changes in skin occur due to external loads, the loads applied would have to be in addition to those normally experienced and thus registered by the body as 'a material increase in pressures'. To facilitate this, short doses of very high pressure (i.e. well outside the normal range for the foot) should be applied.

Thus, to inform device design, knowledge of normal peak vertical plantar pressures under each metatarsal head and contact area are important. Normal peak pressures were obtained from the results of the two aforementioned studies and are displayed as N/m^2 (kPa) (Table 5.1). These pressures were then normalised to kg of bodyweight so that they

could be translated into target pressure for research participants (whose weight would be unknown) (Table 5.2). From the peak pressure data, an estimate of how much pressure might be applied can be calculated as follows:

$$Target\ pressure\ applied\ by\ the\ device = normalised\ plantar\ pressure \times kg\ bodyweight\ of\ participant.$$

For example, a target pressure to apply for a 75kg adult might be 406.86kPa under the second metatarsal head (5.42 multiplied by 75) and 360.86kPa under the third metatarsal head (4.81 multiplied by 75).

Table 5.1 – Normal pressures under each metatarsal head

	Pressure (kPa)					
	1 MPJ	**2 MPJ**	**3 MPJ**	**4 MPJ**	**5 MPJ**	**Weight (kg)**
Bryant et al, 1999	289.6	419.5	362.8	251.1	248.6	70.1
Putti et al, 2008	277	361	330	233	151	74.2

Table 5.2 – Normal plantar pressures per kilogram of bodyweight

	Pressure (kPa) per kg of bodyweight				
	1 MPJ	**2 MPJ**	**3 MPJ**	**4 MPJ**	**5 MPJ**
Bryant et al 1999	4.131241	5.984308	5.175464	3.582026	3.546362
Putti et al, 1999	3.733154	4.865229	4.447439	3.140162	2.03504
Mean	3.932197	5.424769	4.811451	3.361094	2.790701

To calculate an approximate shear pressure that might be experienced under the metatarsal heads, and in the absence of quality data of shear pressure, force plate data was extrapolated and used as a guide. Vertical forces applied to the forefoot are approximately 1.2 times bodyweight, and forefoot shear force is approximately 0.2 times body weight – so approximately one sixth of the vertical force (Richards, 2008). Assuming that the ratio between vertical pressure and shear pressure is the same as between vertical forces and shear forces (merits of this are discussed later), this 1/6 relationship can be used to provide a general approximation of shear pressures the device might apply:

$$Target\ shear\ pressure = \frac{target\ vertical\ pressure}{6}$$

So 406.86 kPa divided by six gives an approximate shear value of 66.72 kPa for the second metatarsal head of a 75kg individual.

To calculate a suitable contact size, work was undertaken to allow a crude measure of metatarsal head diameters, using Vernier calipers and ultrasound, finding that second metatarsal diameters might reach 14mm. A composite, circular contact pad of 15mm diameter ($0.000177m^2$) was chosen as a starting point (although this could be changed later). This would allow enough surface area to compress skin overlying the metatarsal heads, without being so large as to cause some offloading by adjacent metatarsals. To calculate the force the device needed to apply, to achieve the target vertical and shear pressures, the contact area would be used as follows:

$$Force\ (N) = target\ pressure\ (Pa)\ \times contact\ area\ (0.000177)$$

The process above allowed participant-specific plantar and shear pressure to be determined at, or close to, the likely in-vivo levels. However, for the purposes of attempting to induce callus-like skin changes over a time frame that was realistic (i.e. did not over burden participants by requiring a study lasting several months), it was assumed that pressures applied should be above those normally experienced by the participants' feet. While callus is associated with approximately 35% increase in pressure, individuals will be subjected to these pressures during each step they take during the day. As it would only be feasible to apply additional loads at suitably high levels on a regular basis (e.g. daily) to participants for a short window of time (e.g. a few weeks) the initial specification for the load to be applied by the device would need to allow vertical pressures to be two to three times those experienced in vivo (under the second metatarsal head). For the example for a 75kg person the target vertical pressure (three times normal) might therefore be 1,220.58 kPa compression and 200.16 kPa shear. For a heavier individual, of say 90kg, this would be 1,464 kPa compression and 244.11 kPa of shear. Using a 15mm diameter contact pad, a compression force would be required of up to 215.6N and 258.6N for 75kg and 90kg individuals respectively. Likewise, shear force would need to be 35.4N and 43.1N respectively. Through this process a general specification for the device and components (i.e. actuators) delivering the load to the feet was derived.

5.3 Design of loading device

5.3.1 Components

A composite drawing of the device design is shown in Figure 5.1. Table 5.3 shows the device components. The contact pad (A) that contacts the forefoot is fixed to the top of the shear load cell (B). The shear load cell is attached to a slide (C) which moves superiorly and inferiorly along two vertical chrome steel shafts. The vertical force is transferred to the compression load cell (D) through the slide, whose inferior movement during loading compresses the load cell which in turn will measure this compression force. The slide attaches to a bracket (E) that is fixed to the shear actuator (F) via a mounting plate (G). The shear actuator sits on top of the compression actuator (H) and the two are fixed together via a connecting plate (I). The compression actuator is fixed via fixing brackets (J) to a base plate (K).

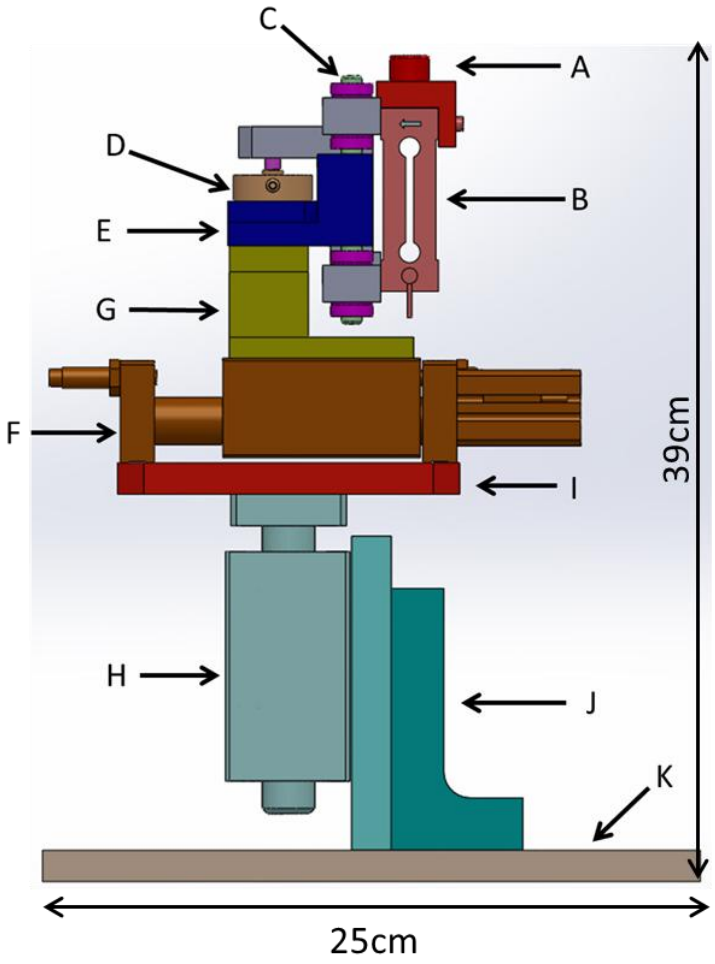


Figure 5.1 – Anatomy of loading device. A: contact pad. B: shear load cell. C: slide. D: compression load cell. E: bracket. F: shear actuator. G: mounting plate. H: compression actuator. I: connecting plate. J: fixing brackets. K: base plate.

Table 5.3 – Details of main device components.

Component	Supplier	Part number	Function
Vertical actuator.	SMC Pneumatics (UK) Ltd.	MGPM32-25A.	Vertical movement.
Horizontal actuator.	SMC Pneumatics (UK) Ltd.	CXTM16-25B.	Horizontal movement.
Compression load cell.	Applied Measurements.	CDFM3-500N.	0 – 500N measurement range.
Shear load cell.	Applied Measurements.	OBUG-20kg.	0 – 200N measurement range.
Load cell digitiser x 2.	Applied Measurements.	DSC-USB.	Supplies power to the load cell and digitises the millivolt signal from it. Allows user to display and log the force values.
Pressure regulators x 2.	SMC Pneumatics (UK) Ltd.	AW20-F02BCE.	Regulation of air pressure to cylinders.
Solenoid valves x 2.	SMC Pneumatics (UK) Ltd.	SY5120-6LOU-C6F-Q.	Control of actuator movements.
Limiter switch x 2	SMC Pneumatics (UK) Ltd.	D-M9BL.	Detects actuator movements.
Base plate, support; mounting and sliding brackets; contact pad.	Ryder and Wallace Ltd.	SGCN 0433-13A to 21A.	Aluminium supporting structure for device.
8mm diameter x 500mm long ground shaft	Hepco Motion.	NIM08-500.	Chrome steel shafts – elastic modulus 200 GPa. Used in slide for movement of shear load cell to transfer load to compression cell.
Computer control box.	Buswell Machine Electronics Ltd.	N/A.	Control of loading sequences. Drives solenoid valves.

The target pressure, force and contact area data described above were used to assist in the development of the device components. These data were a starting point rather than a completed technical specification since there was no previous attempt to do this type of research documented, and thus some unseen challenges were expected in both the device and experimental protocol in its use. In order to deliver forces in both a vertical and horizontal direction, in a controlled and cyclical manner, the decision was made to use actuators powered by pneumatic cylinders. This was deemed a suitable and reliable alternative to using a manually driven device, as had been used in previous projects in the same department (Hashmi et al., 2013). The use of actuators would give a repeatable and adjustable means of applying forces to the foot at a safe magnitude. In order to have optimum control of the forces delivered to the foot via a probe impacting the plantar surface, pressure regulators were fitted to both pneumatic cylinders to allow adjustment of the amount of air pressure delivered to them, and therefore the force resulting from the resistance of the foot. A Jun-Air® Quiet Air 6-15 air compressor base unit was used to deliver pressure to the actuators. This system delivers a maximum of 8 bars (120 PSI) of

pressure and has a 15 litre air tank which makes it ideal for delivering a large range of air pressure values. Velocity of the actuator movements was controlled by air flow restrictors located on the actuators.

It was necessary to have a method of measuring the amount of force delivered so that the air pressure to change the resultant load could be adjusted accurately for each study participant and target plantar pressures achieved with suitable accuracy and repeatability. The use of load cells that could be attached above the actuators and below the contact pad to measure the amount of force delivered by the actuators was deemed a suitable approach. The types of load cells required needed to reflect the types and range of the loads applied. A strain gauge button load cell with a maximum working value of 500N was chosen for compression measurement and a strain gauge single point load cell with a maximum working magnitude of 200N was chosen for shear measurement. Given the contact area of $0.00017m^2$, these sensors could measure up to 2,830.86 kPa of compression and 1,132.34 kPa; 2.3 and 5.6 times the expected maximum compression and shear pressures applied respectively. The compression load cell measures the vertical load applied directly to the cell, while the shear cell, which was positioned longitudinally, measures the amount of horizontal force delivered causing a bend through the strain gauge within the cell. Having developed an outline concept of what the device was required to achieve, the author consulted engineering design support to agree a technical specification, parts list, assembly schedule, and commissioning process. This allowed conversion of device specifications into practicality.

5.3.2 Loading programme sequences

Once the device was built, the issue of how to deliver the loading cycles was advanced. As the device is powered by pneumatic cylinders, there needed to be a system of switches and pneumatic control valves to enable the device to perform movements by allowing and ceasing the air delivery to the pistons controlling the actuators. This could either be performed manually by the investigator, or a series of sequences for loading cycles could be programmed into an electronic computer. For the purposes of efficiency, the latter option was chosen. For the control computer, some basic control functions were needed including: a programme selection; a stop and reset command; a cycle count display; a pause and continue function; and a cycle step function. The sequences that were chosen were based on possible methods of load application that the investigator would use on the foot. The basic load programme sequences are shown below.

1. ‘Compression’ programme: pressure applied to move contact pad vertically to contact the forefoot, then apply pressure in reverse direction to retract the contact pad to start position (the number of vertical movements is selected manually before intervention).
2. ‘Compression and shear’ programme: pressure applied to move contact pad vertically to contact the forefoot; pressure applied to move contact pad forwards; pressure applied in reverse direction to move contact pad backwards (the number of forwards and backwards movements is selected manually, and total number of movements is completed before next step); then apply pressure to retract the contact pad downwards, with horizontal actuator set in starting (backwards) position.
3. ‘Combined’ programme: pressure applied to move contact pad vertically to contact the forefoot; pressure applied to move contact pad forwards; pressure applied to retract the contact pad downwards; pressure applied to move contact pad backwards to starting position (all movements are one repetition, the number of repetitions is preselected).
4. ‘Gait simulation’ programme: pressure applied to move contact pad vertically to contact the forefoot; pressure applied to move contact pad forwards; pressure applied in reverse direction to move contact pad backwards; then apply pressure to retract the contact pad downwards to starting position (all movements are one repetition, the number of repetitions is preselected).

The following paragraphs show a breakdown of the actual sequences for each loading cycle which were based on predefined displacement profiles that were programmed into the device computer. Figure 5.2 shows the positions of the limiter switches which are instrumental in control of the device movements that are explained below.

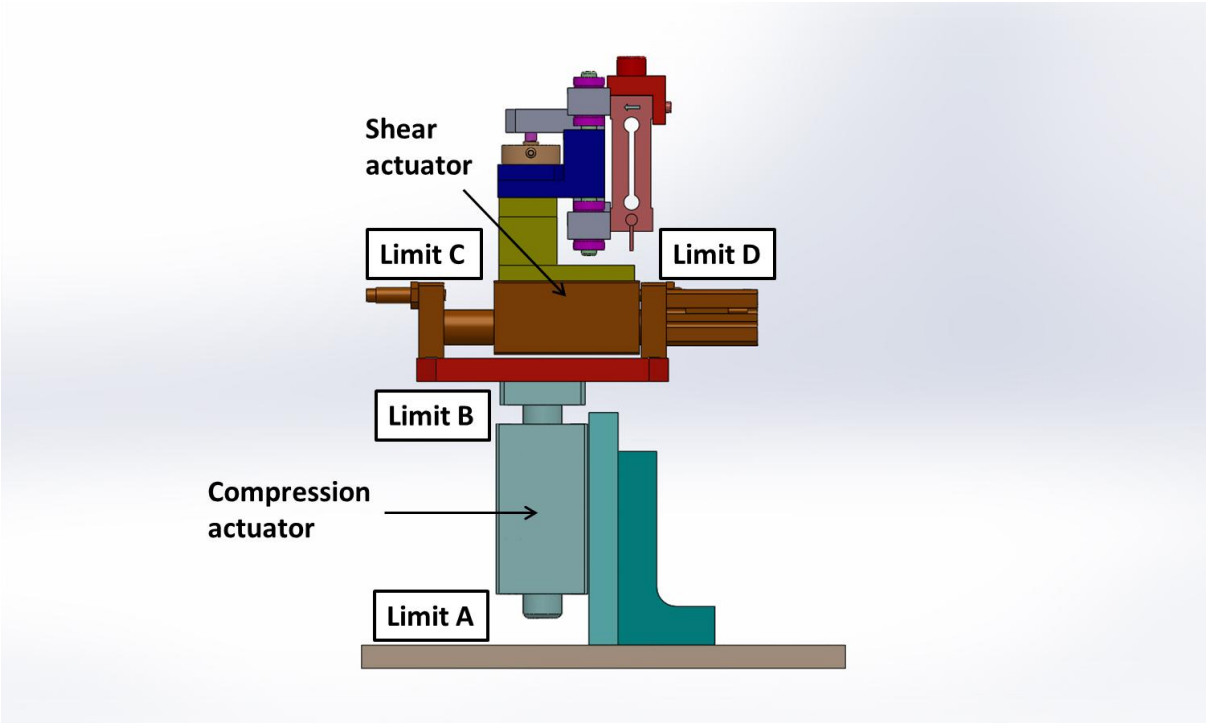


Figure 5.2 – Diagram showing limiter switch positions.

Start conditions:

- Each actuator is controlled by single acting spring return pneumatic 12V solenoid valve.
- Compression actuator retracted, Limit A closed and Limit B open.
- Shear actuator retracted, Limit D closed and Limit C open.
- Select Programme 1, 2, 3 or 4.
- Set number of cycles.
- Set delay on compression actuator in ‘up’ position.
- Set delay on compression actuator in ‘down’ position.
- Set delay on shear actuator in ‘out’ position.
- Set delay on compression actuator in ‘in’ position.

Compression programme sequence:

In this sequence, the skin is compressed in vertical motions where the contact pad contacts and leaves the skin for a set number of cycles.

1. Shear actuator locked at ‘in’ position and Limit D closed (Limit C open).
2. Energise solenoid valve controlling compression actuator.
3. Actuator moves up and Limit B closes (Limit A opens).

4. Programmed time delay.
5. De -energise solenoid valve controlling compression actuator.
6. Actuator moves down and Limit A closes (Limit B opens).
7. Programmed time delay.
8. Repeat steps 3 to 8 for required number of cycles.
9. Test completed, return to start state conditions.

Shear programme sequence:

1. In this sequence, the contact pad moves anteriorly and posteriorly on the skin for a set number of cycles without retracting.
2. Energise solenoid valve controlling compression actuator.
3. Actuator moves up and limit B closes (limit A opens).
4. Energise solenoid valve controlling shear actuator.
5. Shear actuator moves out and limit C closes (limit D opens).
6. Programmed time delay.
7. De-energise solenoid valve controlling shear actuator.
8. Actuator moves in and limit D closes (limit C opens).
9. Programmed time delay.
10. Repeat steps 3 to 8 for required number of cycles.
11. De-energise solenoid valve controlling compression actuator.
12. Actuator moves down and Limit A closes (limit B opens).
13. Test completed, return to start state conditions.

Combined programme sequence:

In this sequence, the contact pad compresses than moves anteriorly on the skin for a set number of cycles. The contact pad then retracts and returns to the start conditions at the beginning of each cycle.

1. Shear actuator locked at ‘in’ position and Limit D closed (Limit C open).
2. Energise solenoid valve controlling compression actuator.
3. Actuator moves up and limit B closes (limit A opens).
4. Programmed time delay.
5. Energise solenoid valve controlling shear actuator.
6. Shear actuator moves out and limit C closes (limit D opens).
7. Programmed time delay.

8. De-energise solenoid valve controlling compression actuator.
9. Actuator moves down and Limit A closes (limit B opens).
10. Programmed time delay.
11. De-energise solenoid valve controlling shear actuator.
12. Actuator moves in and Limit D closes (Limit C opens).
13. Programmed time delay.
14. Repeat steps 3 to 14 for required number of cycles.
15. Test completed, return to start state conditions.

Gait simulation programme sequence

In this sequence, the skin is compressed, the contact head moves anteriorly and posteriorly on the skin, and then retracts. This sequence is repeated for the desired number of cycles.

1. Shear actuator locked at 'in' position and limit D closed ((limit C open)
2. Energise solenoid valve controlling compression actuator
3. Actuator moves up and limit B closes (limit A opens)
4. Programmed time delay (U).
5. Energise solenoid valve controlling shear actuator.
6. Shear actuator moves out and limit C closes (limit D opens)
7. Programmed time delay (O).
8. De-energise solenoid valve controlling shear actuator.
9. Actuator moves in and limit D closes (limit C opens).
10. Programmed time delay (I).
11. De-energise solenoid valve controlling compression actuator.
12. Actuator moves down and limit A closes (limit B opens).
13. Programmed time delay (D).
14. Repeat steps 3 to 14 for required number of cycles.
15. Test completed, return to start state conditions.

To ensure participant safety, the device computer had a preset function which could stop movements of the actuators mid-cycle. The power to the device could also be cut which caused the device to instantly retract downwards from the test subject's foot.

5.3.3 Design of device housing

A stage constructed of 25mm thick plywood was built to house the loading device and prevent any contact with the moving parts which could potentially cause injury to the participant (figure 5.3). This construct also enables the subject to stand above the device which would then apply loads vertically to the plantar aspect of the forefoot. Railings were attached to the stage to assist the participant in stepping onto it and for maintaining balance while having their foot loaded in a standing position. Holes in the sides of the stage were cut to allow the investigator access to the device in order to adjust any settings; and to allow piping and cables to be connected to external devices including an air compressor, laptop computer, and loading cycle computer.

Figure 5.3 – Device housing showing holes in wall and aperture on platform to allow loading of forefoot.

5.3.4 Device modifications

Various minor modifications were made to the device to improve its function and eliminate issues identified through pilot work. The first issue identified was that during the shear programme cycle (and other programmes using the forwards/backwards motion) the compression load cell would measure double the force magnitude at the posterior (starting) position of the shear movement. Through testing of the load cells and actuator movements, this was deemed to be due to compliance occurring through the device which caused excess compression on the compression load cell during the backwards movement. The compliance of the device was very small but given the low deflection range of the load

cell, this was found to be too large. In an attempt to eliminate this, the compression cell was moved to a position beneath the shear load cell to reduce the effect of bending on force measurements; and the device bearings were replaced with larger shafts and secured tightly to increase the device stiffness, eliminating any play (Figure 5.4). This reduced the deviation in compression forces by around 20% but this was deemed to be insufficient. A bending moment was still occurring so the next step was to increase the device stiffness further. This was achieved by replacing the bracket, mounting plate and slide components (figure 5.1 C, E and G) with two pieces of thicker metal and making the shafts thicker, which helped to further eliminate compression force discrepancies (figure 5.4).

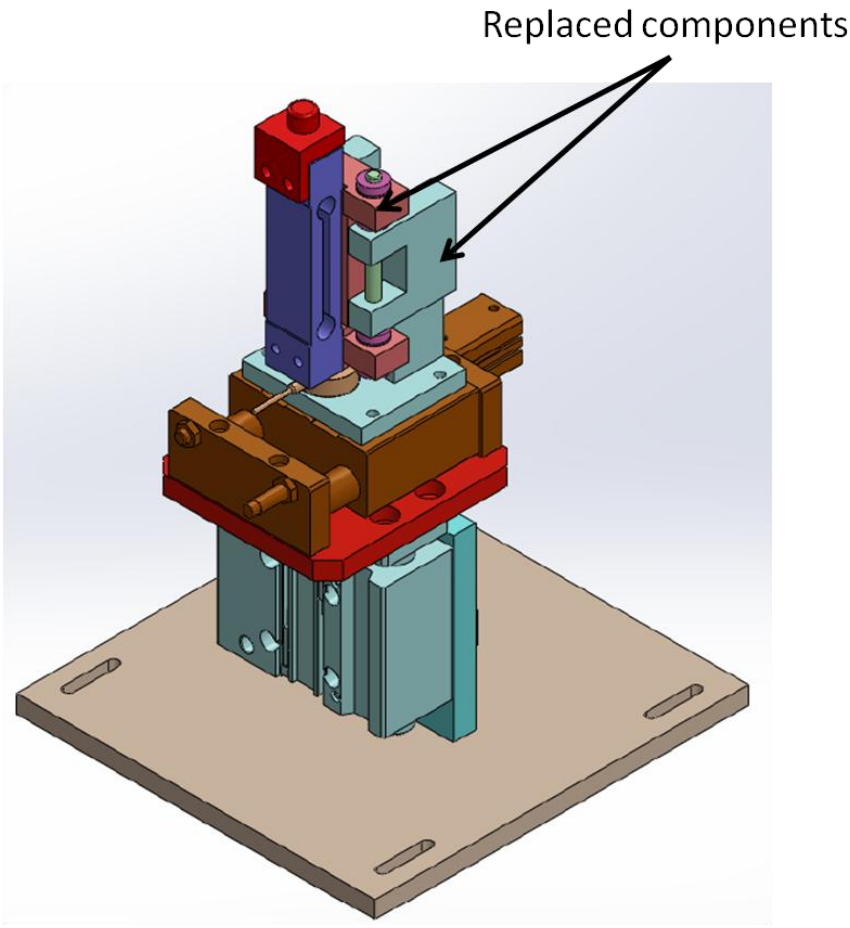


Figure 5.4 – Replaced components.

In order for the device to be used in biomechanics research, it must produce repeatable and consistent loads at different force magnitudes. The accuracy and repeatability of the loads applied should be tested using materials (e.g. wood and EVA)

rather than the foot in the first instance as it would avoid potential safety concerns such as overloading.

5.4 Compression and shear load tests

5.4.1 Rationale and aim

Before any work using the device was conducted, a thorough test of the compression and shear load cells was required to ensure that load measurements were accurate, and to understand if any relationship existed between the compression and shear force measurements when applying only compression loads to the device. The aim of these tests was to test the accuracy of the load cells with different combinations of loads being applied with the device in different positions.

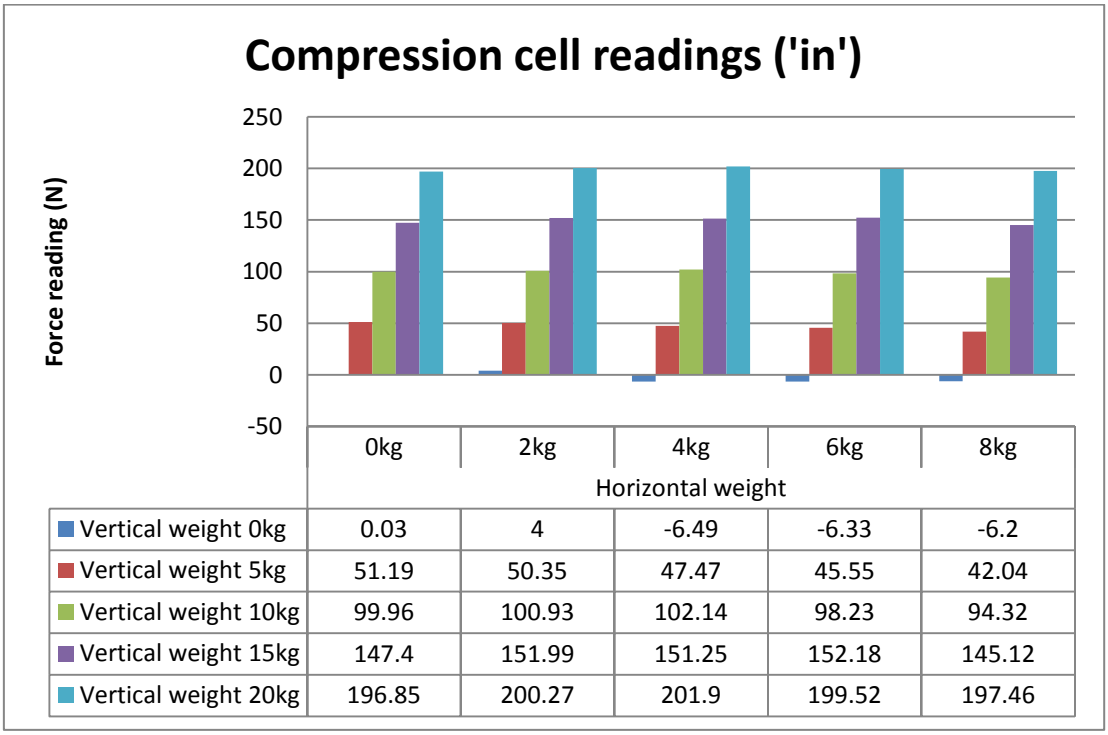
5.4.2 Materials and methods

Four 5kg mass Olympic weights discs, a hanging base weight (1kg) with one 1kg disc and three 2kg discs to make a total of 8kg of mass, a 0.1kg plate and a pulley system designed and built by the author of this thesis were used in these tests. At each test, the data logging software measurements were zeroed before any loads were applied. Loads were then applied to the horizontal pulley system to test the shear load cell and atop the contact pad to test the compression load cell. A small 0.1kg disc was used on top of the contact pad to provide a platform for the weight discs for the vertical cell. The device was set up so that the position of the contact pad was as close to the central longitudinal axis as possible. This setup was used for the remainder of the project. Shear loads were applied in the posterior direction with incremental increases in load on both compression and shear cells. This was conducted with the shear actuator in its backward (or ‘in’) position, then with the shear actuator set in the forward (or ‘out’) position. This aimed to test whether the position of the device in the antero-posterior direction had any effect on the load cell measurements. These tests were then conducted but with shear being applied in the anterior direction. This aimed to test whether there were any major differences in load cell measurements when the loads were applied in the opposite direction. At each magnitude of horizontal mass applied (0kg, 2kg, 4kg, 6kg, 8kg), vertical masses were increased from 0 to 20kg in 5kg increments so there was a matrix of 25 combinations tested.

5.4.4 Results

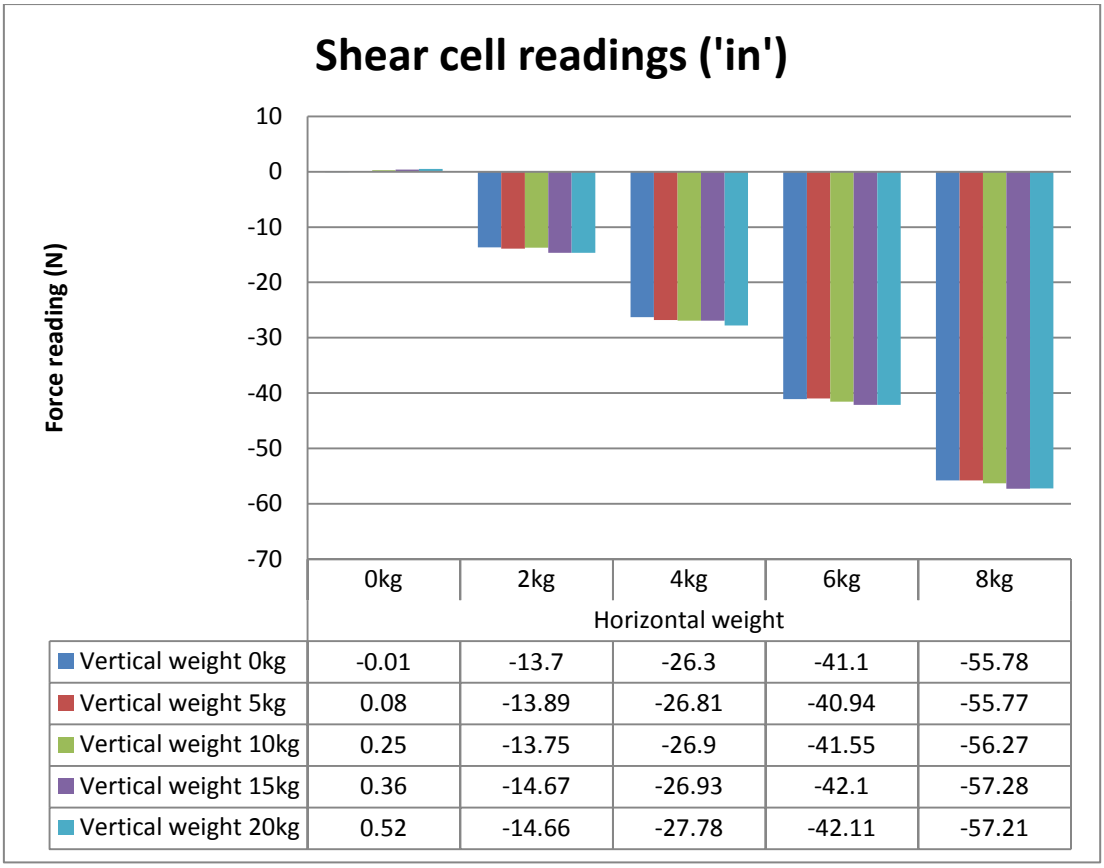
The results of the load cell tests are presented below. Compression and shear load cell measurements were obtained for posterior directed shear in the 'in' and 'out' position (Figures 5.5 – 5.8) and for anterior directed shear in the 'in' and 'out' position (Figures 5.9 – 5.12).

Shear in posterior direction, device locked at 'in' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0.03	4	-6.49	-6.33	-6.2
Vertical weight 5kg	51.19	50.35	47.47	45.55	42.04
Vertical weight 10kg	99.96	100.93	102.14	98.23	94.32
Vertical weight 15kg	147.4	151.99	151.25	152.18	145.12
Vertical weight 20kg	196.85	200.27	201.9	199.52	197.46

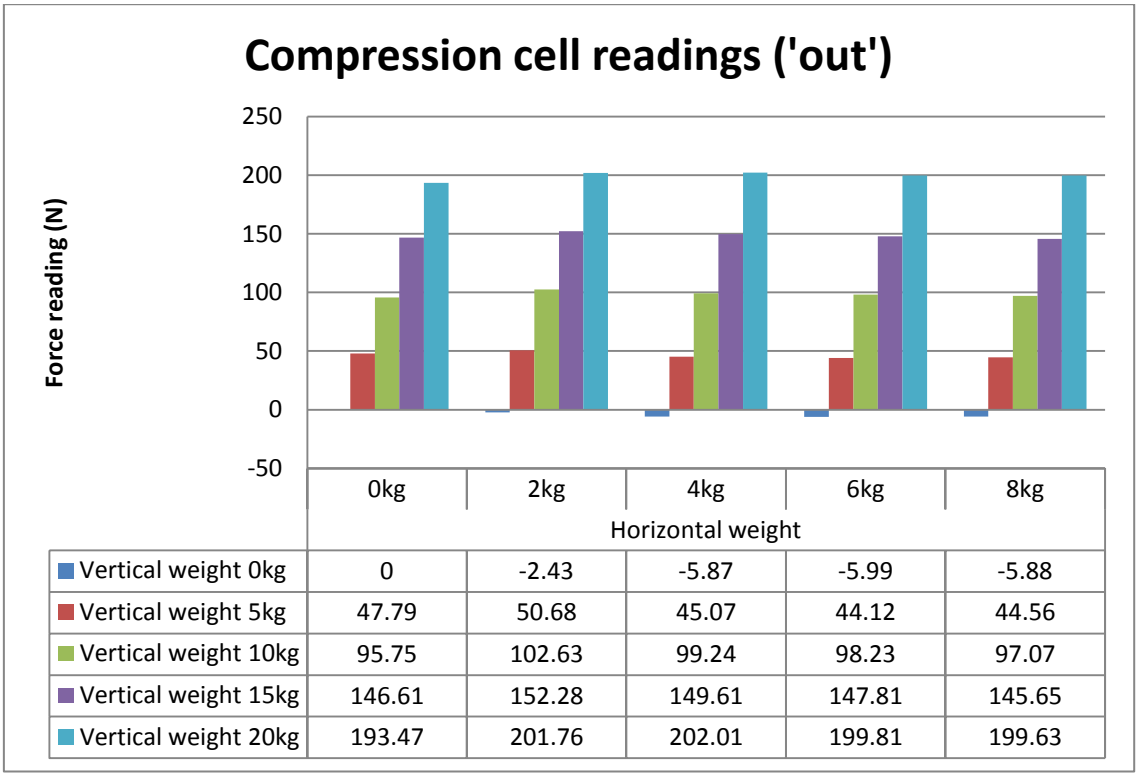
Figure 5.5 – Compression cell measurements: posterior shear, device locked at 'in' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	-0.01	-13.7	-26.3	-41.1	-55.78
Vertical weight 5kg	0.08	-13.89	-26.81	-40.94	-55.77
Vertical weight 10kg	0.25	-13.75	-26.9	-41.55	-56.27
Vertical weight 15kg	0.36	-14.67	-26.93	-42.1	-57.28
Vertical weight 20kg	0.52	-14.66	-27.78	-42.11	-57.21

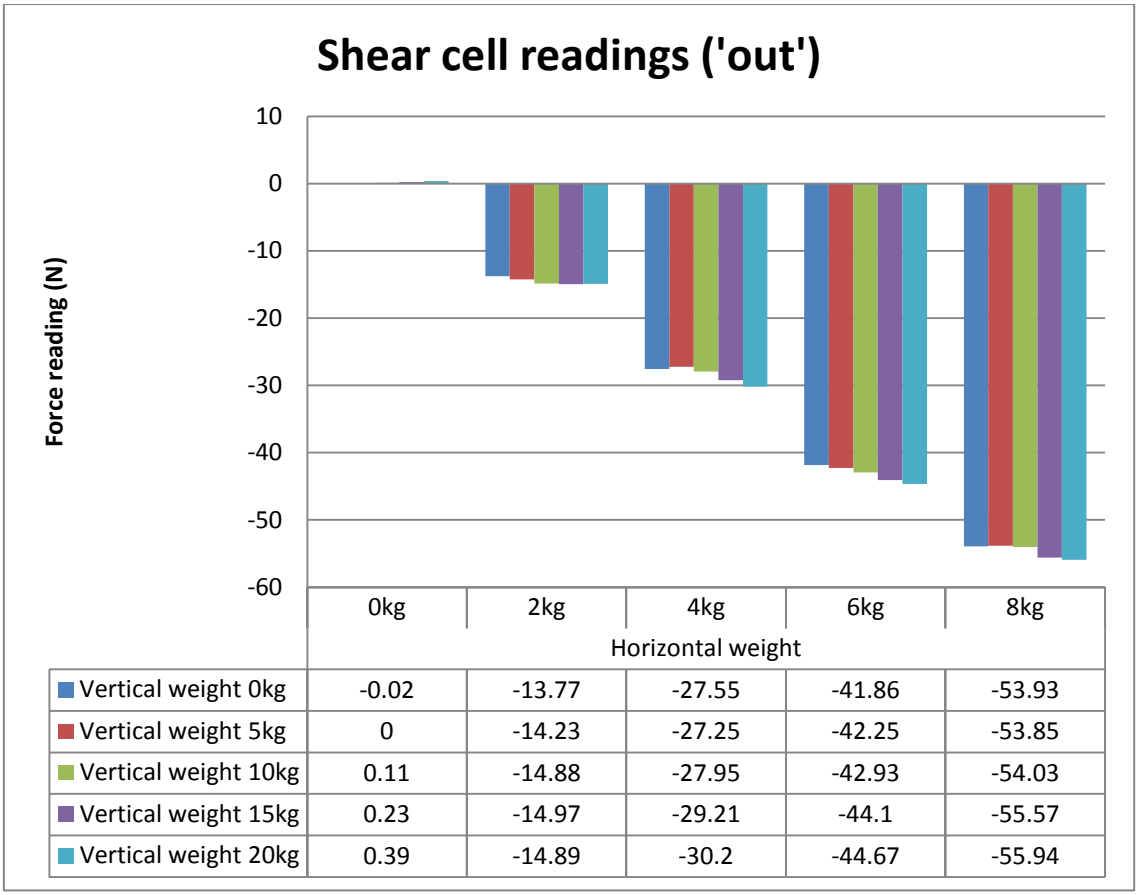
Figure 5.6 – Shear cell measurements: posterior shear, device locked in 'in' position

Shear in posterior direction, device locked in 'out' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0	-2.43	-5.87	-5.99	-5.88
Vertical weight 5kg	47.79	50.68	45.07	44.12	44.56
Vertical weight 10kg	95.75	102.63	99.24	98.23	97.07
Vertical weight 15kg	146.61	152.28	149.61	147.81	145.65
Vertical weight 20kg	193.47	201.76	202.01	199.81	199.63

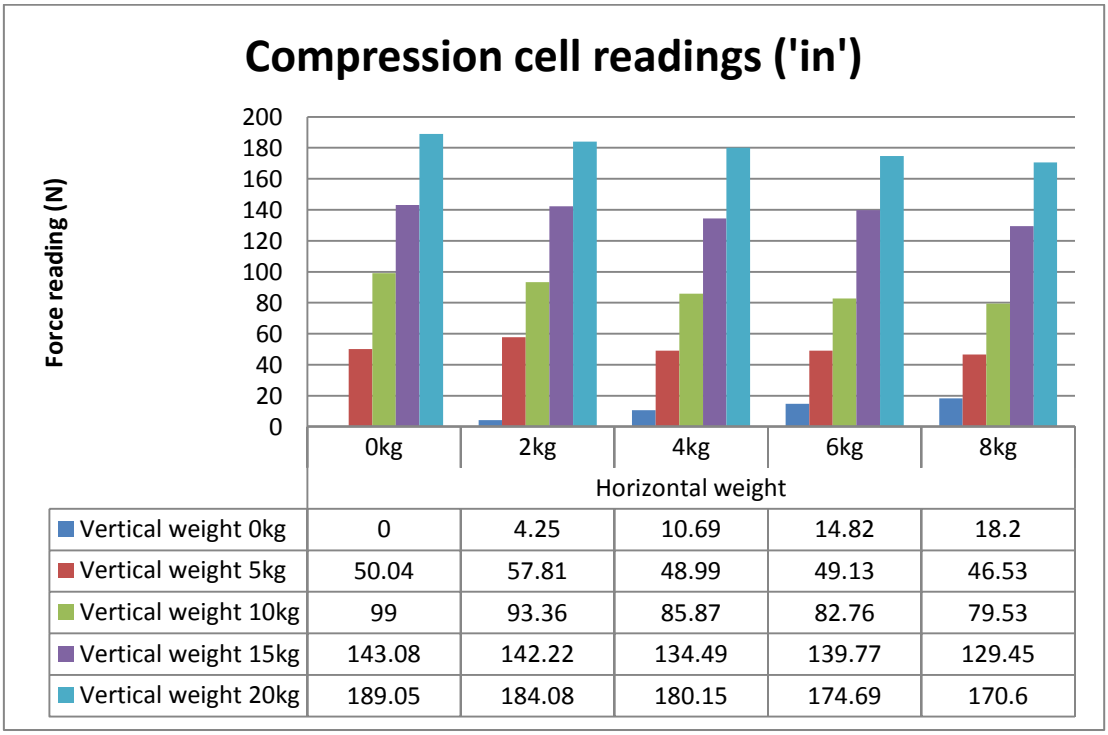
Figure 5.7 – Compression cell measurements: posterior shear, device locked in 'out' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	-0.02	-13.77	-27.55	-41.86	-53.93
Vertical weight 5kg	0	-14.23	-27.25	-42.25	-53.85
Vertical weight 10kg	0.11	-14.88	-27.95	-42.93	-54.03
Vertical weight 15kg	0.23	-14.97	-29.21	-44.1	-55.57
Vertical weight 20kg	0.39	-14.89	-30.2	-44.67	-55.94

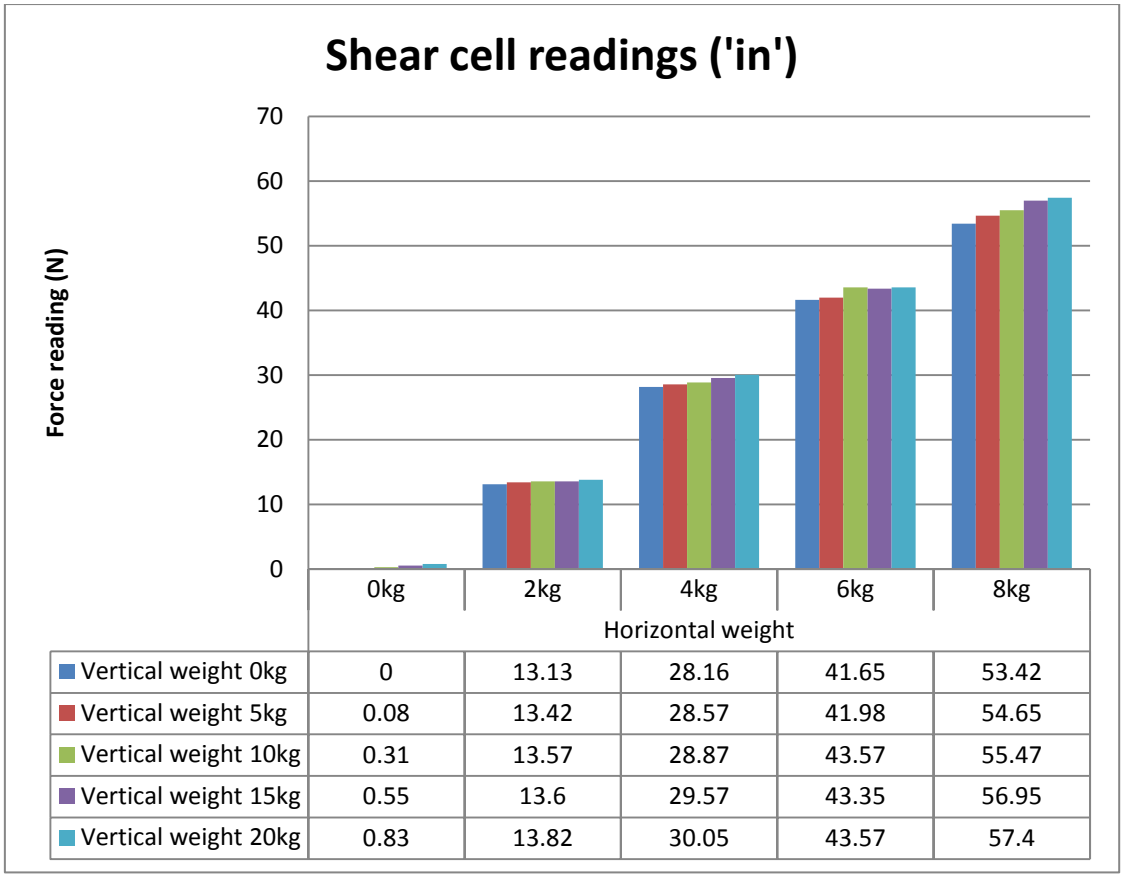
Figure 5.8 – Shear cell measurements: posterior shear, device locked in 'in' position

Anterior shear, device locked at 'in' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0	4.25	10.69	14.82	18.2
Vertical weight 5kg	50.04	57.81	48.99	49.13	46.53
Vertical weight 10kg	99	93.36	85.87	82.76	79.53
Vertical weight 15kg	143.08	142.22	134.49	139.77	129.45
Vertical weight 20kg	189.05	184.08	180.15	174.69	170.6

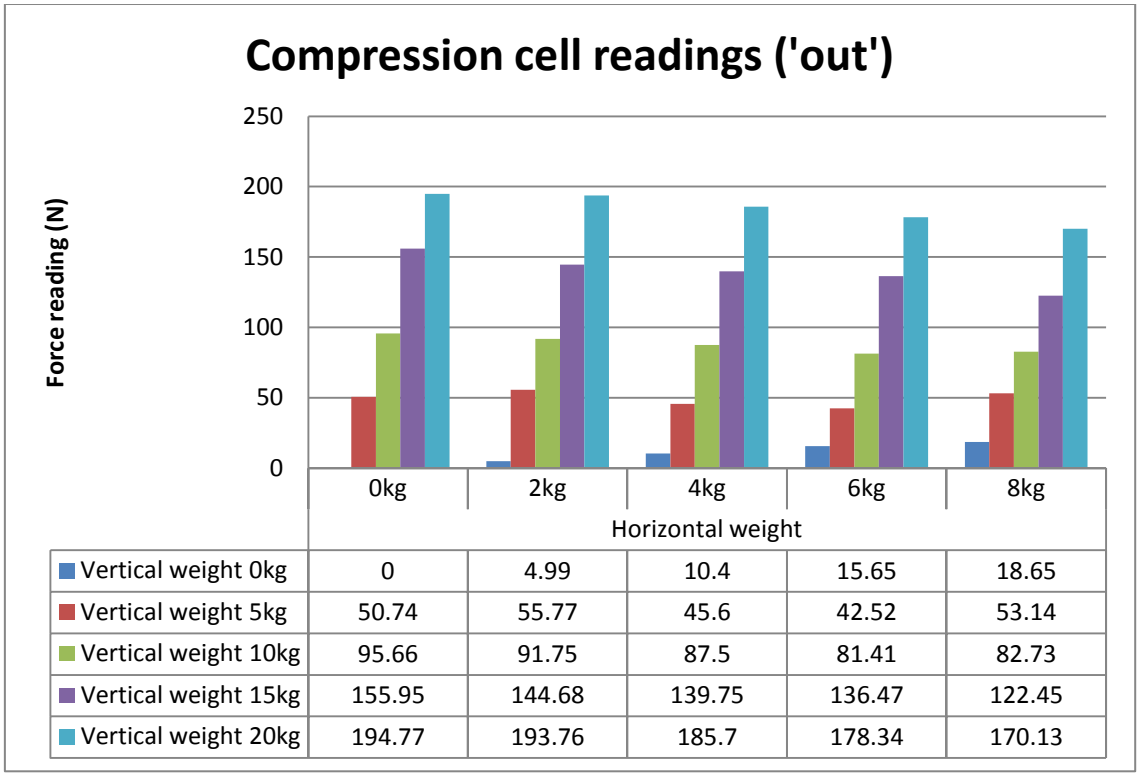
Figure 5.9 – Compression cell measurements: anterior shear, device locked at 'in' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0	13.13	28.16	41.65	53.42
Vertical weight 5kg	0.08	13.42	28.57	41.98	54.65
Vertical weight 10kg	0.31	13.57	28.87	43.57	55.47
Vertical weight 15kg	0.55	13.6	29.57	43.35	56.95
Vertical weight 20kg	0.83	13.82	30.05	43.57	57.4

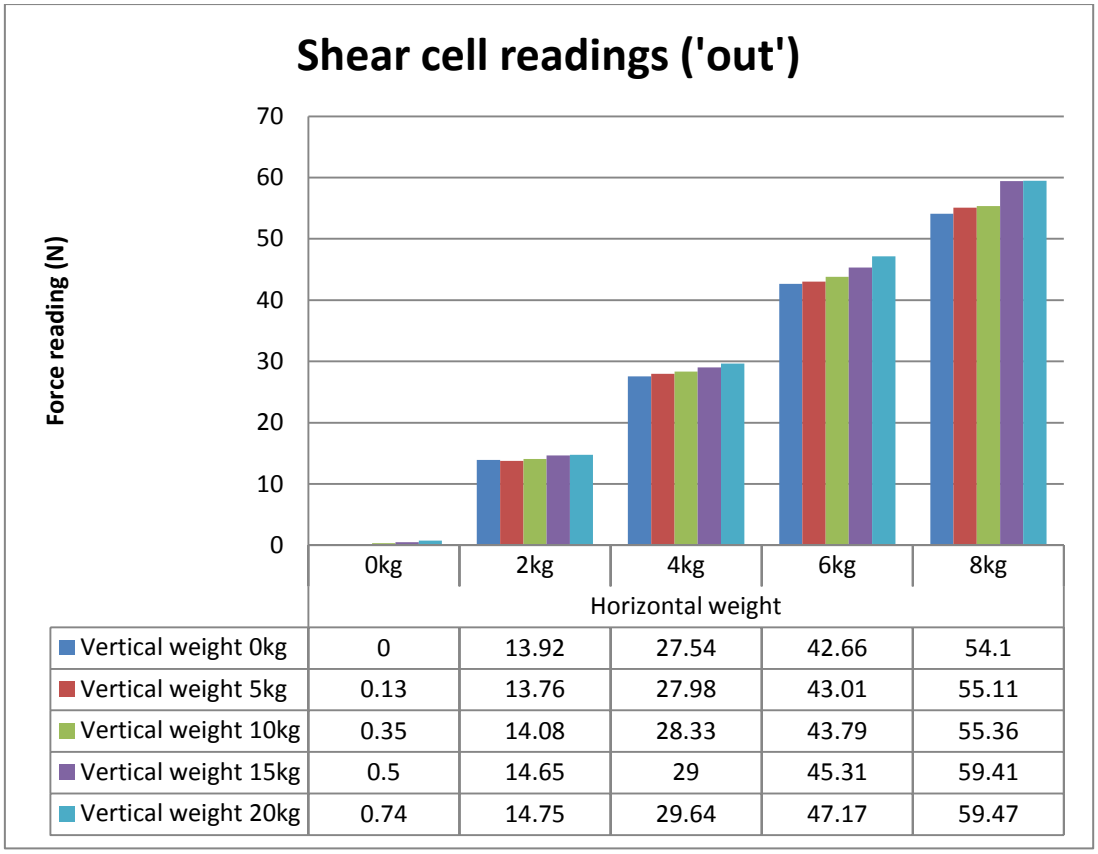
Figure 5.10 – Shear cell measurements: anterior shear, device locked at 'in' position

Anterior shear, device locked at 'out' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0	4.99	10.4	15.65	18.65
Vertical weight 5kg	50.74	55.77	45.6	42.52	53.14
Vertical weight 10kg	95.66	91.75	87.5	81.41	82.73
Vertical weight 15kg	155.95	144.68	139.75	136.47	122.45
Vertical weight 20kg	194.77	193.76	185.7	178.34	170.13

Figure 5.11 – Compression cell measurements: anterior shear, device locked at 'out' position



	0kg	2kg	4kg	6kg	8kg
Vertical weight 0kg	0	13.92	27.54	42.66	54.1
Vertical weight 5kg	0.13	13.76	27.98	43.01	55.11
Vertical weight 10kg	0.35	14.08	28.33	43.79	55.36
Vertical weight 15kg	0.5	14.65	29	45.31	59.41
Vertical weight 20kg	0.74	14.75	29.64	47.17	59.47

Figure 5.12 – Shear cell measurements: anterior shear, device locked at 'out' position

5.4.5 Discussion

At each weight increment, the compression and shear cell measurements were very consistent. When shear is applied in both the posterior and anterior direction, there appeared to be minimal influence of vertical load increase on shear cells measurements. The compression load cell was more sensitive to increases in shear loads, particularly in the anterior direction. The compression cell measurements increased at 0kg of compression loads when the shear loads were applied. This is likely due to the load cell being offloaded slightly in the posterior direction and increased slightly in the anterior direction; a product of the device design where there may be a slight bending moment with the introduction of horizontal loads that affects the compression cell measurement. When shear loads were increased the compression cell measurements tended to show a reduction in measured compression force at 5kg, 10kg, 15kg, and 20kg, particularly when shear was applied in the anterior direction. The reasons for this are unclear. There appears to be no influence of the position of the device, whether in the 'in' or 'out' position, on the load cell measurements. This indicates that play in the shear actuator does not cause discrepancies in data. This means that the device can remain attached to the shear actuator.

The horizontal loads measured by the shear cell appear smaller than the actual load being applied to the pulley. The difference between the measured load and the actual load becomes consistently larger with increases in horizontal loads applied. This could be due to friction in the pulley wheels taking some of the load. This can be tested by removing the device from the housing and hanging weights directly from the device to see if the loads measured are more accurate. If not, it might indicate that the load cell requires calibrating.

5.5 Shear load cell tests

5.5.1 Rationale and aim

Based on the results from the previous tests, it was necessary to assess whether the shear load cells were measuring loads inaccurately, or whether the discrepancies between measured and actual loads were due to friction in the pulley system. The aim of these tests was to assess shear load cell accuracy with load applied directly to it.

5.5.2 Materials and methods

The top part of the device housing the compression and shear cells was removed from the actuator system and attached to a purpose built platform which allowed weights to be applied directly to the device in order to test the shear cell accuracy.

5.5.3 Results

The results are presented in Figure 5.13.

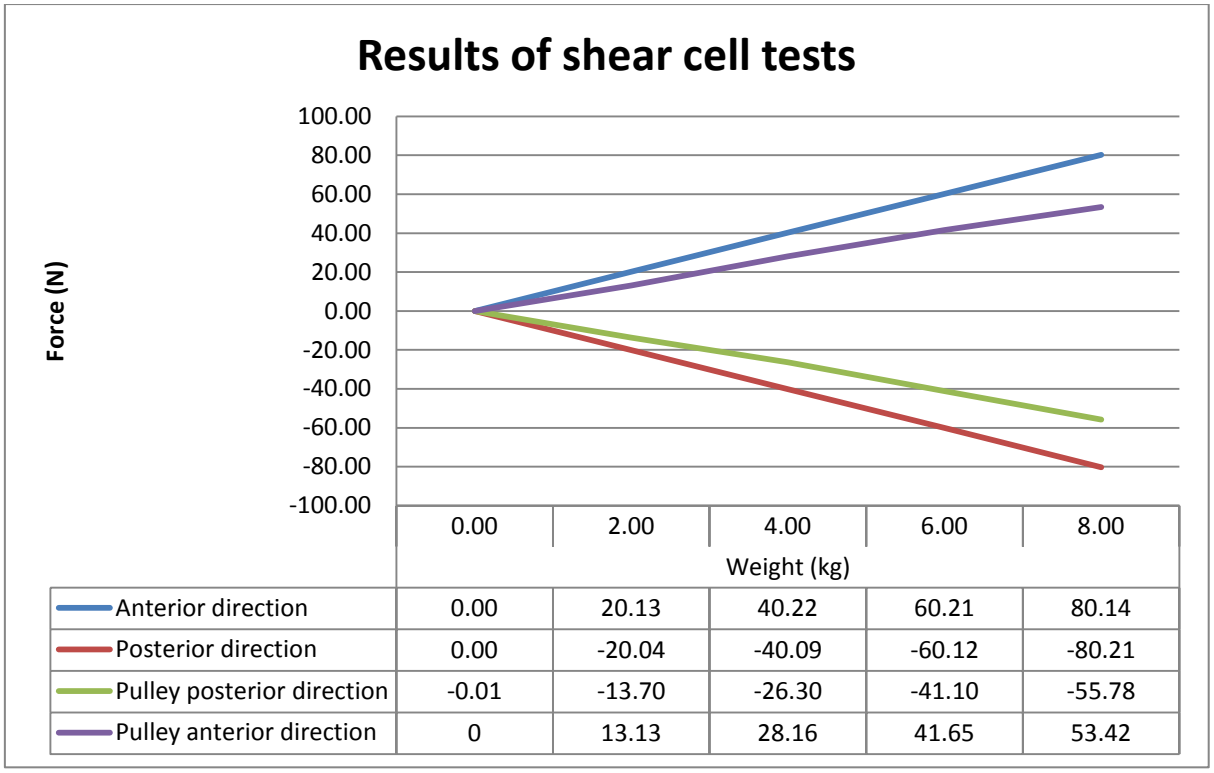


Figure 5.13 – Results of shear cell tests. Weights applied directly to load cell (blue and red lines) show accuracy of measurement compared to when measurements were obtained using pulley system (green and purple lines).

5.5.4 Discussion

It was found that the shear load cell was working correctly because the loads that were applied to the device were measured accurately. Figure 5.13 compares the force measurements in the anterior and posterior direction with those obtained using the pulley system at 0kg of compression force. It shows a systematic effect of the pulley on the force measurements obtained from the shear cell which suggests friction in the pulley system. In both the anterior and posterior shear directions, the loads applied when using the pulley system are 68% of the actual loads.

5.4.6 Conclusion

Both the compression and shear load cells are able to measure loads accurately and consistently. There is no major influence of compression load on shear measurements, but there is some influence of shear loads on compression measurements. The reason for this is unclear but it may mean that some correction for errors in data might be necessary if using the shear actuator. In terms of compression cycles, there will likely be some shear measured by the shear load cell when applied to foot as the metatarsal head is not flat, but

the results of these tests suggest that any compression and shear measurements obtained from plantar skin are likely to be accurate. However, this process also indicates the need for regular check calibrations to ensure that there are no problems with the load cells.

5.6 Compression tests

5.6.1 Aims and methods of compression tests

The first aim of compression tests were to gain an understanding of the relationship between Bars of pressure delivered to the compression actuator, and the resulting forces (Newtons) applied by the device at the contact pad. This would show whether a linear relationship between pressure in the cylinder and force exists, and thus provide information about how to control and adjust the device through the pressure gauge. This is important in being able to apply person-specific plantar pressures, requires that the forces applied by the device can be adjusted accordingly, and they are in part dependent upon the pressure in the cylinder. The second aim was to determine whether or not the device could produce consistent loads during repeated compression cycles. To achieve the aims, the air pressure to the compression actuator was set at two, three, four, five and six Bars. For each test, the device applied 20 cycles of compression with a 10 millisecond delay in the 'up' and 'down' positions. The tests were delivered on plywood, medium density EVA and skin overlying the second metatarsal head of one subject. The following sections show the results from these tests.

5.6.2 Compression test results on plywood

The results of the compression tests on plywood are displayed in Table 5.4 and Figures 5.14 – 5.23. Table 5.4 and Figure 5.14 show that there is a linear relationship between the pressure applied to the compression actuator and the resulting force applied to the plywood. The raw data graphs for each compression test (Figures 5.15 – 5.23) show that the device delivers consistent loads at different magnitudes on hard material. The within-session coefficient of variation (CV) of the force peak magnitudes was shown to be very small ranging from 0.4 – 1.9%. Repeatability was also tested for the between-session mean magnitudes at 2, 3, 4, 5 and 6 Bars of pressure with CV data collected for three datasets. The air pressure was cut between each set of compressions so that the results would reflect how accurately the investigator could set the air pressure. The CV between sessions was found to be low ranging from 0.3 – 5.6%.

Table 5.4 – Results of compression tests on plywood

Pressure delivered to actuator	Resulting compression load (N)	Vertical pressure (kPa) at contact point
2 Bars	99.90	565.61
2.5 Bars	143.99	815.23
3 Bars	183.73	1,040.23
3.5 Bars	214.42	1,213.98
4 Bars	252.56	1,429.92
4.5 Bars	285.61	1,614.04
5 Bars	318.43	1,802.86
5.5 Bars	354.01	2,004.3
6 Bars	382.69	2,166.68

Figure 5.14 – Relationship between cylinder pressure and force delivered by the device to the plywood

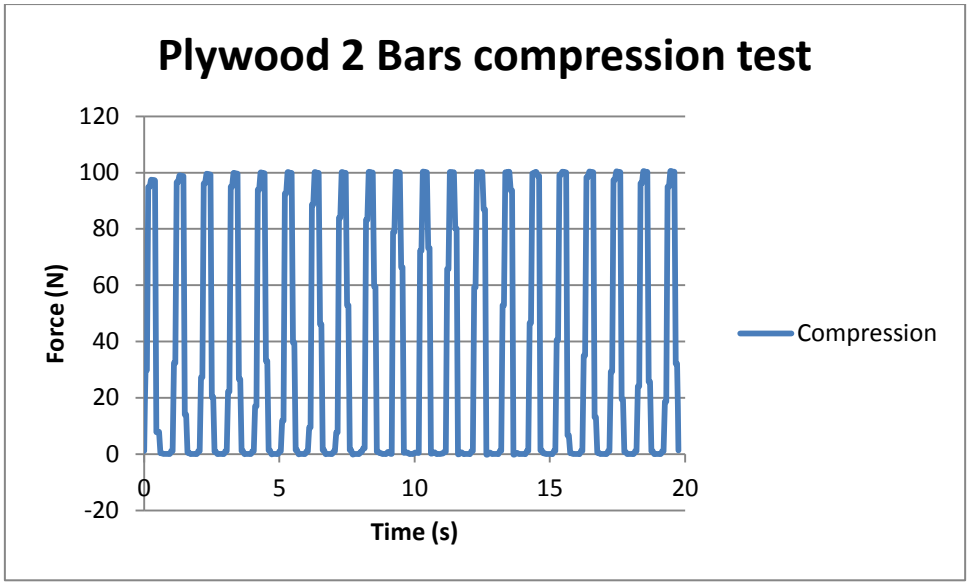


Figure 5.15 – 2 Bars compression test data

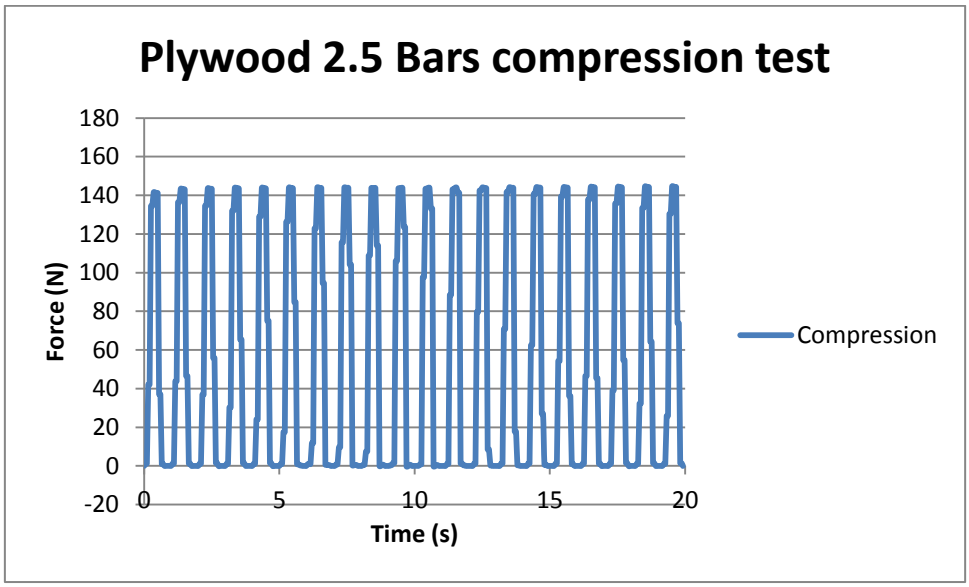


Figure 5.16 – 2.5 Bars compression test data

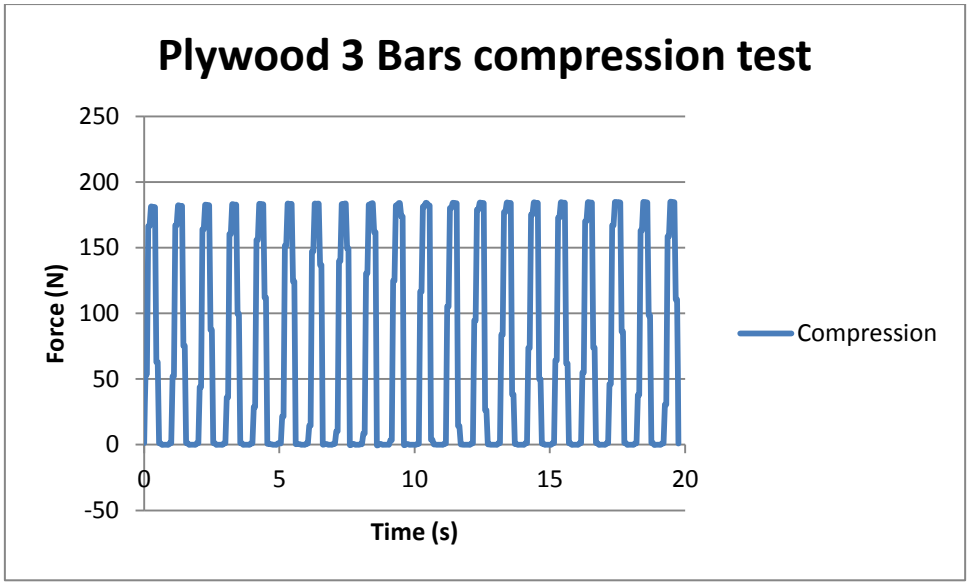


Figure 5.17 – 3 Bars compression test data

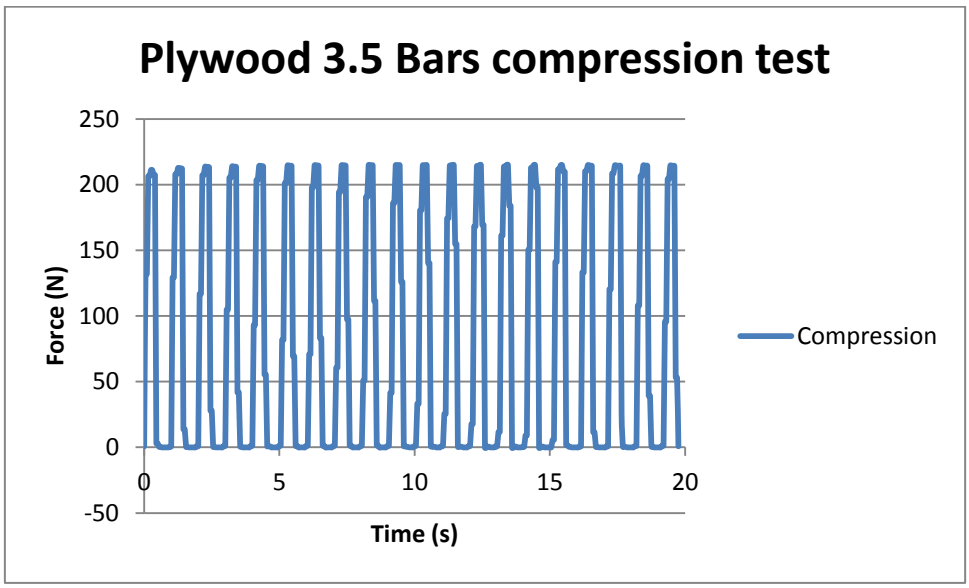


Figure 5.18 – 3.5 Bars compression test data

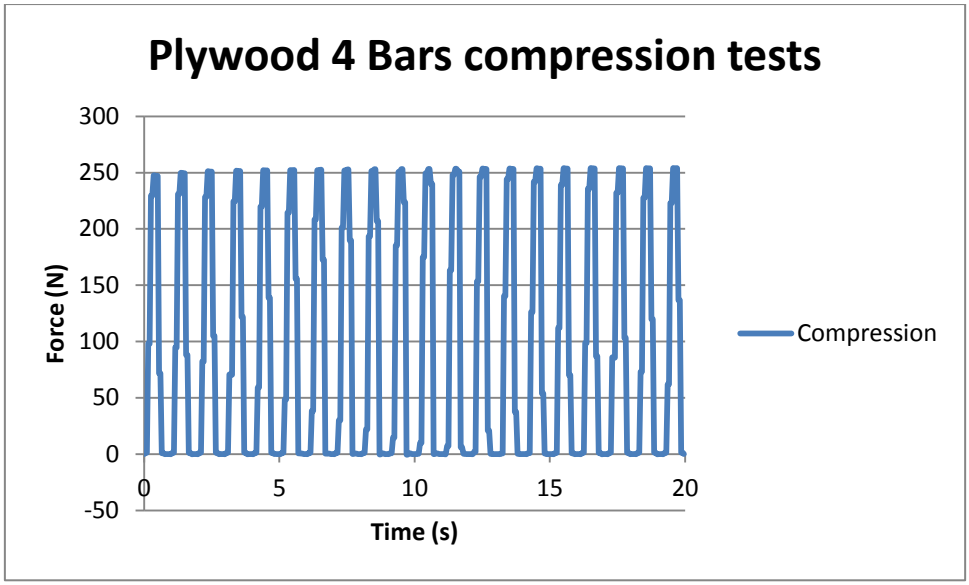


Figure 5.19 – 4 Bars compression test data

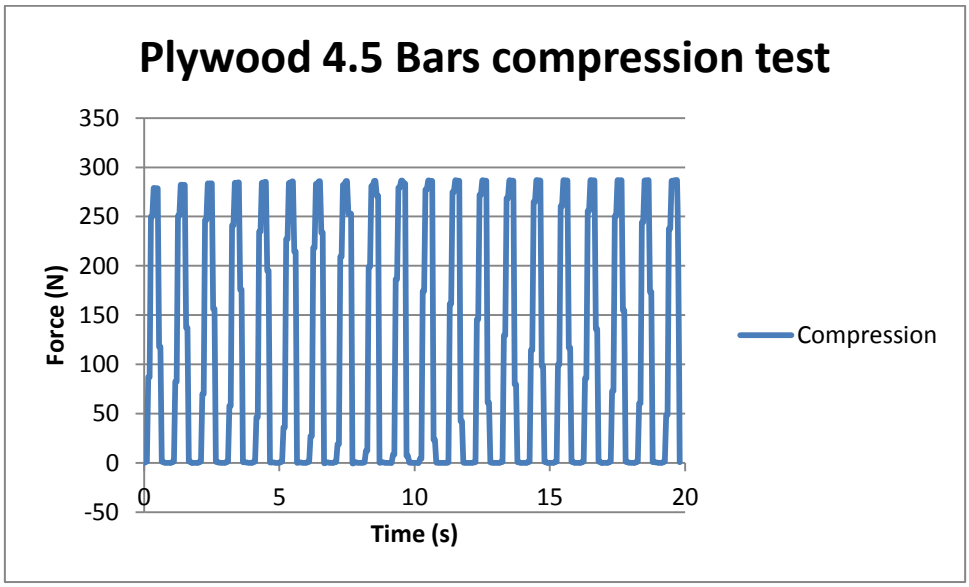


Figure 5.20 – 4.5 Bars compression test data

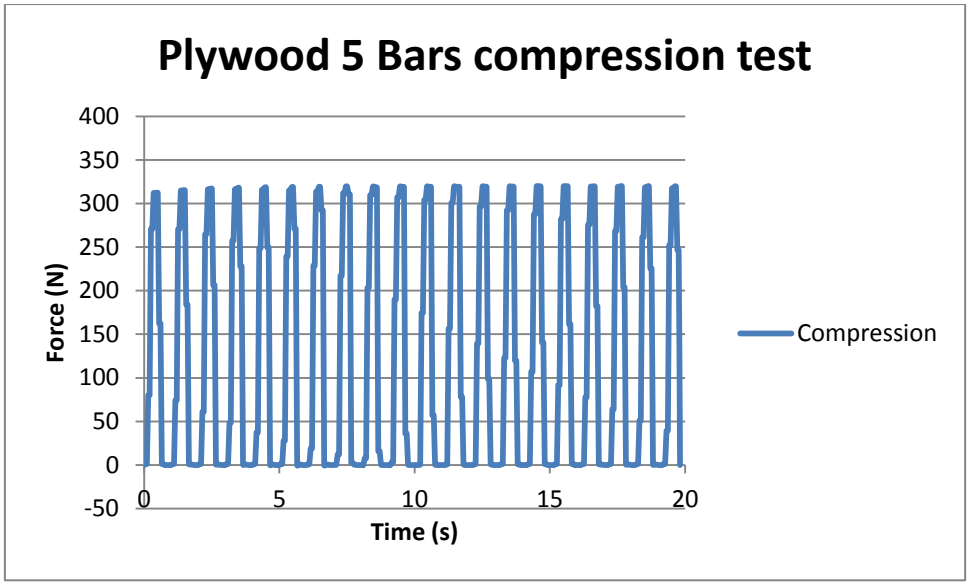


Figure 5.21 – 5 Bars compression test data

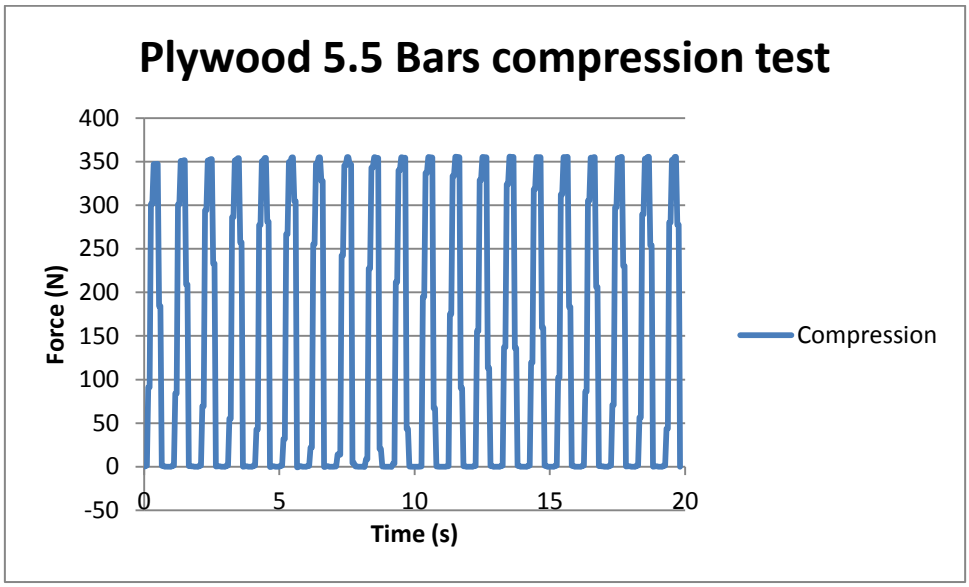


Figure 5.22 – 5.5 Bars compression test data

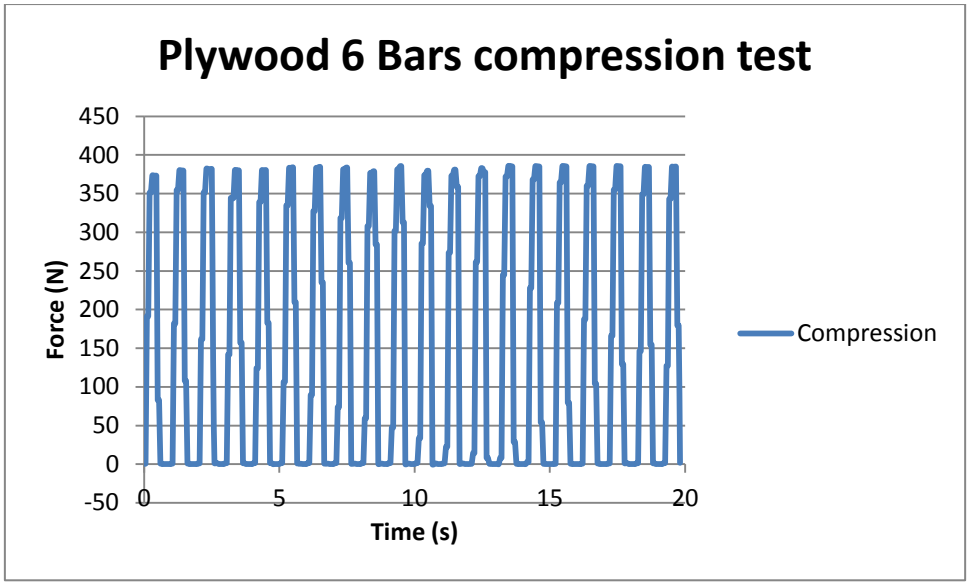


Figure 5.23 – 6 Bars compression test data

5.6.3 Compression test results on medium density EVA material

The results for compression tests on medium density EVA are shown in Table 5.5 and Figures 5.24 – 5.33. Table 5.5 and figure 5.24 show that there is a linear relationship between the pressure applied to the compression actuator and the resulting force applied to medium density EVA. The raw data graphs for each compression test (Figures 5.25 – 5.33) show that the device delivers consistent loads at different magnitudes on EVA. The within-session CV of the force peak magnitudes was shown to be very small ranging from 0.48 – 1.29%. Repeatability was also tested for the between-session mean magnitudes at 2, 3, 4, 5 and 6 Bars of pressure with CV data collected for three datasets. The air pressure was cut between each set of compressions so that the results would reflect how accurately the investigator could set the air pressure. The CV between sessions was found to be low ranging from 0.63 – 4.04%.

Table 5.5 – Results of compression tests on EVA

Pressure delivered to actuator	Resulting compression load (N)	Vertical pressure (kPa) at contact point
2 Bars	107.80	610.33
2.5 Bars	140.90	797.74
3 Bars	175.86	995.67
3.5 Bars	211.42	1,197
4 Bars	242.89	1,375.17
4.5 Bars	273.48	1,548.37
5 Bars	308.23	1,745.11
5.5 Bars	330.97	1,873.86
6 Bars	359.37	2,034.65

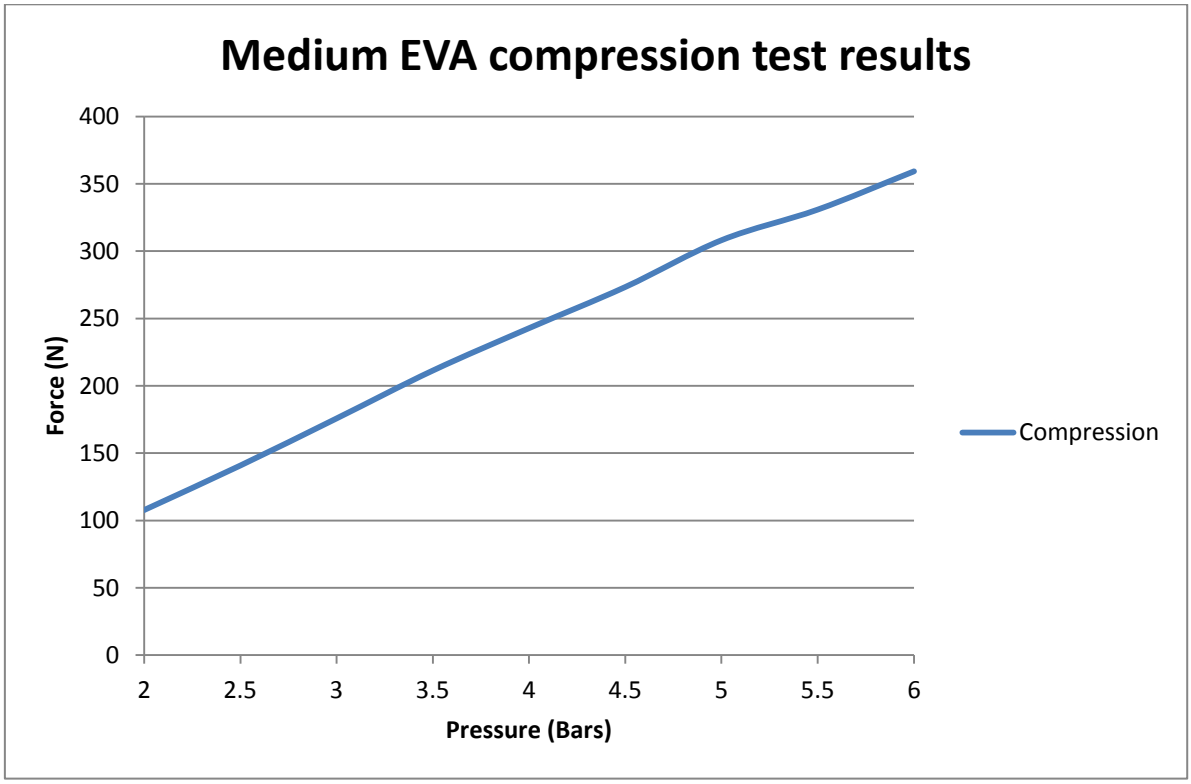


Figure 5.24 – Relationship between cylinder pressure and force delivered by the device to the EVA material

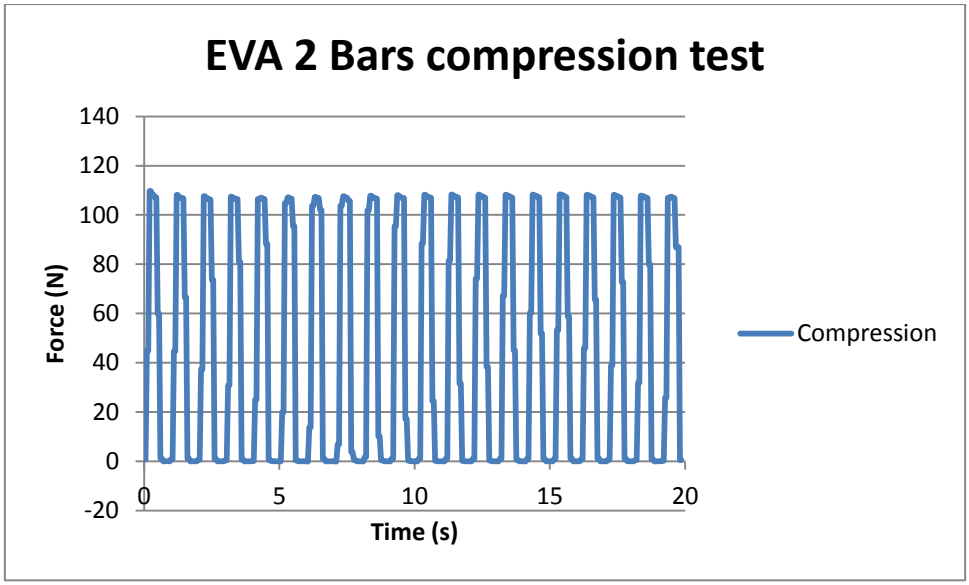


Figure 5.25 – EVA 2 Bars compression test results

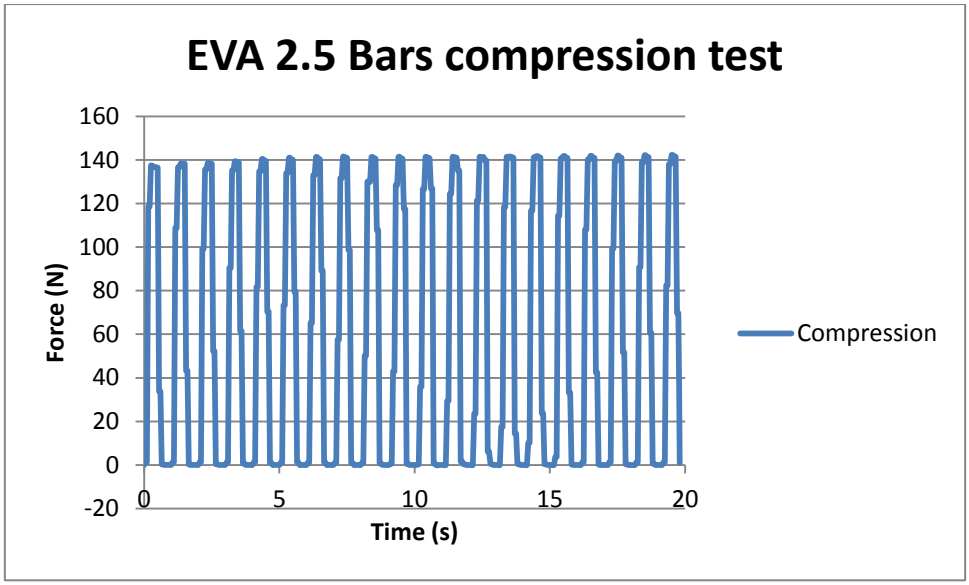


Figure 5.26 – EVA 2.5 Bars compression test results

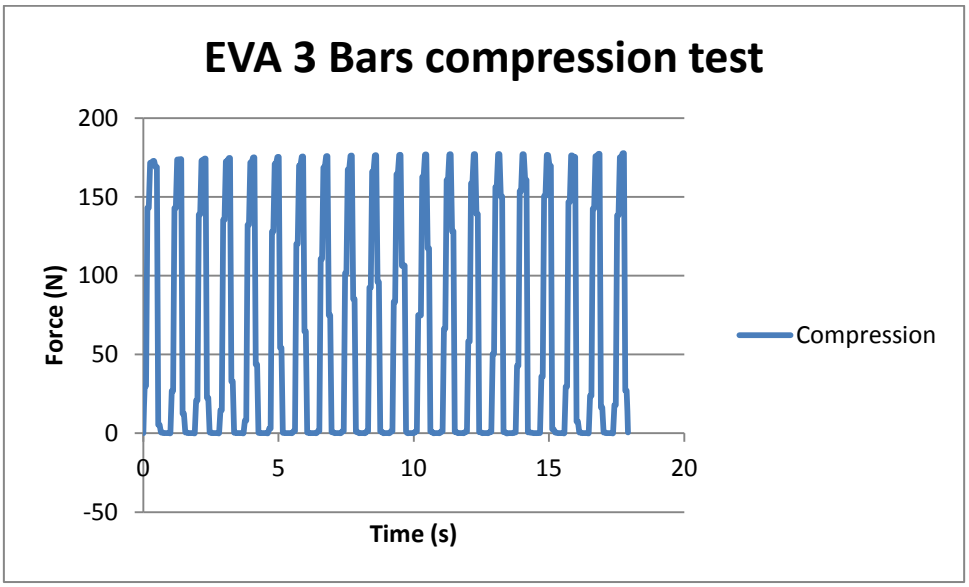


Figure 5.27 – EVA 3 Bars compression test results

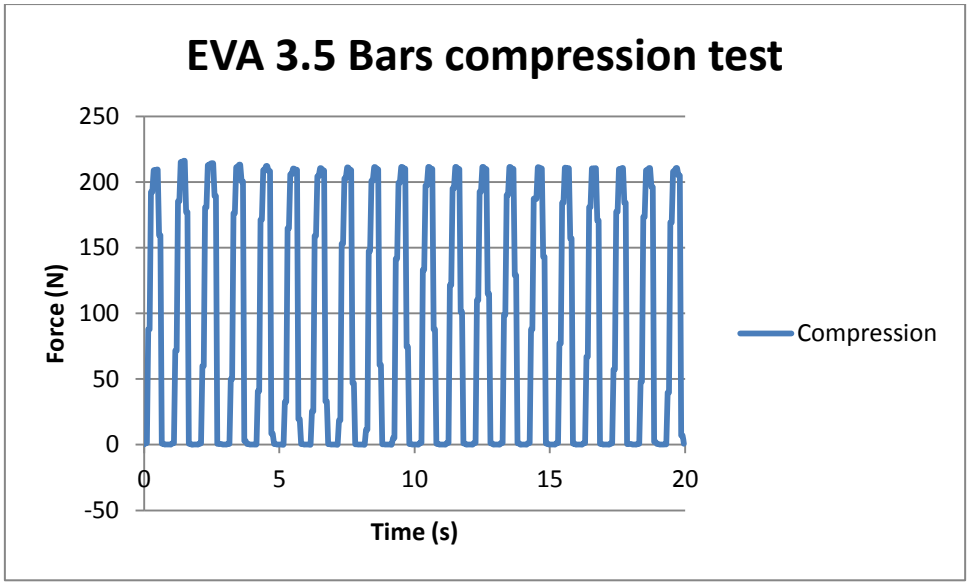


Figure 5.28 – EVA 3.5 Bars compression test results

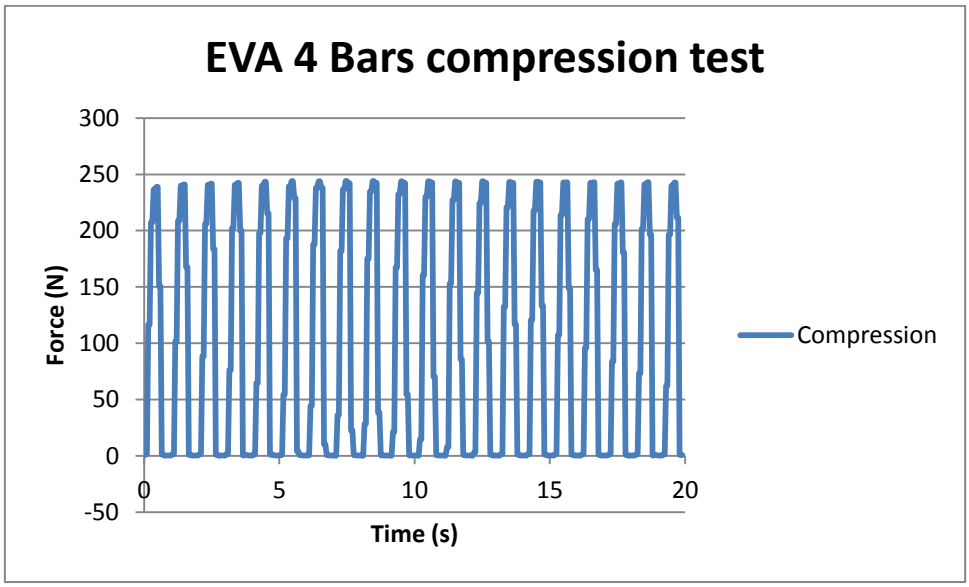


Figure 5.29 – EVA 4 Bars compression test results

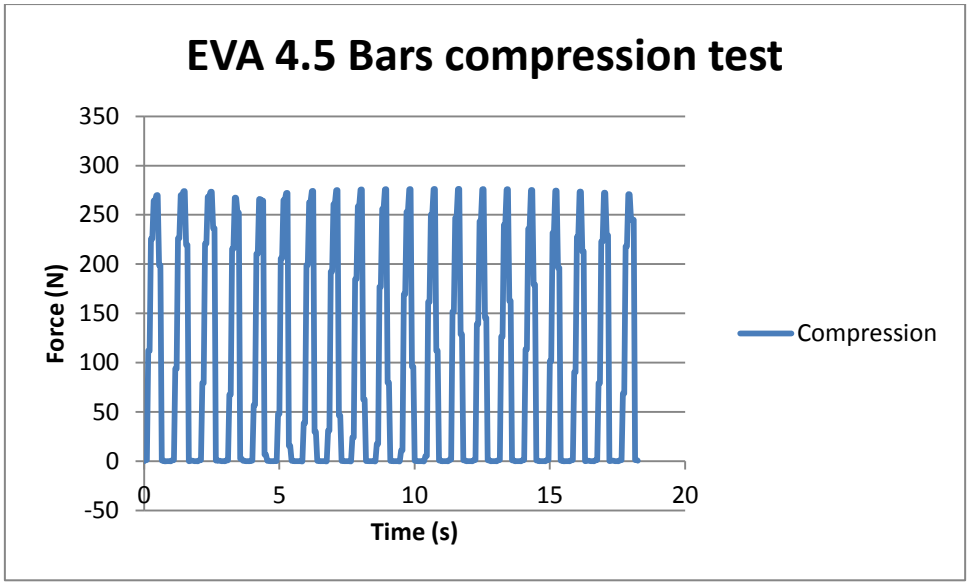


Figure 5.30 – EVA 4.5 Bars compression test results

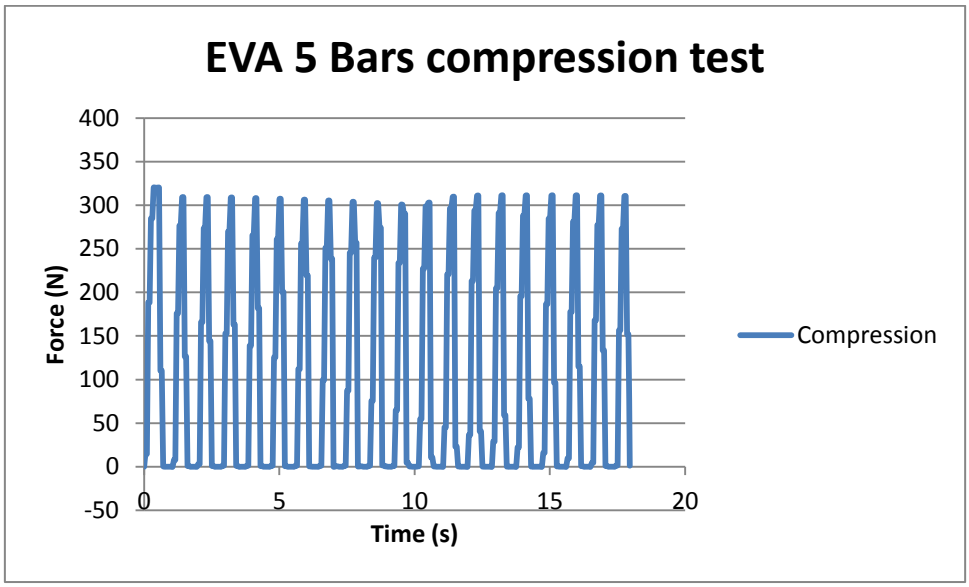


Figure 5.31 – EVA 5 Bars compression test results

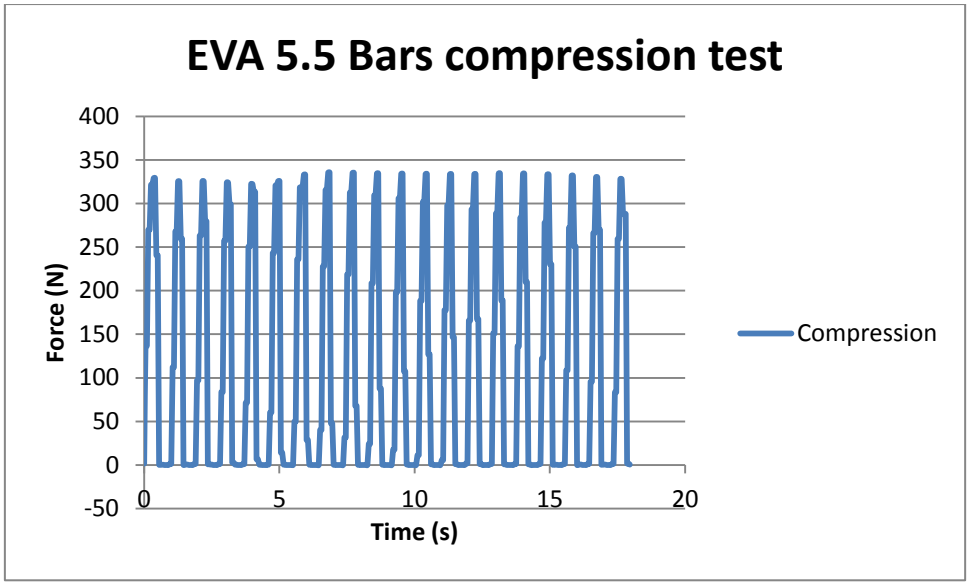


Figure 5.32 – EVA 5.5 Bars compression test results

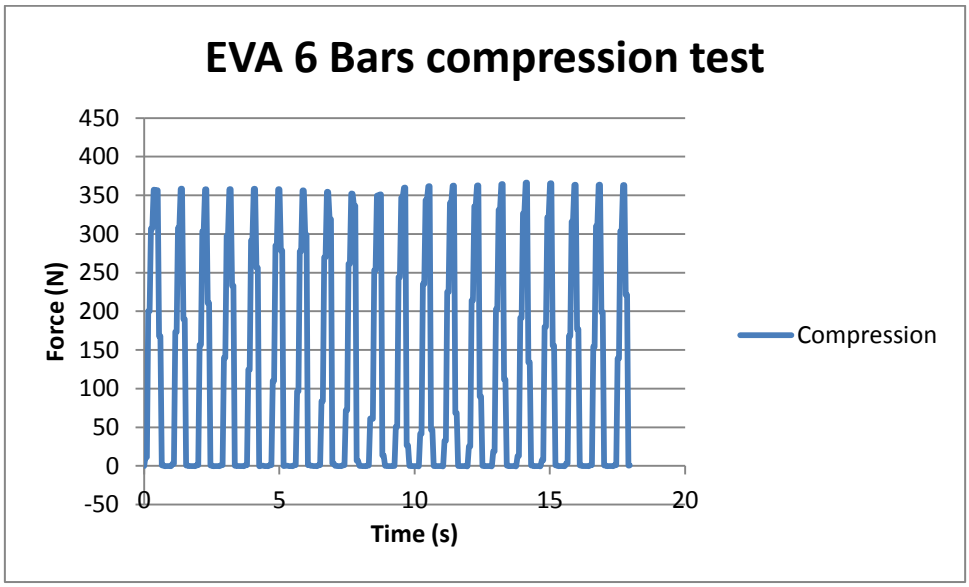


Figure 5.33 – EVA 6 Bars compression test results

5.5.4 Discussion

These compression tests have shown that there is a linear increase in compressive force when the air pressure delivered to the actuator is increased. With the current area of contact head (15mm diameter circular head = 0.000177m^2) the device may be able to apply compression pressures of over seven times greater than what is normally experienced under the second metatarsal head of a 75kg adult. This means it is more than capable of the load requirements for applying four times normal loads to the skin. The

relationship between bars of pressure applied to the device and resulting Newtons of force can be used to control the loads applied to the skin. The CV values obtained from the force peaks show that the within-session and between-session repeatability is excellent for plywood and medium density EVA.

5.5.6 Conclusion

These tests on plywood and medium density EVA have shown the linear relationship between Bars of pressure delivered to the compression pressure gauge and the resulting Newtons of force that are applied to the material. This information can be subsequently used to estimate the amount of compressive force delivered when adjusting the pressure gauge on the device in future tests on the foot and thus to apply person-specific pressures. This satisfies the first aim of compression tests which were to gain an understanding of the relationship between Bars of pressure delivered to the compression actuator, and the resulting force applied.

The data has also shown that the loads applied at repeated cycles are consistent which satisfies the second aim, which was to determine whether or not the device could produce consistent loads during repeated compression cycles. Collectively, these tests show that there are no issues with how the compression actuator operates, therefore the device has been deemed fit for preliminary skin response to load tests. The amount of pressure applied to the materials was large with up to 2,166.68 kPa of pressure applied with the regulator set to 6 Bars on plywood (over a 15mm diameter circular contact area). While these tests demonstrate what the device is capable of, the pressures applied to plantar skin would sit in the lower range of the device's capabilities.

Chapter 6: A pilot study to investigate whether laboratory controlled plantar loading causes callus-like thickening in healthy skin overlying the plantar metatarsal area

6.1 Introduction

The review of the literature (Chapter 2) discussed the impact that external loads have on skin. In-vivo human (Rubin, 1949, Goldblum and Piper, 1954, Pinkus, 1952, Brophy and Lobitz, 1959) and animal studies (Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998, Sanders et al., 2002) have shown how skin adapts to friction, shear and compression loads. Under small to moderate loads over a period of up to 35 days, the skin's mitotic levels increase, and increases in epidermal thickness has been documented. However, these studies have been cited in the callus literature, but in reality cannot be applied to plantar skin, due to the anatomical and functional differences plantar skin has to the areas tested in these papers (e.g. leg, back, mouse ears etc.). Numerous studies have reported increased vertical pressures under callus (Springett, 1993, Pitei et al., 1999, Potter and Potter, 2000b, Abouaesha et al., 2001, Pataky et al., 2002, Menz et al., 2007), which highlights that there is likely to be a relationship between loading of the skin and callus development. However, no work has been done to further address the relationship between loads and callus development. Furthermore, the available evidence is limited in that plantar pressure studies only state the magnitude of pressure beneath the callused skin and not the accumulative increase in load, which is necessary to further understand any link between callus and pressure. This is essential if an understanding of plantar callus, and thus treatment, is to progress. The work outlined in this chapter is the first to ever attempt to cause callus like skin changes using compression pressure application, and to express this in terms of accumulative weekly loads.

Having established that the loading device detailed in Chapter 5 is fit for purpose, protocols for its use in an investigation of whether external loads lead to responses in the skin required development. Whilst some assumptions were already made in chapter 5, questions include the magnitude of pressure to apply, the site over which to apply this pressure, the number of cycles (loading dose) and the number of days per week to apply the loads. This chapter aims to further investigate the relationship between increased plantar loading and callus development by undertaking a pilot study to test skin properties pre and post a period of increased plantar loading (delivered with the loading device

developed in chapter 5). A pilot study was required because load application on plantar skin has never been conducted in this manner before, so feasibility would have to be proven and safety concerns, particularly the potential for biochemical changes leading to callus growth (and the possibility of continued callus development post-study), would have to be determined. The aim of this study was not to create callus, but rather to alter normal PMA skin in the direction of callus using data in Chapter 4 as a guide. The author hypothesises that increases in pressure beneath the metatarsal heads causes a hyperkeratotic response akin to callus development, which is consistent with current opinion (Singh, 1996, Freeman, 2002, Helfand, 2003, Grouios, 2004). It is hypothesised that a decrease in skin hydration, a decrease in skin distensibility, a decrease in skin homogeneity, and an increase in skin variance parameters will result from increased loading at the PMA skin site in comparison to the control sites. If these changes occur, it could be attributed to a callus-like skin response.

6.2 Preliminary tests

A series of short preliminary tests were conducted to develop and test detailed aspects of the protocol to be used in the later pilot study of how increases in plantar pressure might affect plantar skin.

6.2.1 Variability of positioning foot in insole

Foot positioning was tested on four healthy volunteers. The aim was to check the repeatability of positioning a metatarsal head over the loading device contact pad. A Salfordinsole™ Firm insole was fitted for each volunteer. This insole was chosen because it is made of a rigid plastic and has a well-defined heel cup, which is beneficial for positioning the foot repeatably. The second metatarsal head was identified through palpation and marked using black ink. Each volunteer was then asked to carefully stand on the insole heel first and then slowly place their forefoot down onto the insole, ensuring their foot position corresponded, as closely as possible, to the contours of the insole at the midfoot and heel. The ink mark from the metatarsal head was transferred to the corresponding location on the insole. Using a Stanley knife, a 40mm diameter circle was cut with the ink mark in the centre of the circle which was deemed large enough to allow adequate access for foot marking from beneath while participants stood on the platform. The volunteers were then asked to stand on the platform and the foot was secured to the

platform to limit dorsiflexion. The investigator then used a ball point pen to mark the edges of the cut-out in the insole on the volunteer's foot. This process was performed a total of three times for each participant with a different colour of pen used on each occasion. It was found that a mean of 1.3mm in variation existed in the antero-posterior position and 1.2mm in the medio-lateral position, measured using Vernier calipers. This small amount of positional variation was deemed acceptable for the main pilot study.

6.2.2 Adjustment of plantar pressures applied

Using two participants, a test was performed to observe how accurately loads could be delivered using the pneumatic cylinder pressure regulator controls and sandbags to constrain foot movement. The aim was to establish the ability to fine tune the precise plantar pressure values applied to the foot. Using previously measured in-shoe pressures, a target pressure was set for each participant and the corresponding forces calculated to reach these pressures. The initial load was applied in real-time using the air pressure regulator with the device computer set to deliver a single constant compression load. Once the force applied to the foot was at an acceptable magnitude, a 10 cycle compression dose (at approximately 1Hz) at this setting was applied and the load cell data logged. The process was repeated once more. The results showed that compression cycles could be applied with loads within 10% of the calculated target load.

6.2.3 Duration of pressures applied

Further testing in the same participants above aimed to determine the magnitude of pressure that could be applied without causing discomfort. Using subjective feedback from repeated tests, it was found that applying no more than three times normal pressure in compression cycles would be appropriate. This could be tolerated over a period of 20 minutes (1,200 compressions at 1Hz). However, because this duration was nearing the limit of tolerability, it was determined that any longer duration of load application over a period of weeks could risk unintended discomfort, so limiting the duration to 1,200 compressions was appropriate. Any changes to load application could be done on a participant by participant basis during the study as necessary.

6.3 Pilot study methodology

Following the preliminary tests, a pilot study was conducted to determine whether increased plantar pressures could achieve a skin response akin to callus development, and if so, whether this skin change continues after load application ceases. This is important to determine before any larger-scale study is carried out as there are no other reports of research like this being undertaken in plantar skin. This pilot study is presented as a series of case studies where load applied with the device is expressed within the context of estimated weekly loads at the skin site of interest. As stated in Chapter 2 and earlier in this chapter, expressing applied loads in the context of estimated weekly loads addresses an important omission from the literature, as pressure studies only state the pressure beneath the callused site and not the accumulative effect of this. A case study design allows a more in-depth, meticulous approach which is important because this type of work has never before been conducted. Ethical approval for this study was granted by the University of Salford's College Research Ethics Panel (application number HSCR14/37).

6.3.1 Participant selection

The intended inclusion criteria for this study were individuals over the age of 18 with skin prone to callus (i.e. with callus on some areas of the foot), but with enough non-callused skin over the metatarsal areas to allow a loading and control site, so at least two metatarsal heads free from callus. This inclusion criterion was chosen because having plantar skin prone to callus would provide a better chance of observing a callus-like skin response during the study.

6.3.2 In-shoe pressure measurement

In-shoe pressure data collection in the participants' own shoes using a Novel Pedar-X in-shoe pressure system (Novel, Germany) was undertaken to aid in setting the target pressures for the loading device. Pedar insoles have a matrix of 99 sensors (which vary in size according to the size of insole). The in-shoe pressure measurements were obtained using the participants' own shoes that they wore most often. The participants were asked to walk along an eight metre walkway, four times at a self-selected walking speed, which was chosen as it would give the most realistic pressure profile for each participant. Timing gates were used to record speed to ensure consistency in the walking trials.

The pressure data was analysed using InShoe Pressure Analyser© version 1.0 (2012), a pressure analysis programme written in the Foot and Ankle Research department at the University of Salford. The code separates the walking trial into blocks of steps. The first and last three steps were then removed as they represent gait initiation and end. The code then takes the peaks from all the sensors in each mask and then means them, so there is a mean peak pressure for each step, and then it gives a mean peak pressure in each mask for the entire walking trial. The masks employed included the first metatarsal head, second to fourth metatarsal heads, fifth metatarsal head, hallux and heel and is based on the work of Bontrager et al. (1997) who used Harris Mat imprints and is therefore anatomically accurate (Figure 6.1). For this study, peak pressure data was collected from the mask overlying the second to fourth metatarsal heads. These heads lie very close together and are therefore difficult to separate using the Pedar® sensors, particularly as the area is covered by an array of 16 sensors (4 x 4) and cannot be evenly split into three regions. Because the loading site in this study was only ever the second or fourth metatarsal head, using the Pressure Analyser software, the author equally divided the second to fourth metatarsal head mask into two smaller masks of a 4 x 2 sensor array, and the section overlying the area of interest (the second or fourth metatarsal head) was used. The peak pressure was then used as a guide target compression pressure for the study. Pressures at two to three times normal were deemed suitable because they were tolerable and did not lead to excessive discomfort, which was found when using higher loads in preliminary tests. In addition to peak pressure, pressure time integral (PTI) using the non-zero mean from all the sensors within the mask, and the contact time were also obtained for use later in the study.

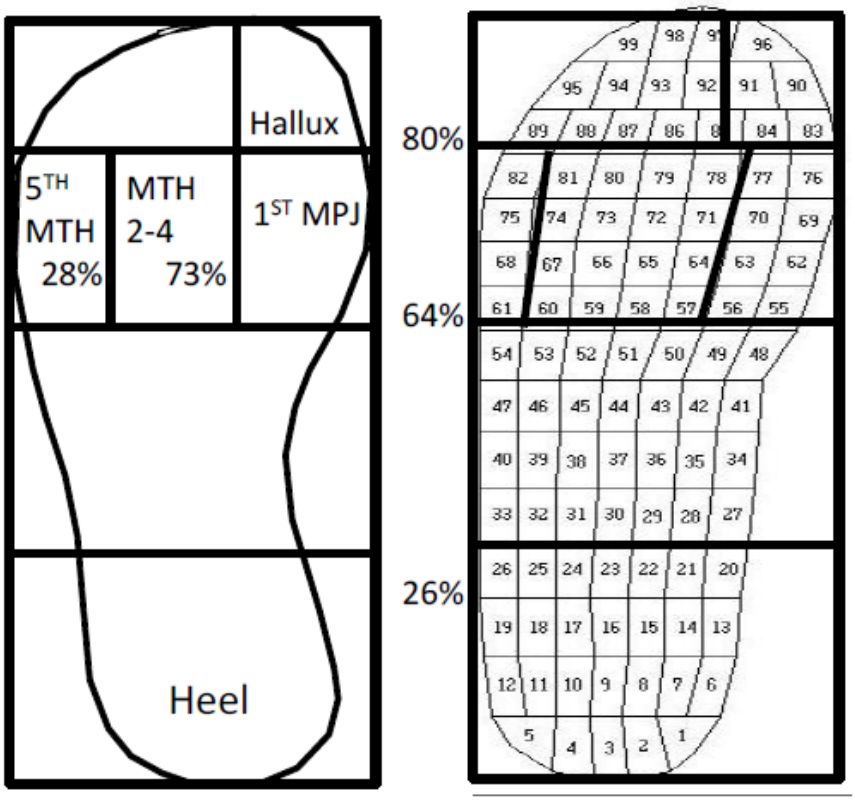


Figure 6.1 – Bontrager et al. (1997) Pedar® mask used in study using percentages of insole length and width (Chapman, 2014, p.26). Region borders are placed to the nearest whole sensor.

6.3.3 Positioning the foot in relation to the loading device

As described previously, having had the metatarsal head of choice marked with a pen, the participant was asked to carefully stand over the insole heel first and gradually loading the forefoot over the insole to transfer the pen mark to the corresponding location. To ensure the loading device accurately contacted the skin overlying the correct metatarsal head without contacting the insole at full extension, the head of the device which houses the contact pad was removed and used as a stencil. With the pen mark of the metatarsal head on the insole, the aperture where the pin of the contact pad is inserted was used as a guide to visualise the pen mark, then the outer edges of the device head were marked on the insole. This was then cut out using a Stanley knife leaving a 30mm diameter square aperture. This ensured that in full extension, the contact pad of the device would contact the desired area of skin each time the foot was placed on the insole but without making contact with the insole, which would absorb some of the load. The foot was secured to the platform using a sandbag strapped to the dorsal aspect of the foot to limit dorsiflexion and superior movement of the forefoot, as described previously. A short loading test was performed to determine the actual loading point on the foot so as to measure the skin in the

precise area being loaded. Using the imprint from the contact pad of the device, the position was measured and noted, and would serve as the site of interest for all of the subsequent skin tests.

6.3.4 Measurement of skin biophysical properties

During the pilot study, a minimum timeframe of six weeks was chosen. This was deemed appropriate as it would potentially allow at least two plantar stratum corneum cell turnovers (Thomas et al., 1985) and exceeded the timeframe of load application in previous skin studies, optimising the chance of skin change occurring. Testing of biophysical skin properties was performed on the first day of a week prior to any load application and then four weeks after the cessation of the minimum six weeks of load application. The post load measures were therefore taken at a minimum of 10 weeks after the start of the study. Biophysical skin data was collected by one investigator. The skin measures included skin hydration (Corneometer®), skin distensibility (Cutometer®) and skin surface topography measures of homogeneity and variance (Visioscan®). The skin sites measured included the intervention site – non-callused skin overlying the metatarsal head of choice; and two normal weight bearing skin sites including (1) a plantar metatarsal head, and (2) skin overlying the base of the fifth metatarsal. The skin sites were marked with a ball point pen. The first metatarsal was used as a control site unless any callus lay over this site, in which case the fourth metatarsal head was used. The fifth metatarsal base of the same foot was used as a second control site. Both control sites were identified through palpation and marked. The distance of the radii of the measurement probes were marked over the centre of each skin measurement site to allow accurate probe placement (Figure 6.1).

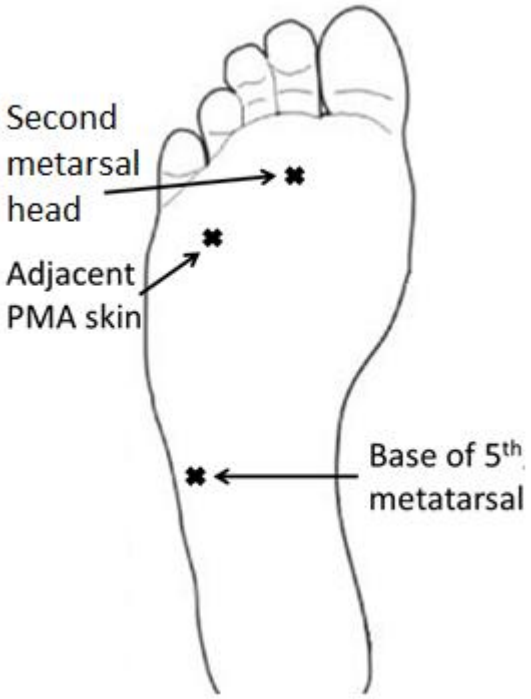


Figure 6.2 - The skin measurement sites included the load site (in this case the second metatarsal head) and skin overlying the PMA and base of the 5th metatarsal. A skin site on the opposite foot corresponding to the intervention site was also chosen (adapted from Hashmi and Malone-Lee, 2007 p.253).

Measurements were taken based on manufacturers' instructions but adjusted for the needs of plantar skin testing as follows. 10 measurements were taken per skin site using the Corneometer® and one image was taken per site for the Visioscan®. For the Cutometer®, 500mbar of negative pressure was applied over a period of 30 seconds, and skin relaxation time was set to 30 seconds each per skin site, as described in previous chapters.

6.3.5 Activity monitoring

In order to express the applied load to the plantar skin in terms of accumulative weekly load, it was necessary to determine what the weekly load for each participant would be. Therefore, in order to estimate this, activity monitoring was employed. Approximately halfway through the study period, each subject was asked to wear an activPAL3™ activity monitor for a period of seven days to record activity profile. The activPAL3™ is a small device which contains an accelerometer and records data relating to body posture while worn on the anterior aspect of the thigh. The data is then analysed through software which classifies the data into sitting, standing and stepping categories, giving a profile of activity data over the duration which it was recorded. Parameters including stepping, standing and

sitting duration, number of steps, sitting to standing transfers, and cadence can be extracted. The activPAL™ has previously been validated for walking and posture activity measurement in adults (Grant et al., 2006, Ryan et al., 2006, Godfrey et al., 2007) and infants (Davies et al., 2012) and has shown good reliability. Inter-device reliability has been shown to be very high (ICC > 0.99) and percentage differences in step number and cadence between device and observation have been reported at less than 1% difference (Ryan et al., 2006). The measurement accuracy between sitting, lying, standing and walking has been shown to be 98% in comparison to an accelerometer (Godfrey et al., 2007). In the study outlined in this chapter, step count, and stepping and standing time were the parameters of interest, best representing the cumulative loads that would be applied to the foot.

6.3.7 Barefoot pressure measurement

In addition to the in-shoe pressure data collected before the start of the study, barefoot pressure data was also collected from each participant during the study period, in order to estimate the accumulative pressure caused by walking barefoot, for example, when at home. The system used in this study was the Novel Emed® barefoot pressure platform which has recently shown good intra- and inter-platform reliability with ICC values greater than 0.7 showing high agreement (Hafer et al., 2013). The participants were asked to walk along a six metre walkway at a self-selected speed, measured by timing gates to ensure consistency, facing straight ahead. The platform was positioned in the centre of the walkway and participants struck the platform with the foot of interest while walking normally. This is known as the midgait method and has shown to be very reliable with ICC values exceeding 0.75 with three or more trials used (Hughes et al., 1991, McPoil et al., 1999). Static pressures were also collected for each participant.

The pressure data was analysed using a MATLAB programme written in the Foot and Ankle Research department at the University of Salford. The programme calculates the peak pressure from masks inside the boundaries of the plantar surface of the foot. The mask dimensions and position are predefined and can be visually evaluated on the foot map to assist with measurement accuracy. For this study, a mask of consistent size was positioned over the region of interest for each study participant. In addition to peak pressures, the mean pressure data over each time point in the trial is also calculated within the mask and is exported to Microsoft Excel. This data was used to calculate PTI using the

non-zero mean of all the sensors within the mask at the region of interest. Contact time was also calculated in this programme.

6.3.8 Foot loading data

During each loading session the device and foot were set up as previously described and loading programme 1 ‘Compression’ was selected. Setting of the pressure values to be applied to the foot was achieved by first setting the pneumatic cylinder at 0 bars and gradually increasing pressure until the intended force had been reached. Once this was achieved, the device head was retracted and the loading cycles initialised.

The intended loading dose for each participant was 1,200 cycles of compressions at a maximum of three times normal pressure, over three sessions per week over a minimum period of six weeks. The loading dose of a maximum of three times normal pressure at 1,200 cycles was chosen as it was found to be tolerable in preliminary test sessions (section 6.2.3). Three loading sessions per week was chosen as it was the maximum that any of the participants could commit to. Load cell data was collected from the device which gave force and time values throughout the session. From this data the peak pressure, PTI and contact time for each loading session was calculated. The data from each session was used to calculate an overall peak pressure, PTI and contact time per compression for analysis. The activity monitoring data was used so that the loads applied by the device could be contextualised.

6.3.9 Data analysis

The design of this study is based on individual cases so significance tests are not appropriate. For skin data, in addition to plotting the trends from baseline on a line graph, the smallest detectable change (SDC) was calculated to help contextualise changes in skin biophysical data (as described in more depth in Chapter 3). The SDC is calculated from the SEM as follows:

$$SEM = \frac{SD_{Differences}}{\sqrt{2}}$$

(de Vet et al., 2006, p.1037)

$$SDC = 1.96 \times \sqrt{2} \times SEM$$

(de Vet et al., 2006, p.1038)

The step count from the activity data collected from each participant was divided by two to get the number of strides for each foot. The stride number was multiplied by the peak pressure (PP), pressure time integral (PTI) and contact time (CT) of the barefoot and in-shoe pressure data. To estimate the total accumulative peak pressure, PTI and contact time for the week, it was assumed that 10% of walking occurred barefoot with the other 90% shod so this was applied to the barefoot and in-shoe data (0.1 and 0.9 times the total accumulative pressures respectively). These values were then summed for the overall weekly values.

$$Barefoot\ estimated\ PP, PTI\ or\ CT\ per\ week = 0.1\ \times (PP, PTI\ or\ CT\ \times Stride\ number)$$

$$Shod\ estimated\ PP, PTI\ or\ CT\ per\ week = 0.9\ \times (PP, PTI\ or\ CT\ \times Stride\ number)$$

$$Total\ accumulative\ plantar\ PP, PTI\ or\ CT\ per\ week = Barefoot\ estimated\ PP, PTI\ or\ CT + Shod\ estimated\ PP, PTI\ or\ CT$$

The loading device peak pressure, PTI and contact time per compression were multiplied by the number of compressions (3,600) applied to the foot each week to calculate the estimated average weekly compression dose. The weekly compression data was added to the overall estimated weekly accumulative peak pressure, PTI and contact time to calculate the total load beneath the region of interest over the week. The percentage of this total load applied by the device was calculated for these pressure parameters.

$$Average\ weekly\ compression\ dose = Average\ PP, PTI\ and\ CT\ per\ compression\ \times 3{,}600$$

$$Total\ estimated\ weekly\ load\ beneath\ load\ site = Average\ weekly\ compression\ dose\ of\ PP, PTI\ and\ CT + Total\ accumulative\ plantar\ PP, PTI\ or\ CT$$

$$Percentage\ of\ weekly\ pressure\ from\ load\ device = \frac{Average\ weekly\ compression\ dose}{Total\ estimated\ weekly\ dose\ beneath\ load\ site} \times 100$$

For example, an individual with 150 kPa shod peak pressure at the region of interest would have a device peak pressure maximum target of 450 kPa (= 150 x 3). The device compression dose per week is 1,620,000 kPa (3,600 compressions x 450 kPa). If this person takes 25,000 steps per week on this foot, estimated accumulative shod peak pressure would be 3,375,000 kPa (25,000 steps x 150 kPa x 0.9 [estimated time shod]). Estimated accumulative barefoot peak pressure would be 500,000 kPa (25,000 steps x 200 kPa x 0.1 [estimated time barefoot]). This gives a total accumulative peak pressure of 3,875,000 kPa. The sum of the accumulative device load and the stepping load is 5,495,000 kPa. The load applied by the device therefore accounts for 29.48% of peak pressure load per week.

6.4 Case study – Subject 1

6.4.1 Participant profile

Subject 1 was a 45 year old, white British female with a BMI of 26.2. She was generally healthy, engaged in regular running and weight-lifting activities and had no medical conditions. On examination, bilateral callus beneath the second metatarsal heads and diffuse callus type skin over the first metatarsal heads was observed. It was determined that the most appropriate site for skin loading was the right fourth metatarsal head. The right foot was chosen as there was more 'normal' non-callused skin to provide control measurements. The control sites were the third metatarsal head, as it was callus free; the fifth metatarsal base, a semi weight-bearing site which seldom develops callus; and the fourth metatarsal head on the left foot to provide direct comparison with the loading site on the right foot.

6.4.2 Loading profile

Table 6.1 shows the loading profile for the loading site on Subject 1's foot which includes activity data, dynamic pressure data and pressure data collected from the loading device. It also shows the estimated accumulative dynamic loads and how much of this was

contributed by the loading device. The mean load applied by the device was approximately 2.9 times higher than normal pressure at the foot site.

Table 6.1 Subject 1 loading profile

Activity profile	
Number of strides per week	26,032
Time stepping (s)	40,320
Time standing (s)	120,240
Plantar pressure per step at load site	
In-shoe PP (kPa)	117.20
Barefoot PP (kPa)	138.00
In-shoe PTI (kPa / s)	21.72
Barefoot PTI (kPa / s)	24.49
In-shoe contact time (s)	0.51
Barefoot contact time (s)	0.44
Estimated plantar pressure per week at load site	
PP (kPa)	3,105,097
PTI (kPa / s)	572,525
Contact time (s)	13,039
Mean plantar pressure applied per device compression at load site	
PP (kPa)	334.72
PTI (kPa / s)	112.86
Contact time (s)	0.57
Mean plantar pressure applied by device per week at load site	
PP (kPa)	1,204,992
PTI (kPa / s)	406,297
Contact time	2,058
Total weekly plantar pressure experienced under load site (% applied with device)	
PP (kPa)	4,310,089 (28.0)
PTI (kPa / s)	978,822 (41.5)
Contact time (s)	15,097 (13.6)

6.4.3 Skin data

Figures 6.2 – 6.5 and tables 6.3 – 6.6 show the raw data and smallest detectable change (SDC) collected from the skin for hydration, distensibility, homogeneity and variance respectively.

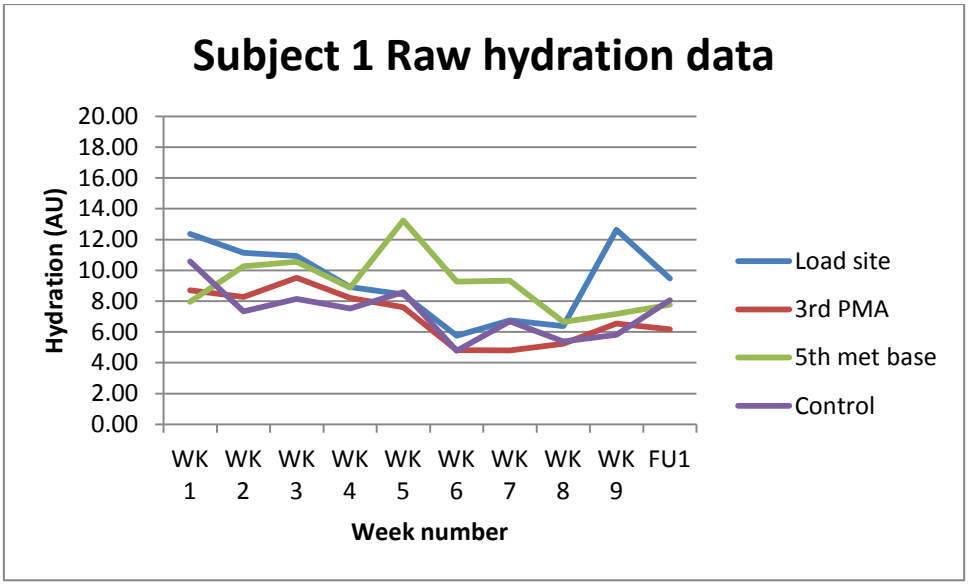


Figure 6.3 – Subject 1 raw hydration data.

Table 6.2 Subject 1 smallest detectable change for hydration

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	3.43	1.91	1.28	3.66
SD of differences between time points	2.16	1.93	1.99	1.41
SEM	1.53	1.37	1.41	1.00
SDC	4.23	3.79	3.90	2.76

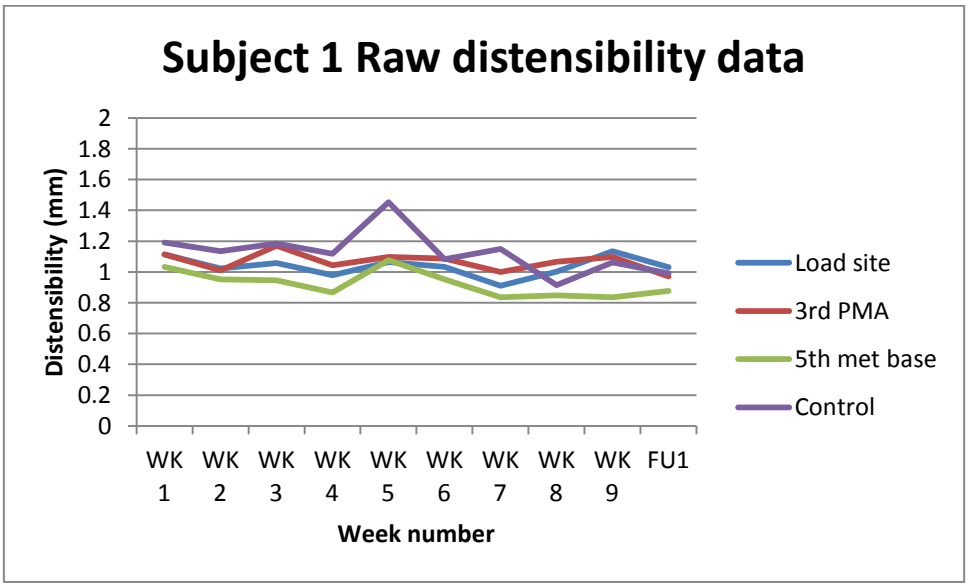


Figure 6.4 – Subject 1 raw distensibility data.

Table 6.3 Subject 1 smallest detectable change for distensibility

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.09	0.05	0.12	0.07
SD of differences between time points	0.05	0.06	0.08	0.16
SEM	0.04	0.04	0.06	0.11
SDC	0.10	0.11	0.16	0.31

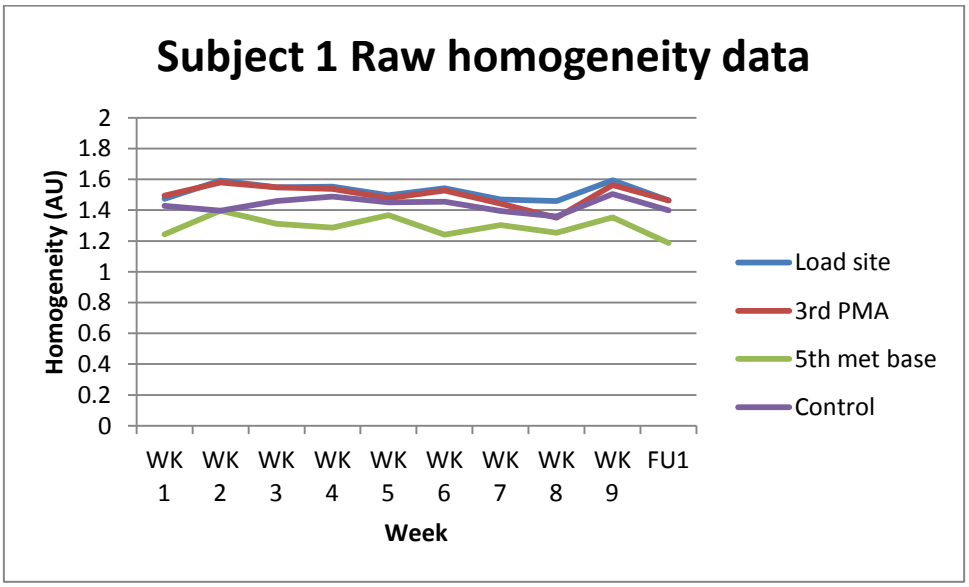


Figure 6.5 – Subject 1 raw homogeneity data.

Table 6.4 Subject 1 smallest detectable change for homogeneity

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.05	0.00	0.06	0.01
SD of differences between time points	0.05	0.08	0.06	0.05
SEM	0.03	0.06	0.04	0.03
SDC	0.10	0.15	0.11	0.09

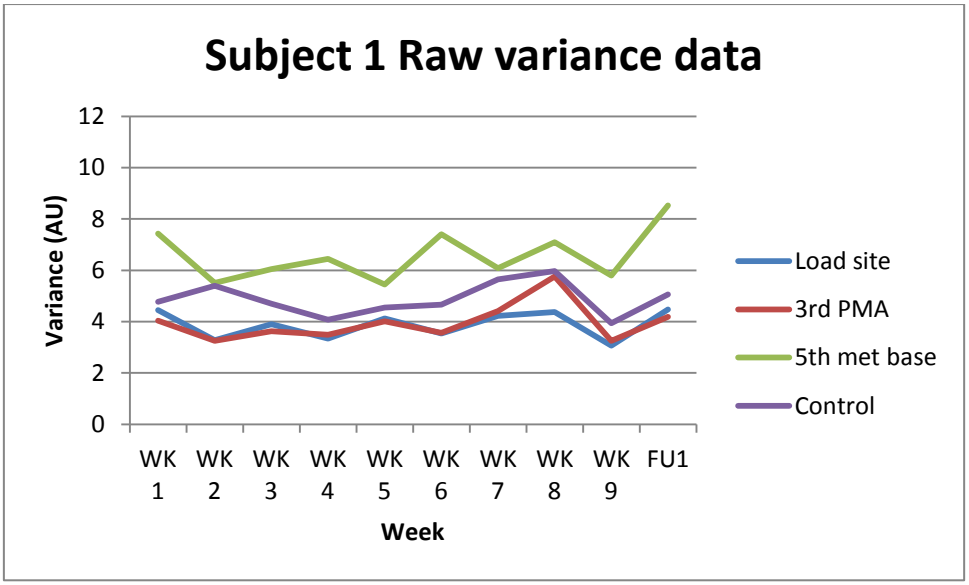


Figure 6.6 – Subject 1 raw variance data.

Table 6.5 Subject 1 smallest detectable change for variance

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.64	0.09	0.95	0.12
SD of differences between time points	0.44	0.86	0.75	0.68
SEM	0.31	0.61	0.53	0.48
SDC	0.86	1.69	1.46	1.33

6.5 Case study – Subject 2

6.5.1 Participant profile

Subject 2 was a 44 year old female with ancestry from the Indian Subcontinent with a BMI of 26.3. She did not undertake in regular physical activity and had no medical issues to contra-indicate participation in this study. On examination, bilateral callus beneath the second and third metatarsal heads was observed. The left foot had scaly skin over the metatarsal area, midfoot and heel, but the right foot had normal skin on callus-free areas. The right fourth metatarsal head was chosen as the loading site with controls including the first metatarsal head, which was callus free, fifth metatarsal base, and fourth metatarsal head of the left foot for direct comparison. The subject withdrew from the study after two appointments due to discomfort during the load application cycles.

6.6 Case study – Subject 3

6.6.1 Participant profile

Subject 3 was a 25 year old female of white Greek and Canadian descent. She was generally healthy with a BMI of 22.3, participating in regular physical activity, and had no medical issues to contra-indicate participation in this study. On examination, bilateral callus was observed beneath the second metatarsal heads with the rest of the forefoot showing healthy skin. The right fourth metatarsal head was chosen as the loading site with the first metatarsal head, fifth metatarsal base, and fourth metatarsal head of the left foot serving as control sites.

6.6.2 Loading profile

Table 6.6 shows the loading profile for the loading site on Subject 3's foot which includes activity data, dynamic pressure data and pressure data collected from the loading device. It also shows the estimated accumulative dynamic loads and how much of this was contributed by the loading device. The mean load applied by the device was approximately 2.2 times higher than normal pressure.

Table 6.6 Subject 3 loading profile

Activity profile	
Number of strides per week	46,043
Time stepping (s)	55,440
Time standing (s)	90,000
Plantar pressure per step at load site	
In-shoe PP (kPa)	186.33
Barefoot PP (kPa)	192
In-shoe PTI (kPa / s)	31.96
Barefoot PTI (kPa / s)	35.04
In-shoe contact time (s)	0.59
Barefoot contact time (s)	0.6
Estimated plantar pressure per week at load site	
PP (kPa)	8,605,299
PTI (kPa / s)	1,485,574
Contact time (s)	27,111
Mean plantar pressure applied per device compression at load site	
PP (kPa)	411.02
PTI (kPa / s)	144.8
Contact time (s)	0.57
Mean plantar pressure applied by device per week at load site	
PP (kPa)	1,479,672
PTI (kPa / s)	521,262
Contact time	2,036.82
Total weekly plantar pressure experienced under load site (% applied with device)	
PP (kPa)	10,084,970 (14.7)
PTI (kPa / s)	2,006,837 (26.0)
Contact time (s)	29,148 (7.0)

6.6.3 Skin data

Figures 6.7 – 6.10 and Tables 6.7 – 6.10 show the raw data and SDC collected from the skin for hydration, distensibility, homogeneity and variance respectively.

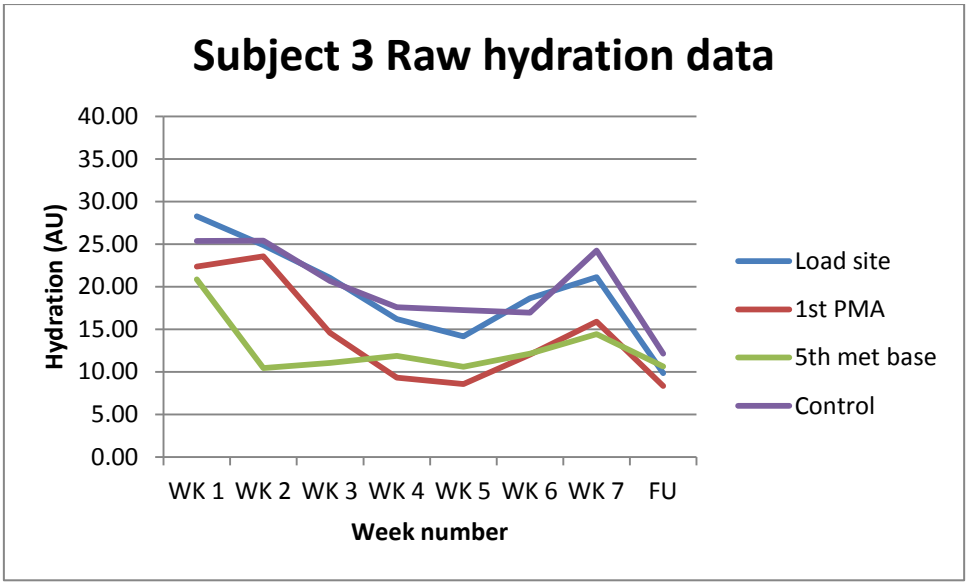


Figure 6.7 – Subject 3 raw hydration data.

Table 6.7 Subject 3 smallest detectable change for hydration

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	10.29	9.19	9.27	6.20
SD of differences between time points	5.03	5.45	1.41	4.61
SEM	3.56	3.85	0.99	3.26
SDC	9.86	10.67	2.75	9.04

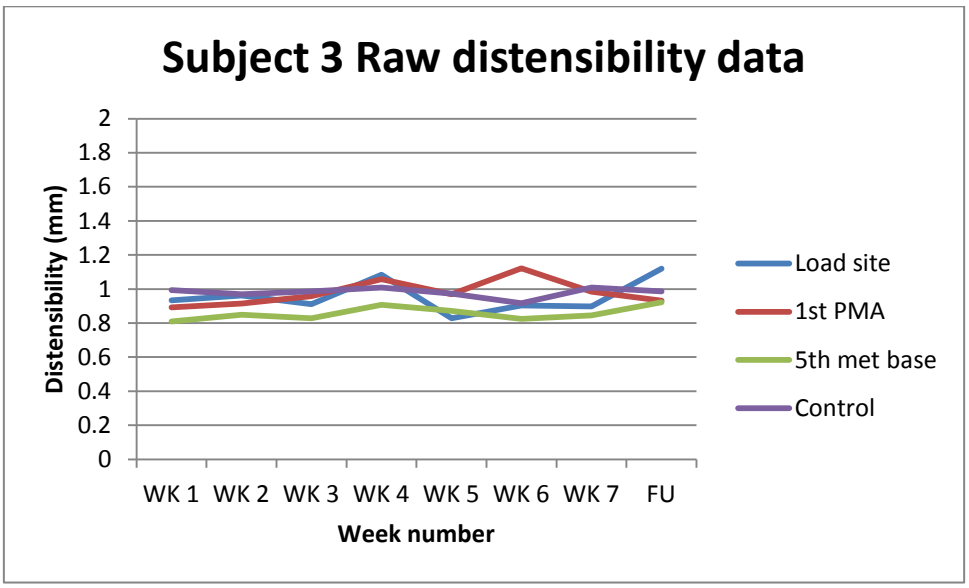


Figure 6.8 – Subject 3 raw distensibility data.

Table 6.8 Subject 3 smallest detectable change for distensibility

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.02	0.10	0.05	0.01
SD of differences between time points	0.11	0.07	0.04	0.03
SEM	0.07	0.05	0.03	0.02
SDC	0.21	0.14	0.08	0.06

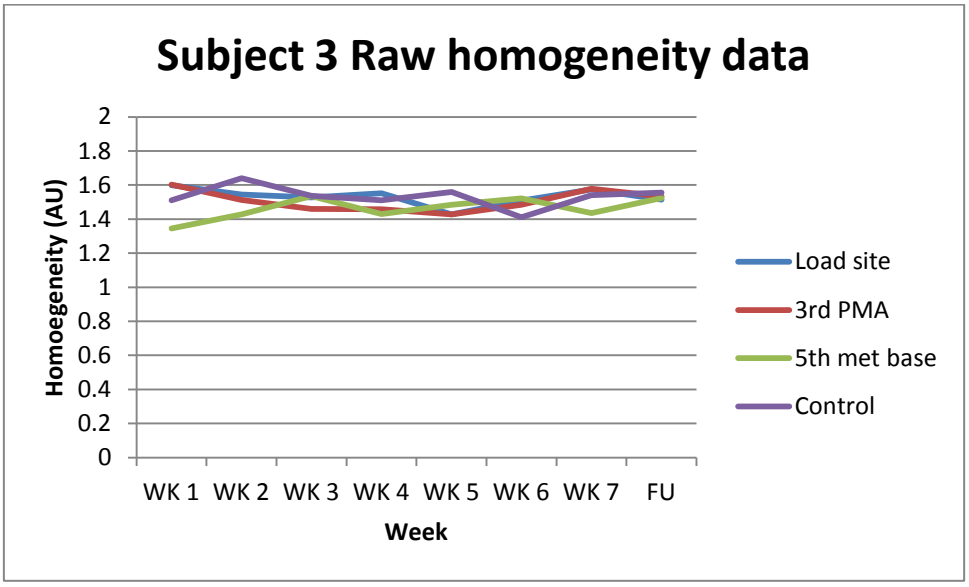


Figure 6.9 – Subject 3 raw homogeneity data.

Table 6.9 Subject 3 smallest detectable change for homogeneity

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.08	0.11	0.13	-0.03
SD of differences between time points	0.05	0.05	0.05	0.07
SEM	0.03	0.04	0.03	0.05
SDC	0.09	0.10	0.09	0.13

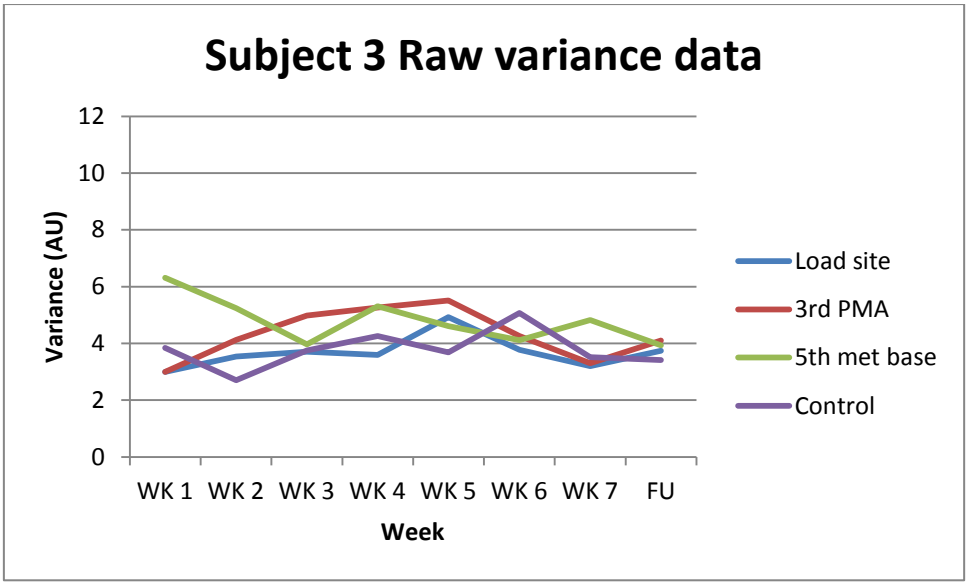


Figure 6.10 – Subject 3 raw variance data.

Table 6.10 Subject 3 smallest detectable change for variance

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.79	1.51	1.74	0.07
SD of differences between time points	0.54	0.78	0.58	0.74
SEM	0.38	0.55	0.41	0.52
SDC	1.06	1.53	1.14	1.44

6.7 Case study – Subject 4

6.7.1 Participant profile

Subject 4 was a 23 year old female of white Irish descent. She undertook in regular physical activity including distance running and cycling, and had a BMI of 22.0. On examination, bilateral callus was observed beneath the second metatarsal heads with healthy skin over the rest of the forefoot. The right fourth metatarsal head was chosen as the loading site and the control sites included the first metatarsal head, fifth metatarsal base, and fourth metatarsal head of the left foot.

6.7.2 Loading profile

Table 6.11 shows the loading profile for the loading site on Subject 4's foot which includes activity data, dynamic pressure data and pressure data collected from the loading device. It also shows the estimated accumulative dynamic loads and how much of this was contributed by the loading device. The average load applied by the device was

approximately 1.5 times higher than normal pressure. Loads higher than this could not be tolerated.

Table 6.11 Subject 4 loading profile

Activity profile	
Number of strides per week	34,211
Time stepping	35,169
Time standing	103,392
Plantar pressure per step at load site	
In-shoe PP (kPa)	247.66
Barefoot PP (kPa)	266.00
In-shoe PTI (kPa / s)	46.09
Barefoot PTI (kPa / s)	37.68
In-shoe contact time (s)	0.71
Barefoot contact time (s)	0.61
Estimated plantar pressure per week at load site	
PP (kPa)	8,535,439
PTI (kPa / s)	1,548,132
Contact time (s)	24,036
Mean plantar pressure applied per device compression at load site	
PP (kPa)	374.47
PTI (kPa / s)	132.31
Contact time (s)	0.58
Mean plantar pressure applied by device per week at load site	
PP (kPa)	1,348,092
PTI (kPa / s)	476,308
Contact time	2,072
Total weekly plantar pressure experienced under load site (% applied with device)	
PP (kPa)	9,883,531 (13.6)
PTI (kPa / s)	2,024,441 (23.5)
Contact time (s)	26,108 (7.9)

6.7.3 Skin data

Figures 6.11 – 6.14 and Tables 6.12 – 6.15 show the raw data and SDC collected from the skin for hydration, distensibility, homogeneity and variance respectively.

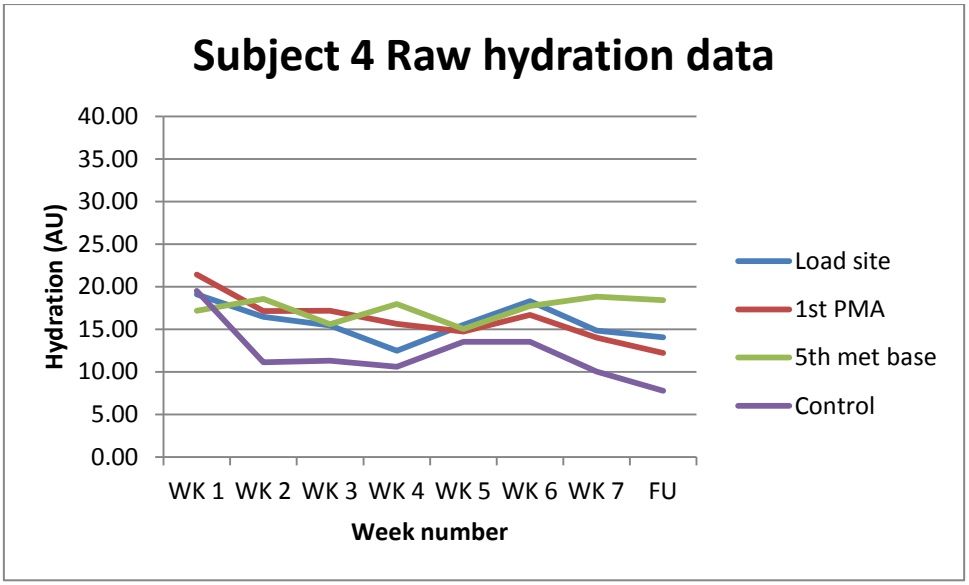


Figure 6.11 – Subject 4 raw hydration data.

Table 6.12 Subject 4 smallest detectable change for hydration

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	3.81	6.05	0.28	8.39
SD of differences between time points	1.83	1.84	1.51	2.00
SEM	1.30	1.30	1.07	1.42
SDC	3.60	3.61	2.95	3.92

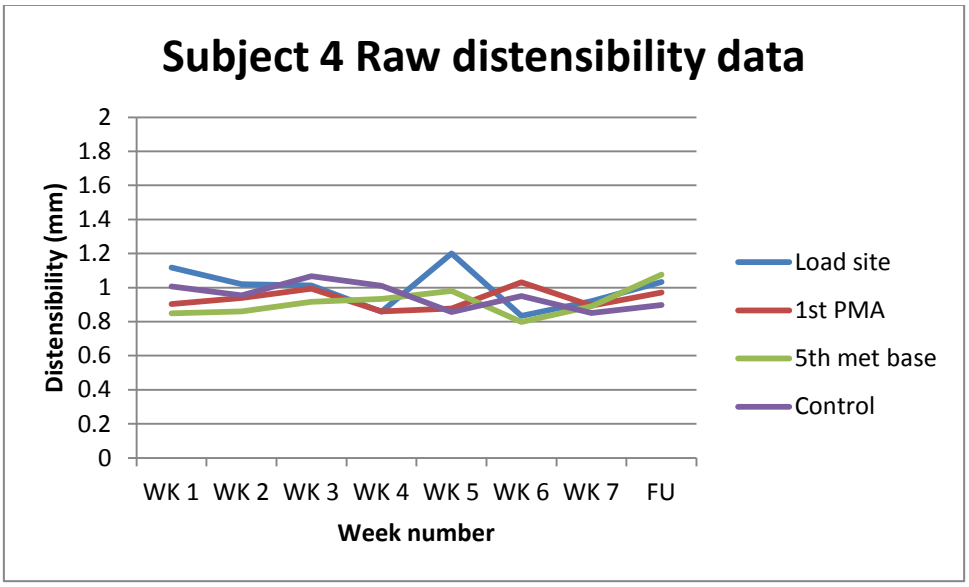


Figure 6.12 – Subject 4 raw distensibility data.

Table 6.13 Subject 4 smallest detectable change for distensibility

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.14	0.03	0.07	0.07
SD of differences between time points	0.12	0.06	0.09	0.08
SEM	0.09	0.05	0.06	0.06
SDC	0.24	0.13	0.17	0.16

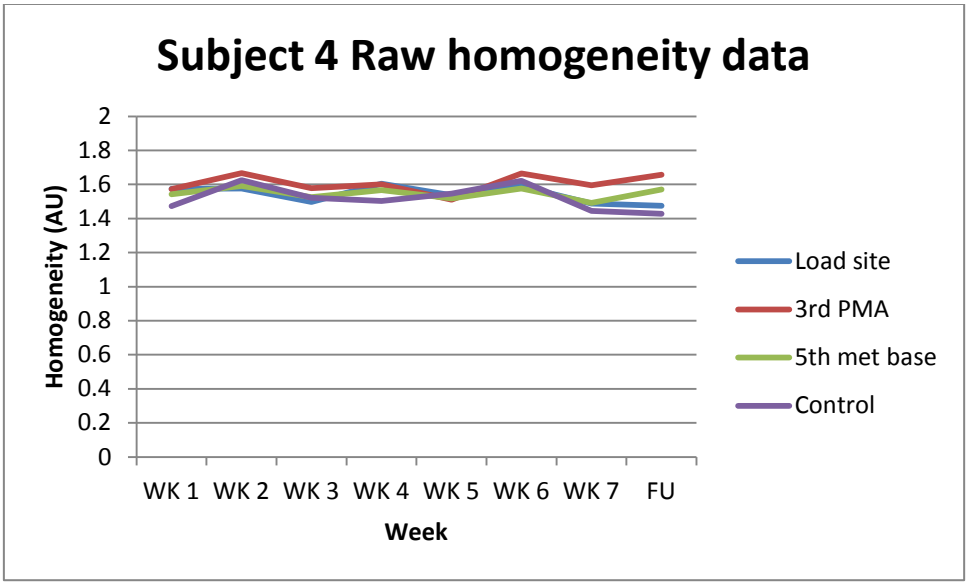


Figure 6.13 – Subject 4 raw homogeneity data.

Table 6.14 Subject 4 smallest detectable change for homogeneity

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.04	0.04	0.01	0.05
SD of differences between time points	0.05	0.06	0.04	0.08
SEM	0.04	0.04	0.03	0.05
SDC	0.10	0.11	0.07	0.15

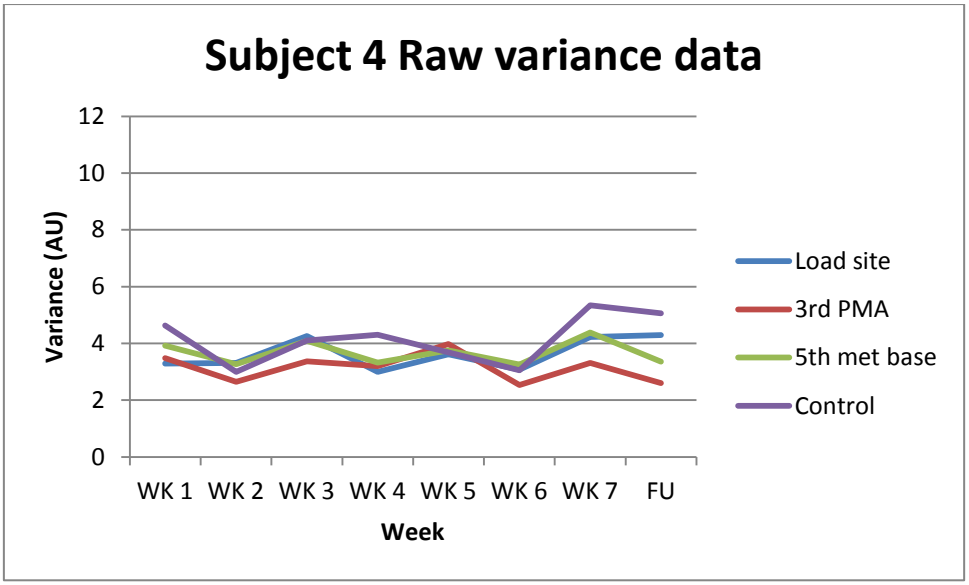


Figure 6.14 – Subject 4 raw variance data.

Table 6.15 Subject 4 smallest detectable change for variance

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.40	0.39	0.28	0.56
SD of differences between time points	0.57	0.53	0.45	0.91
SEM	0.41	0.37	0.32	0.64
SDC	1.13	1.04	0.89	1.79

6.8 Case study – Subject 5

6.8.1 Participant profile

Subject 5 was a 20 year old, white British female. She was generally healthy and participated in regular sporting activities including netball and gym work. On examination, bilateral callus beneath the fifth metatarsal heads was observed with healthy skin on the rest of the forefoot. The right second metatarsal head was chosen as the loading site, and the first metatarsal head, fifth metatarsal base and second metatarsal head of the left foot were chosen for control measurements.

6.8.2 Loading profile

Table 6.16 shows the loading profile for the loading site on Subject 5's foot which includes activity data, dynamic pressure data and pressure data collected from the loading device. It also shows the estimated accumulative dynamic loads and how much of this was contributed by the loading device. The average load applied by the device was approximately 2.1 times higher than normal pressure.

Table 6.16 Subject 5 loading profile

Activity profile	
Number of strides per week	34,562
Time stepping	44,640
Time standing	159,840
Plantar pressure per step at load site	
In-shoe PP (kPa)	182.19
Barefoot PP (kPa)	291.00
In-shoe PTI (kPa / s)	29.69
Barefoot PTI (kPa / s)	62.10
In-shoe contact time (s)	0.49
Barefoot contact time (s)	0.58
Estimated plantar pressure per week at load site	
PP (kPa)	6,672,919
PTI (kPa / s)	1,138,116
Contact time (s)	17,222
Mean plantar pressure applied per device compression at load site	
PP (kPa)	384.87
PTI (kPa / s)	128.41
Contact time (s)	0.58
Mean plantar pressure applied by device per week at load site	
PP (kPa)	1,385,532
PTI (kPa / s)	462,278
Contact time	2,096
Total weekly plantar pressure experienced under load site (% applied with device)	
PP (kPa)	8,058,451 (17.2)
PTI (kPa / s)	1,600,394 (28.9)
Contact time (s)	19,318 (10.9)

6.8.3 Skin data

Figures 6.15 – 6.18 and Tables 6.17 – 6.20 show the raw data and SDC collected from the skin for hydration, distensibility, homogeneity and variance respectively.

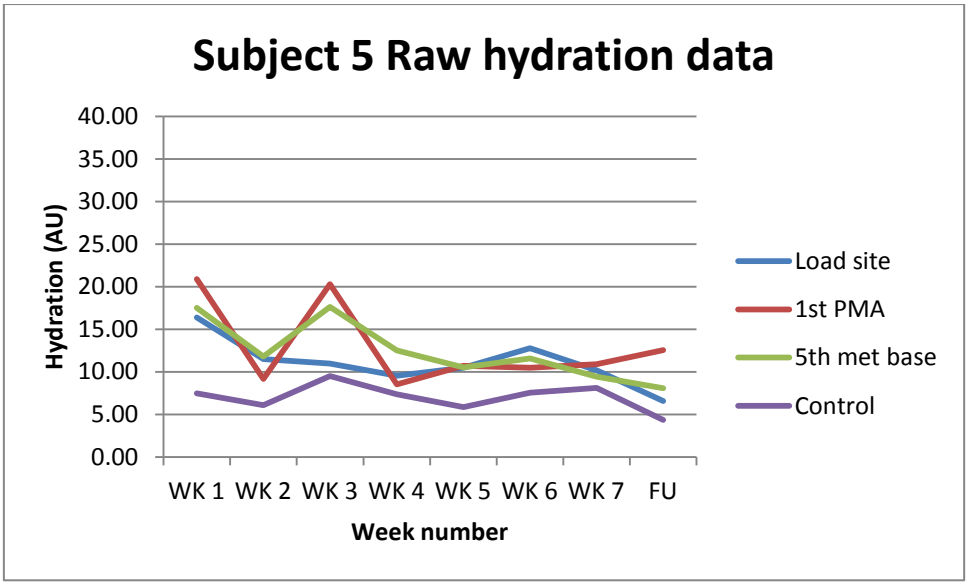


Figure 6.15 – Subject 5 raw hydration data.

Table 6.17 Subject 5 smallest detectable change for hydration

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	6.11	9.11	5.85	0.50
SD of differences between time points	1.94	3.97	3.04	1.69
SEM	1.37	2.81	2.15	1.19
SDC	3.80	7.78	5.96	3.30

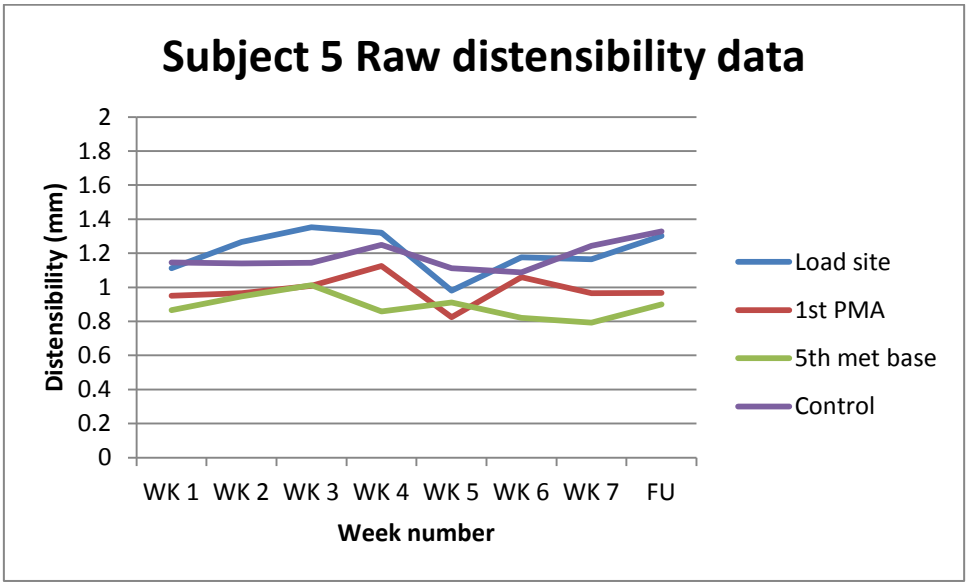


Figure 6.16 – Subject 5 raw distensibility data.

Table 6.18 Subject 5 smallest detectable change for distensibility

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.11	0.04	0.03	0.04
SD of differences between time points	0.13	0.09	0.08	0.09
SEM	0.09	0.07	0.05	0.06
SDC	0.25	0.18	0.15	0.17

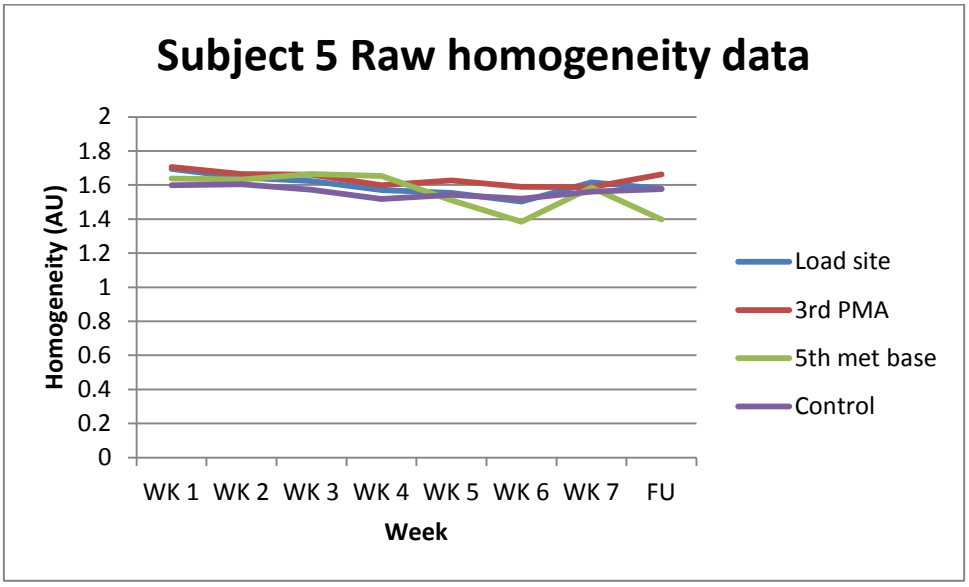


Figure 6.17 – Subject 5 raw homogeneity data.

Table 6.19 Subject 5 smallest detectable change for homogeneity

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	0.11	0.09	0.07	0.05
SD of differences between time points	0.05	0.04	0.11	0.03
SEM	0.04	0.02	0.08	0.02
SDC	0.10	0.07	0.21	0.06

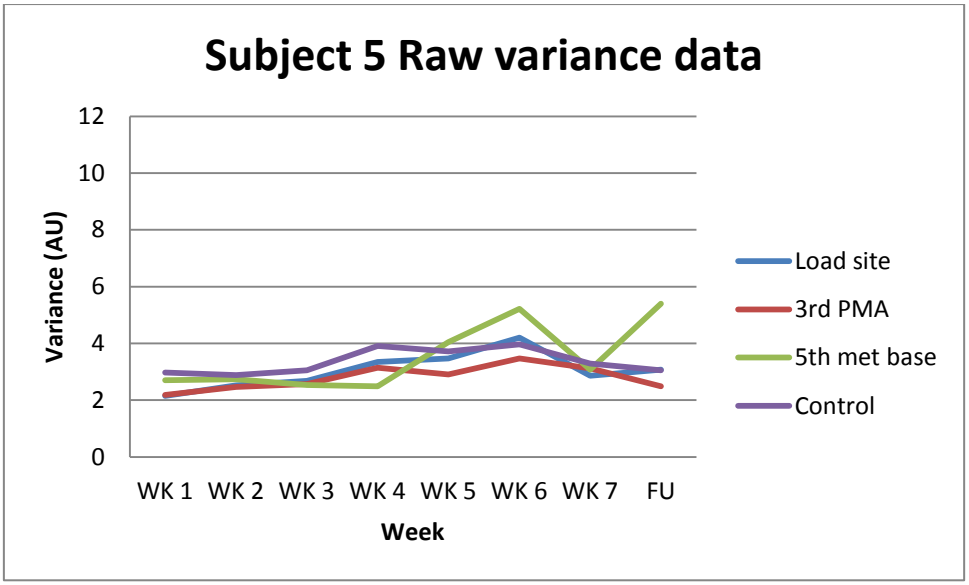


Figure 6.18 – Subject 5 raw variance data.

Table 6.20 Subject 5 smallest detectable change for variance

	Load site	PMA	Base of 5th metatarsal	Opposite foot
Mean difference between time points	1.03	0.77	0.65	0.50
SD of differences between time points	0.62	0.38	1.08	0.46
SEM	0.44	0.27	0.76	0.32
SDC	1.22	0.74	2.12	0.90

6.9 Discussion

Contrary to the widely held hypothesis, the data suggest that there was no skin response at the area of additional loading. There are a number of points related to methodology and implications which must be considered in the context of this unexpected nil-response.

Tables 6.1, 6.6, 6.11 and 6.16 show the estimated percentage of total weekly dynamic loading delivered during the loading sessions by the device to each participant's plantar skin. These show that while only three appointments per week were possible, the volume of load administered in relation to what the site of load might normally experience (albeit estimated) was relatively high. The percentage of peak pressure ranged from 13.6% to 28%; PTI, the sum of total loading 23.5% to 41.5%, and contact time 7% to 13.6%. It is important to highlight that the results of this study only reflect estimated loads due to walking and not due to standing. The percentage load contributed by the device to the total accumulative loads experienced by the foot site would be much lower if the periods of standing were taken into account. The activity data shows that the duration of standing is considerably higher than that of stepping; for Subjects 4 and 5 almost three times as much.

It is possible that much longer periods of loading per day and week provide optimum conditions for stimulation of callus, rather than cyclical loading during stepping activities. If so then the loads added to the feet of participants is relatively small compared to the loads experienced each week, perhaps too little additional load to stimulate a skin response, thus explaining the results. It might be that duration of loading as opposed to magnitude of peak pressure is the main factor in callus development. An increase in pressure resulting, for example from an anatomical deformity which might lead to callus-type skin, is present in the individual's foot all the time, and in cases where footwear might be a factor, a period of several hours for at least several days per week. This amount of time would not be feasible in a laboratory controlled study and it would be unethical to request such a commitment from participants.

Conversely, if volume of accumulative load is the factor irrespective of duration of load, an increase in the level of pressure applied by the device at each loading session may have accelerated a skin response as it would have increased this volume of total load. However, increasing the pressure applied to the foot by the device was not appropriate. Only two fold normal in-shoe plantar pressure values could be tolerated by most of the participants for a short period of time. Higher pressures were tested in preliminary testing sessions and were found to cause discomfort, so for this, and also safety reasons, a maximum of three fold normal peak pressure was considered appropriate, but could be reduced if necessary to suit the participants. In these sessions, it was also deemed that 20 minutes was an appropriate timeframe for each session of loading. This was due to time commitment required and also comfort. In one case, Subject 2, it still proved to be a magnitude of load that was too great and could not be tolerated. Furthermore, only three days per week of loading was possible. All the individuals recruited were from the University and worked or studied in relatively close proximity to the author of this thesis. This was considered important to allow for frequent loading sessions with minimal disruption of work commitments, thus adding recruitment and retention of participants. Recruiting outside the institution would not have proved effective since the burden on participants would have been too great.

Other difficulties in the recruitment process involved the recruitment criteria. Originally, the criterion was for healthy females above the age of 40 years. Previous research has suggested that older females are more likely to develop callus (Menz et al., 2007) and while an inclusion age of 40 is not by any means elderly, having an age range too narrow would make it difficult to reach the intended sample size in a population of

individuals working or studying at the University. However, due to the poor initial response, the inclusion criteria were changed to individuals of any age with the presence of callus somewhere on the foot. This was deemed to be acceptable because the presence of callus would suggest that the individual's skin would be prone to such a response with the correct conditions.

A potentially important factor in eliciting a skin response might have been the type of load administered to the plantar skin. This study focussed purely on application of vertical pressure, due to the fact that several studies (Potter and Potter, 2000b, Pataky et al., 2002, Duffin et al., 2003) have reported significant increases in vertical plantar pressures beneath callused skin compared to normal skin and control subjects. Further studies have investigated changes in normal pressure in response to treatment (Pitei et al., 1999, Potter and Potter, 2000b, Woodburn et al., 2000, Davys et al., 2005). However, shear force might also have been a beneficial topic to study in place of or in addition to vertical pressures, given that several studies have noted skin changes at a histological level resulting from shear loads (Rubin, 1949, Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998). Given that these studies have shown skin responses in human and animal non-plantar sites in a relatively short timeframe (between seven and 35 days), it could be argued that a similar approach could have reaped a similar response in plantar skin. However, it must be stressed that these studies only investigated non-plantar skin sites, areas that are anatomically and functionally different from plantar skin, and are not designed for bearing loads, so this could explain why these studies found it relatively easy to drive skin change in a short timeframe. As plantar skin is designed for bearing loads, given that literature already supported the hypothesis of vertical pressure being related to plantar callus, the need to test the skin response to vertical pressure application took precedence over shear pressure. Vertical pressure is much easier to measure, and administer repeatably and safely. It is also expected to be in the region of six times greater than shear pressure, and therefore could be argued to be more worthy of investigation, at least initially. Shear pressure has been shown to be difficult to measure and studies have found conflicting results regarding the locations of peak shear pressures beneath the foot (Pollard et al., 1983, Tappin and Robertson, 1991, Hosein and Lord, 2000, Perry et al., 2002, Yavuz et al., 2007, Stucke et al., 2012, Mori et al., 2012).

While applying shear pressure using the loading device used in this chapter is technically possible, it is very challenging because the nature of the compression application required to allow the shear actuator to move whilst in contact with the skin is

difficult to achieve. Therefore, the repeatability of compression to shear ratios within and between loading sessions could be poor with the current device. Also, if one were to apply similar levels of compression and shear as is present during gait (1.2 and 0.2 times bodyweight respectively (Richards, 2008)) a very large shear actuator and a more robust device design might be needed, perhaps with a more sophisticated pressure control system than manually adjustable pressure regulators. Applying such large shear forces artificially with a metal device could be potentially dangerous with the risk of skin tearing a possibility. For these reasons, the most feasible starting point for studying the relationship between loading and callus was vertical pressure. However, addressing the role of shear could be a potentially beneficial study to undertake at a later time, and is certainly an important area of study.

One challenge for this study and indeed any study of factors affecting skin properties over time is the apparently normal large variation of skin properties over time. As shown in the biophysical measurements data, the skin experiences apparently normal changes in its properties over time under typical circumstances; indeed this study is perhaps the first to reveal this in pedal skin. The reasons for this could include changes in footwear (and thus occlusion), weather and activity over time. The SDC values for skin hydration suggest that relatively large changes in skin properties at single skin sites are necessary in order for the change to be considered 'real' and not due to random error (i.e. statistically significant at 95% confidence level). However, the relatively large SDC values are likely to be a product of skin variation over time. The values might suggest that skin changes may be somewhat hidden by measurement error but control sites would also be susceptible to these changes, so comparing the load site to controls would still allow an effect to be identified.

Certainly, Studies on callus and heel fissure treatments that the author of this thesis co-authored at this institution (Hashmi et al., 2015; under review) clearly showed an obvious skin change in the regions of interest in comparison to the control sites which could not be attributed to normal variation over time. These studies showed that increases in hydration of up to 152%, and distensibility of up to 100% in callused skin sites, between baseline and post-treatment, were possible after podiatry treatment for callus. This magnitude of skin change is far larger than can be attributed to normal skin variation, and these changes were obvious in relation to the control site. In other studies of pedal skin properties, large changes in hydration within a smaller timeframe than the work in this chapter have been demonstrated post-treatment with topical moisturisers. Garrigue et al.

(2011) observed a 48.9% and 57.3% increase in hydration after 14 and 28 days ($p = 0.0002$ and < 0.0001 respectively), and Papanas et al. (2011) observed an 8.9% and a 20.6% increase after seven and 14 days respectively ($p < 0.001$). In the context of the study in this chapter, if normal skin were to become 'callus-like', it should clearly show in the data. The fact that it has not suggests that, in this case, the loading protocol was not enough to elicit a skin change. It might be that this study was not conducted over a long enough period for any possible changes to manifest in relation to the control sites and that skin changes, if very small, were masked beneath normal week to week variation. However, as previous studies have shown obvious skin changes within 35 days (Rubin, 1949, Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998), the period of loading used in this study was deemed a suitable timeframe for identifying skin changes. Because no skin change due to loading was identified, a future study investigating vertical pressure and skin response might have to be conducted over a period of many more months before a noticeable change can be detected. It might be that a large timeframe is the only way to cause a biophysical change in plantar skin, a site that is anatomically designed for load bearing. Such studies will prove very demanding for participants and researchers alike.

One area that must be addressed before concluding future study is safety. Preliminary tests were conducted, as discussed previously, to determine the loading dose and frequency of loading sessions for the study. However, these aspects were not completely rigid for the entire study and could have been subject to change if need be. At each session, the load was set for each participant and during the loading cycle, feedback was sought regarding comfort. This was important because it ensured participant safety. The second participant in the study was required to withdraw because the sensation of having the probe head applied to the skin was uncomfortable and caused a small amount of bruising to the skin. This was identified by participant feedback and highlights its importance. No further data was collected from this participant, but on examination it was noted that she had particularly thin tissue overlying the metatarsal heads compared to the other participants. The participant also reported not undertaking regular exercise, leading a relatively sedentary lifestyle compared to Subjects 1, 3, 4 and 5 who all undertook regular, strenuous exercise including running, sports and gym sessions. This might have meant that the tissues in her foot were not accustomed to higher loads and were therefore more sensitive and prone to injury. This could be a potential factor to consider in recruitment – seeking individuals who are physically active so they can tolerate higher loads. However, it seems logical that individuals that are sedentary might show a greater skin response to

laboratory-induced loads due to the fact that their skin is not as used to intense loading. The ability to apply person specific loads using the device used here addresses this concern to some degree, because even if high pressures are common for a specific foot, at least two fold these high pressures can be applied. Careful application of loads, beginning from a small load, such as applying the same level of pressure they would normally experience beneath the area of interest, and building up to a higher, but tolerable load, after several weeks in the study might be of benefit to pre-condition the skin. However, this would also further increase the burden on research participants.

Regular examination of the feet is also recommended to identify any inadvertent bruising or inflammation caused by the device, or any abrasions or blistering that could occur. Subject 5, during the second week of loading, developed a small abrasion at an adjacent area to the loading site, caused by the probe rubbing on the skin. The adjacent skin in question had a small patch of callus and was therefore stiffer than the healthy skin of the loading site. This stiffness of the adjacent skin was likely responsible for the small abrasion so the participant was sent for a podiatry assessment and had the hard skin debrided. The next loading session was delayed to allow time for the skin to heal, and then commenced as normal. Regular examination of the foot was undertaken for each participant subsequently to ensure safety.

In addition to those highlighted above, this study has several other limitations which need to be taken into account. Firstly, regarding activity monitoring, it is important to note that it only represents data collected in a given week and not necessarily every week. Levels of activity inevitably will vary from week to week so the estimated percentage of loading beneath the foot delivered via the loading device will be different each week. For optimum accuracy, one would have to wear the monitor for the entire duration of the study.

Another limitation is that pressures from barefoot and shod walking were used to create a target plantar pressure that would be applied using the device. Activity monitoring data assists in this profile but does not give an indication of how much time is spent walking shod and barefoot in real life. In this study, the author had to make an assumption of shod walking accounting for 90% and barefoot 10% of physical activity. It is likely the actual ratio between the two is different for different people and not consistent on a day-to-day basis.

When processing pressure data collect from the Pedar® and Emed® devices, a mask was used to overlay the loading site in order to capture the data. Due to the difference in

pressure sensors and software, the masks may have varied slightly in size. For Pedar® the sensor sizes are relatively large so potential error exists in that adjacent metatarsal data may have been collected within the mask. The metatarsal heads cannot be visualised using Pedar® and the sensors are too large to be able to have a mask overlay the exact area. The mask used was the only one underpinned by research relating to foot anatomy (Bontrager et al., 1997) – the mask in question overlaying the second, third and fourth metatarsal heads with 16 pressure sensors in an array of 4 x 4 (Figure 6.1). In this study, either the second or the fourth metatarsal head was used as the loading site so the mask was divided into two with the area overlying the region of interest used. There is a small possibility of some third metatarsal head data being collected within this mask, but if the mask was further reduced making it narrower, it may have overlaid the wrong area completely. For the Emed® MatLab software, it is possible to approximate the region of each metatarsal head visually from the pressure footmap which aids in creating the mask. Due to these factors, there was possibly a discrepancy between data collected between the two systems. If peak pressure data was actually from an adjacent metatarsal head as opposed to the area of interest, this could have led to over or underestimation of pressure magnitude. For PTI, the mean pressures of all the sensors within the mask were used. This was deemed a safer option in the case that other, unwanted metatarsal heads strayed into the mask. However, it may have had the effect of underestimating the PTI that was actually occurring at the load site. Using a PTI with the summed pressure values of all the sensors in the mask would have been most accurate provided the mask exactly overlaid the area which the contact pad of the device contacted and had the same surface area. However, this would have been exceptionally difficult due to the size and location of the Pedar® sensors. With advanced software, one might be able to achieve something similar in Emed® but as of yet, none is available.

6.10 Conclusion and implications for further research

Overall, while no real skin response was observed for any participant, this study proved to be a useful exercise towards improving our understanding of how external loads might affect plantar skin properties. The device and pilot work represent the design and implementation of a novel research study that has never been attempted on plantar skin previously.

Several possible reasons have been identified for the nil-response shown in this chapter, and recommendations for others attempting this type of research are highlighted.

The load dose used in this study may not have been high enough or administered over long enough duration of time to elicit a callus-like skin response. However, increasing this loading dose any more would make recruitment exceptionally difficult and raise possible safety concerns. Recruitment was undertaken to allow the greatest chance of a skin response (e.g. feet known to be able to produce callus), but a narrower recruitment criteria such as older females might have been beneficial (due to changes in skin properties with age). However, this too may make implementation of the study more difficult. The direction of pressure administered to the skin may not have provided optimum conditions for skin response. Research supports increased vertical pressure beneath callus, which was the rationale for further investigating this phenomenon, but shear pressure application might have been proven effective as has been highlighted in dermatology studies. In this case shear was too difficult to administer and vertical pressures were deemed a more suitable initial focus. Safety considerations including small initial doses of load, particularly for individuals leading a sedentary lifestyle whose feet are not used to being vigorously loaded and regular examination of the feet have also been recommended for skin loading research.

This project, to the author's knowledge, is the first of its kind investigating the effect of regular load doses on plantar skin and using biophysical skin measurement devices to record skin response. It is also the first to relate activity monitoring data to plantar pressure data to estimate accumulative loads. This is an approach that could be beneficial in other areas of research, such as assessment of loading profiles of individuals at risk of ulceration in diabetes and rheumatoid arthritis. The type of pressure research conducted in a laboratory, while useful, provides only a very narrow snapshot of individuals' loading profiles, and activity monitoring could allow a greater understanding of this and possibly identifying those at risk of ulceration or explain the presence of existing lesions.

To summarise, this study investigated the relationship between plantar loading and callus, implemented a novel loading device for the first time, and sought to track changes in plantar skin through a pilot study. The non-response observed in the skin is interesting because it points to the need for perhaps greater vertical plantar loads or need for shear loads to trigger skin responses. The next step in understanding the relationship between loading and callus is to investigate how existing calluses respond to pressure reduction; do the lesions regress and become more like 'normal' plantar skin? This will be investigated in the final study, Chapter 7.

Chapter 7: The effect of pressure reduction on the biophysical properties of plantar callus

7. 1 Introduction

Orthotic insoles have been used as a conservative pressure reduction modality in podiatry and other physical therapy professions for many years. One of their key uses is to offload pressure areas on the plantar foot, which is particularly beneficial for individuals with diabetes and rheumatoid arthritis, as high plantar pressures have been associated with ulceration (Boulton et al., 1983, Murray et al., 1996). As a result, much of the research into orthotic insoles has focussed on these groups with minimal studies focussing on their use in treating pressure related lesions. However, in order to understand how insoles work it is necessary to explore the literature on their design and common uses. This chapter will begin with a review of the literature into insole design to set the context for the research before detailing the study.

7.2 Review of the literature

7.2.1. Efficacy of customised insoles

In the literature, the benefits of the use of custom moulded insoles to reduce plantar pressures under the forefoot have been widely reported. Raspovic et al. (2000) assessed the effect of using customised orthoses as a pressure relieving measure in individuals ($n = 8$) with healed diabetic neuropathic ulcers. F-Scan in-shoe pressure data revealed that customised insoles reduced peak pressure ($p < 0.01$) and pressure time integral (PTI) ($p < 0.05$), and increased the contact area ($p < 0.01$) compared to the shoe-only condition. The actual pressure reductions ranged from 6% to 93%. They authors highlighted that the extent of pressure the reduction was highly variable between individuals.

Several studies have reported significant reductions in peak pressure and PTI with increases in surface area in custom moulded insoles compared to flat insoles. In 42 participants with metatarsalgia, Postema et al. (1998) reported a significant reduction in peak pressure at the distal central and lateral forefoot of 18.2% and 10.8% ($p = 0.000$ and 0.03 respectively) and significant reductions of force impulse at the distal central and lateral, and proximal central forefoot ($p = 0.006$, 0.005 and 0.000 respectively) with a custom insole compared to a standard insole. Birke et al. (1999) found a 55% reduction in pressure ($p < 0.05$) beneath high pressure areas in 19 participants with diabetes with a

history of ulceration in their own shoes with customised insoles compared with a standardised shoe alone and with flat Poron insoles of different densities. In 21 subjects with diabetes presenting with neuropathy plus foot deformities, Bus et al. (2004b) found a 15.6% decrease in peak pressure over the first metatarsal head ($p < 0.05$) but no significant reductions beneath the other metatarsals. However, they did report a significant 10.1% decrease ($p < 0.05$) in force/ time integral beneath the lateral metatarsal heads. The peak pressure and force/ time integral were transferred to the medial midfoot. Tsung et al. (2004) found, in a group consisting of 8 healthy individuals and 6 individuals with diabetes, that custom moulded insoles manufactured using scans taken at different loading conditions significantly reduced the peak pressures and increased the surface areas over the forefoot compared with shoe only conditions. The pressure reductions reported ranged from 2.6% to 17.9%. Mueller et al. (2006) studied the effects of pressure relieving modalities in a sample of individuals suffering from diabetes with a history of neuropathy and ulceration ($n = 20$). They reported a significant reduction in peak pressure ($p < 0.03$) and PTI ($p < 0.036$) with total contact insoles compared to shoes in each metatarsal head. Pressure reductions of between 19% and 24% were reported. In a randomised controlled trial (Burns et al., 2009), it was reported that customised insoles significantly decreased peak pressures in the forefoot ($p = 0.034$) and rearfoot ($p < 0.001$) compared to sham orthoses in individuals with diabetes with peripheral arterial disease and foot pain. The pressure reductions are the forefoot were 16.3% in the customised insole group and 10.6% in the sham group when compared to shoe only conditions (Burns et al., 2009).

However, not all studies have found significant improvements in using customised orthoses over prefabricated. Novak et al. (2009) conducted a randomised controlled study comparing the pressure reduction effects of customised orthoses versus flat insoles in a sample of rheumatoid arthritis patients ($n = 40$). They reported no significant differences in pressure redistribution qualities between the insoles. Redmond et al. (2009) compared the pressure reducing effects of prefabricated and customised insoles in randomised cross-over trial with individuals presenting with flat feet. They found that while both sets of insoles reduced peak pressure, mean pressure, PTI and force/ time integral at the forefoot compared to using a shoe only ($p < 0.05$), there was no significant difference noted between the insoles. In a group of 18 runners tested in normal and custom fit additive manufactured insoles, Salles and Gyi (2013) reported no significant differences in forefoot pressures between the insoles, noting only differences at the heel region.

The efficacy of full contact insoles versus flat insoles has also been explored using computational analysis. Chen et al. (2003) found, comparing finite element models of two different full contact insole types against a flat insole, that peak and normal stresses were reduced in all areas of the plantar foot except for the medial midfoot where the stresses increased as a result of stress redistribution.

These studies show that generally full contact customised insoles are more effective than no insole or flat insoles in reducing peak pressures. This is due to the fact that pressures are redistributed to the midfoot region, therefore offloading regions with higher pressures, such as the metatarsal heads.

7.2.2. Methods of insole design and manufacture

There are several papers exploring different methods of insole design. Tsung et al. (2004) explored the pressure relieving effects of insoles moulded using different loading conditions including non-weight-bearing, semi-weight-bearing and full-weight-bearing. They found that all conditions were significantly more effective than using flat insoles with reductions ranging from 2.6% to 17.9% across the metatarsal heads. When comparing the three different loading conditions, it was found that the insole defined using the semi-weight-bearing foot shape was the most effective at reducing peak pressures at the medial forefoot with reductions of 17.9% at the first to third metatarsal heads. The insole defined using the non-weight-bearing foot shape was more effective at reducing peak pressure at the lateral metatarsal heads (14.8%). This insole also reduced PTIs at the forefoot up to 20.9%, and increased it at the midfoot by 23.7%, which the authors stated was due to the greater arch support compared to the other insoles.

Another study has compared the pressure relieving effects of custom moulded versus insoles fabricated from material inserted in participants' shoes and moulded through walking (termed dynamic impression insoles) (Chang et al., 2012). Beneath the forefoot, the custom moulded and dynamic impression insoles reduced peak pressures by 34.3% and 46.3% respectively compared with the flat insole ($p < 0.05$). The dynamic impression insoles significantly reduced peak pressure by 18.2% compared to the full contact insole ($p < 0.05$). The authors also observed significant reductions in PTI ($p < 0.05$) and increased contact areas ($p < 0.05$) for both insoles compared to the control, and for dynamic impression insoles compared to custom moulded insoles at the forefoot.

One study has compared CAD-CAM designed insoles with insoles made using foot shape, captured using a foam impression box (Ki et al., 2008) in 30 individuals. It was

reported that the peak pressures and force were significantly reduced in the heel region ($p = 0.000$ and 0.000 respectively) and increased in the midfoot region ($p = 0.004$ and 0.000 respectively) for both customised insoles compared to a flat insole. The PTI was also increased in the midfoot for both insoles. When comparing the CAD-CAM with the foam moulded insoles, the CAD-CAM insole delivered a 15.3% lower peak pressure beneath the mid forefoot region than the foam moulded insole ($p = 0.01$).

Further research has looked into CAD-CAM customised insoles. Owings et al. (2008) explored the effect of using pressure data combined with foot shape data in CAD-CAM insoles in 20 participants suffering from diabetes. They sent foam impressions from each participant to three insole companies for CAD-CAM of insoles and one company also received barefoot plantar pressure data to accompany the foam box impressions. From plantar pressure analysis of the forefoot using the three pairs of insoles, the insoles which were manufactured using pressure data plus foot shape derived from the foam box showed significantly lower peak pressures at 14.2% and 30.7% reduction compared to the two insoles that used foam box only ($p < 0.0001$). The force/ time integral ($p < 0.0001$) was also significantly lower at the forefoot, but increased at the midfoot ($p < 0.01$) for the insoles developed from foam box and plantar pressure data.

This section has highlighted the potential benefit of using semi-weight-bearing loading conditions and/or created from dynamic material impressions to relieve peak pressure. The use of CAD-CAM as an insole fabrication method may give quality insoles for pressure reduction and this could be further enhanced by the use of pressure data (Owings et al., 2008).

7.2.3. Insole materials

There is an abundance of studies addressing the efficacy of different materials in plantar pressure reduction and comfort. An early study by Leber and Evanski (1986) compared the pressure reduction qualities of seven different insole materials on patients with high plantar pressures ($n = 26$). The materials included latex foam, Plastazote, Dynafoam, Orthofelt, PPT, Spenco and Molo. Using the Harris and Beath footprinting technique, they observed that all conditions significantly reduced pressure with reductions ranging from 28% - 53% ($p < 0.01$) compared with the no-material control footprint. PPT, Plastazote and Spenco were significantly more effective at reducing pressure than the other materials, reducing the pressure between 51% and 53% compared to the control ($p < 0.01$). Barrow et al. (1992) further investigated the pressure relieving properties of PPT and Spenco.

Participants (n = 7) with high plantar pressure beneath the second metatarsal head wore a total of four insoles for one month each. The insoles included two PPT and two Spenco with a U-shaped cut-out beneath the second metatarsal head and a sponge-filled U-shaped cut-out beneath the second metatarsal head. Pressures were measured using pedobarography at monthly intervals to allow data to be collected with new insoles, then insoles after one month of wear. The authors found that all four insoles significantly reduced pressure (percentage reductions not published) ($p < 0.0001$) and there was no significant difference between the pressure relief of PPT and Spenco. There was no significant difference between new and old insoles in pressure relief. The Spenco insoles with the sponge filled cavity slightly offloaded pressure beneath the second metatarsal head, but this was not evident for the PPT insoles.

Birke et al. (1999) compared the pressure relieving effects of flat Poron insoles at different densities in 19 individuals with diabetes with a history of ulceration. They found that medium density Poron (shore 22, 27 and 32) was significantly more effective at relieving mean pressures (range 36% - 39.5% reduction) than soft density (shore 14 and 17) and high density (shore 40 and 55) Poron (range 20% - 33.7% reduction, $p < 0.05$). However all Poron densities were significantly more effective than a shoe only condition but not as effective as a moulded insole in the participants' own shoes which gave 55% reduction in mean pressure ($p < 0.05$).

In a population of German soldiers (n = 26), Hinz et al. (2008) compared conventional army boot insoles with prefabricated, contoured EVA and Neoprene insoles to assess their pressure relieving effects for use in injury prevention. They found that forces were significantly reduced in the Neoprene insoles compared with the conventional insoles ($p < 0.0006$) across each metatarsal head and the EVA insoles ($p < 0.00001$) across metatarsal heads three to five. Compared with the other insoles, peak pressures were significantly lower in the Neoprene insoles in the second metatarsal head ($p < 0.003$) and the third to fifth metatarsal heads ($p < 0.0001$) as was the force/ time integral across all metatarsal heads ($p < 0.0002$). Peak pressure reductions were shown to range from 20.6% to 31.4% for the Neoprene insoles compared to the conventional types beneath the second and third metatarsal heads.

Tong and Ng (2010) investigated the pressure reduction effects of slow recovery Poron, standard Poron, Poron with firm Plastazote and Poron with soft Plastazote. Simple 6.5mm thick insoles were made from the materials and pressures were measured using an F-Scan system in 5 subjects. They found that all materials could reduce pressure compared

with no insole, but only the Poron with high density Plastazote reached significance reducing the mean peak pressure by 27% ($p < 0.03$).

Healy et al. (2012) studied EVA and PU materials in custom and flat insoles in 10 subjects. All materials significantly reduced peak pressures at the first and lateral metatarsal heads for both insole types with reductions ranging from 2.1% to 18.3% for the flat insoles and 6.5% to 18.6% for the custom insoles. Peak pressures were also significantly reduced at the heel for the custom moulded insole type. Comparing the materials, the authors found that PU was effective at reducing PTI and increasing contact area, while medium density EVA showed higher pressures in flat insoles than the other materials.

Two studies have assessed the effects of wear on pressure reduction capabilities of insoles. Rogers et al. (2006) investigated how two insole compositions, Poron and a mixture of Poron and Plastazote, reduced pressure before and after 50,000 steps assessed with a pedometer in 19 subjects. They observed significant reductions in peak pressures before ($p < 0.05$) and after ($p < 0.05$) 50,000 steps for both insole compositions compared to a shoe only condition. The pressure reductions were 29.9% and 28% before 50,000 steps; and 18.3% and 25.3% reduction after 50,000 steps for Poron and Poron/ Plastazote respectively. The Poron insoles' pressure reduction qualities were significantly reduced after 50,000 steps (16.6% increase in pressure compared to measurements taken before 50,000 steps; $p < 0.05$), but there was no significant impairment in pressure reduction for the Poron and Plastazote insole. Cronkwright et al. (2011) found similar results with a prefabricated, dual density Formthotic insole before and after one year. Compared to shoe only, the new insole reduced pressures beneath the forefoot and heel by 12% and 23% respectively ($p < 0.05$ and 0.01). The old insole still reduced pressures in these regions compared to the shoe, but only the heel reduction was significant ($p < 0.01$). There was no significant change in the forefoot between old and new insoles but the heel pressures were significantly higher in the old insole ($p < 0.01$). The maximum force was significantly increased in the forefoot and decreased in the heel in the old and new insole compared to the shoe only ($p < 0.01$), and significantly increased in the forefoot in the old insole ($p < 0.05$). Compared to the new insole, the force was significantly higher in the forefoot ($p < 0.05$) and the heel ($p < 0.01$) in the old insole. Contact areas were also significantly increased in the new and old insoles compared to the shoe ($p < 0.01$) but not between insoles.

These studies show that most insole materials are capable of reducing peak pressures compared to wearing a shoe without an insole. The most effective materials are arguably PPT, Spenco, Poron combined with Plastazote, Neoprene and PU and these could be particularly effective in a customised insole. Insole wear also appears not to significantly affect pressure reduction in most cases, even after one year of wear.

7.2.4. Pressure relieving additions to insoles

There is a wealth of literature focussing on pressure relieving additions to insole design. These include metatarsal domes, pads and bars which are designed to offload all or individual metatarsal heads and relieve pressure and pain. Specific pressure reductions are shown in Table 7.1.

Table 7.1 – Pressure changes beneath forefoot using pressure relieving additions reported in the literature

Study	Modality	Pressure without modality	Pressure with modality	Pressure reduction (%)
Holmes and Timmerman (1990)	Metatarsal pad - met head 1	2.43 kg/cm^2	2.16 kg/cm^2	-11.1
	Metatarsal pad – met head 2	4.31 kg/cm^2	3.12 kg/cm^2	-27.6
	Metatarsal pad – met heads 3 & 4	2.91 kg/cm^2	2.25 kg/cm^2	-22.7
	Metatarsal pad – met head 5	1.92 kg/cm^2	1.6 kg/cm^2	-16.7
Poon and Love (1997)	Metatarsal dome	2833 g/cm	2366 g/cm	-16.5
Hodge et al. (1999)	Orthosis with dome	25.87 N/cm^2	21.82 N/cm^2	-15.7
	Orthosis with bar	25.87 N/cm^2	20.57 N/cm^2	-20.5
Jackson et al. (2004)	Orthosis with dome	274.5 kPa	242 kPa	-11.8
	Orthosis with bar	274.5 kPa	216.1 kPa	-21.3
Kang et al. (2006)	Orthosis with dome	225.8 kPa	199 kPa	-11.9
Lott et al. (2007)	Orthosis with dome	176 kPa	143 kPa	-18.8
Lin et al. (2013)	Orthosis with cut-out at region of interest	262.5 kPa	149.9 kPa	-42.9
	Orthosis with cut-out plus arch support	262.5 kPa	135.6 kPa	-48.3
Lee et al. (2014)	Orthosis, dome 10mm proximal	399 kPa	364.7 kPa	-8.6
	Orthosis, dome 5mm distal	399 kPa	331.9 kPa	-16.8
	Orthosis with bar	399 kPa	358.2 kPa	-10.2

Pressure changes calculated by new value – old value / old value x 100.

The use of metatarsal pads/ domes have been found to offer significant reductions in peak pressure and pain scores in individuals with metatarsalgia (Poon and Love, 1997);

diabetes and a history of neuropathy and ulceration (Lott et al., 2007, Guldemond et al., 2007, Mueller et al., 2006); rheumatoid arthritis (Jackson et al., 2004, Kang et al., 2006) and asymptomatic individuals (Holmes and Timmerman, 1990). Pads may also decrease soft tissue strain in healthy feet (Ibrahim et al., 2013) and increase tissue thickness in diabetic feet (Mueller et al., 2006). Extra arch support may decrease the peak pressures further in individuals suffering from diabetic neuropathy (Guldemond et al., 2007). Metatarsal bars also offer significant pressure reductions comparable to metatarsal pads/ domes (Hodge et al., 1999, Jackson et al., 2004) and may offer greater impulse reductions beneath the second metatarsal head (Deshaies et al., 2011).

The longitudinal placement of the metatarsal pad/ dome also has an influence on the extent of pressure reduction. One study (Hsi et al., 2005) reported that metatarsal pads placed 4.4mm proximal to the target area of pressure reduction was the most effective position, causing significant pressure reduction ($p < 0.05$) directly below the area of peak pressure at the second metatarsal head. Positioning the pad 8.8m proximal, or directly at the site of peak pressure would lead to significant reductions 4.4mm distal to those points. Hastings et al. (2007) observed that pads placed 6.1 – 10.6mm proximal to the metatarsal heads may reduce pressure; pads placed between 1.8mm distal and 6.1mm proximal, and between 10.6 – 16.8mm proximal can have a variable pressure reducing effect; and pads placed more than 1.8mm distal or more than 16.8mm proximal to the metatarsal heads can lead to increased pressure (Hastings et al., 2007). Another study, however, found that placing the pad 5mm distal was a more effective method of reducing pressure beneath the metatarsal heads than 10mm proximal to the metatarsal line (Lee et al., 2014). The differences between results could be attributed to materials used: Hastings et al. (2007) used a metatarsal pad made of cork while Hsi et al. (2005) used foam rubber and Lee et al. (2014) used PPT foam. The measurement devices were also different.

One other study has also investigated the effect of longitudinal position of the metatarsal pad (between 0 – 25mm proximal to metatarsal line) combined with the use of two different thicknesses of pad (5mm and 10mm) (Brodtkorb et al., 2008). In contrast to the two studies highlighted previously, these authors found no significant impact of longitudinal axis position on mean plantar force values. While both pad thicknesses significantly decreased force beneath the second metatarsal head ($p < 0.05$), the 10mm pad was significantly more effective than the 5mm pad ($p < 0.05$).

While metatarsal pads and bars are very well publicised, another pressure reduction modality which exists is the use of cavities in the insole. Actis et al. (2008) designed a

cavity insole using finite element analysis based on the plantar geometry of two individuals with high peak plantar pressures. The insole in question had a cavity which could be filled with pressure reducing material positioned below the high pressure areas. It was found that the insole with a cavity filled with a matrix of soft Poron plugs that were 4mm in diameter was more effective at reducing high peak pressures than using a total contact insole, an insole with a forefoot inlay, and an insole with the forefoot area inserted with a matrix of 4mm plugs. A more recent study (Lin et al., 2013) assessed the pressure relieving effects of insoles with removable plugs at a region of interest. The subjects in question were 26 individuals presenting with diabetes and neuropathy. The forefoot area with the highest peak pressures as defined from in-shoe pressure tests were used as the regions of interest. The insoles were tested before and after plug removal, then with additional arch support. Peak pressures were found to be significantly reduced after insole plug removal ($p < 0.001$) and were further reduced when arch support was added ($p < 0.001$). In the adjacent areas which were not regions of interest, there was no significant change between pre and post-plug removal. For both regions of interest and non-regions of interest, there was a significant reduction in peak pressures from baseline (6mm flat EVA insole) and the insoles with removable plugs ($p < 0.001$).

These studies show that the use of insole additions including metatarsal bars and pads to offload the metatarsal heads are effective in terms of pressure and pain reduction. This may be further enhanced with additional arch support (Guldemond et al., 2007). However, if using a pad, care should be taken to ensure optimal positioning in the longitudinal axis to achieve the best results (Hsi et al., 2005, Hastings et al., 2007, Lee et al., 2014). With these modalities, one should bear in mind that they are designed to relieve the pressure from several metatarsal heads and not specific points of high pressure such as an area of callus. For localised pressure reduction, insoles with cavities are a viable option (Actis et al., 2008, Lin et al., 2013).

7.2.5. Insoles used to treat plantar callus

Two studies have investigated the use of insoles for the treatment of callus (see Chapter 2). Briefly, Colagiuri et al. (1995) investigated the effect of a yearlong treatment using custom moulded plastic insoles worn seven hours per day in individuals with diabetes presenting with callus ($n = 9$). Clinical assessment of photographs taken before and after the study was used to assess callus grade and thus changes in callus. Results showed that there was a significant improvement ($p = 0.02$) in callus appearance compared with patients treated by

scalpel debridement (n = 11). However, plantar pressures were not assessed so whether or not these reductions in clinical appearance of callus can be attributed to pressure reduction is unclear.

Duffin et al. (2003) assessed pressure reducing measures a group of adolescents with type 1 diabetes with (n = 17) and without callus (n = 17). The pressure reduction measures included a custom moulded semi-rigid PE insole, PPT cushioning and both insole and cushioning combined. Cushioning reduced pressure by 21.8% and 20.2% ($p = 0.001$), the orthosis alone reduced pressures by 17.7% and 14.3% ($p = 0.05$) and the cushioning and orthoses combined reduced pressures by 27.7% and 31% ($p < 0.001$) in the groups with callus and high plantar pressure respectively. All pressure reduction measures also significantly reduced PTI ($p < 0.05$). Combined cushioning with orthoses was significantly more effective at reducing pressure than orthoses ($p < 0.05$) and cushioning ($p < 0.001$) alone. Out of their sample, they recruited 23 subjects who used custom made orthotic insoles and 67 control subjects who did not use insoles and reassessed them after a year. A significant reduction in pressure was found ($p = 0.0003$) with pressure changes ranging between +41% to -35% (mean reduction of 12 N/cm^2 / 120kPa) in the orthoses group; but no significant difference was found in the control group. In the insole intervention group, six participants had callus at the beginning of the trial but at the end of the trial two calluses had resolved. In the control group, seven had callus at the beginning of the trial and no calluses resolved. There were no quantitative measures of skin properties taken or any attempt to quantify the effect of pressure reduction on callus.

For the purposes of this thesis, these studies are very important as they highlight a possible link between pressure reduction and callus regression, but their methodologies are severely limited in that they do not use quantitative measures of skin properties. Also, it is unknown whether individuals were permitted to use foot treatments or whether there were any other factors which may have affected the skin. Furthermore, Duffin et al. (2003) provided no information as to whether the insoles were still delivering pressure reductions at the 12 month follow-up. Combined with skin biophysical measurements, a study which quantitatively measures pressure reduction using insoles would be beneficial to further understand the effect of offloading on the skin, but also for the efficacy of insoles as a pressure reduction measure in callused feet.

7.2.6. Conclusion

This review of the literature has highlighted that most commonly used insole materials are able to significantly reduce pressures, custom moulded insoles are particularly effective in offloading the metatarsal heads, particularly when used with a pressure relieving aid that is adequately positioned. The use of semi-weight-bearing or dynamic impressions and CAD-CAM fabrication are likely to result in the most effective pressure reduction. Offloading beneath callused skin may result in an improvement in skin characteristics. The subsequent study will provide participants with plantar pressure relief beneath forefoot calluses using the aforementioned literature as a guide, and investigate whether the callused skin changes over time.

7.3 Study aims and objectives

The aim of this study was to determine the effect on biophysical plantar skin properties of reducing pressure under the forefoot in individuals who present with plantar callus beneath the metatarsal heads. The objectives were to provide healthy participants with a plantar forefoot callus with a pressure relieving insole and assess the biophysical properties of the callused skin sites at baseline, and six and 12 weeks after using the insole.

7.4 Methodology

Ethical approval for this study was granted by the University of Salford's College Research Ethics Panel (application number HSCR14/41).

7.4.1. Subjects

The sample size for this study was informed using a paired T-test power calculation (http://biomath.info/power/prt.htm) using the skin characterisation data (presented in Chapter 4) from central callus and callus edge regions. The callus edge data was used because this skin site displays properties similar to central callus while still being significantly different to both this and normal skin, so in effect a halfway point between plantar callus and normal PMA skin. This would be an appropriate level of change to be confident of a skin response from pressure reduction. The results from the calculation suggested 16 datasets would be needed to determine whether or not significance would be achieved from this method of treatment.

Participants were recruited via flyers displayed in the waiting area of the Podiatry Clinic site and areas around the School of Health Sciences buildings at the University of

Salford. Recruitment also involved radio advertisement, the Research Database at the University, and through an advert in a local newspaper. Participants presenting with plantar forefoot callus were included provided they did not have any of the following exclusion criteria which could potentially affect the skin's properties: compromised cardiovascular or neurological status, connective tissue disorders (such as lupus erythematosus), diabetes, autoimmune disorders (such as rheumatoid arthritis), peripheral vascular disease, or wounds/ ulcers of the legs and feet, and eczema, psoriasis or other dry skin disorders affecting the plantar skin.

Before being enrolled into the study, the foot was assessed for neuropathy and peripheral vascular disease. Pulses from the posterior tibial and dorsalis pedis arteries were assessed by palpation. Presence of neuropathy was assessed by vibration perception using a 128MHz tuning fork and 10g monofilament test on the hallux, first and fifth metatarsal heads. If these tests, skin assessment and medical history were satisfied, the volunteers were recruited to participate in the study.

7.4.2. Insole design and manufacture

Following recruitment and initial screening the participants attended a clinical screening which included an assessment of the foot. To allow a custom orthotic to be made, a 3D scan of the participants' feet were taken. The orthotic interventions were then designed and manufactured using CAD-CAM technology by a biomechanics researcher specialising in insole design and manufacture. This study formed part of a larger project comparing insole design, manufacturing methods and materials, and two designs and two materials were used in this study to enable reductions in forefoot plantar pressure. The insole materials used were medium density EVA and rubber, and the designs included a total contact insole or a total contact insole plus a cut-out area beneath the callused skin, both based on the 3D plantar surface geometry. The insoles were designed using the Custom 3Din plugin for Rhinoceros 5, with the basic design based on default templates within the programme. The basic design was using 3mm material with the top surface contoured based on the geometry of the individual's foot. The cut-out design was similar to this but with an extra 2 to 5mm of material added to the existing arch, up to a maximum height of 25mm, and an oval shaped cut-out positioned at the area of peak pressure corresponding to the site of callus. The latter was obtained from a pressure map image from the Emed® scan which was overlaid onto the CAD model of the insole. The cut-out dimensions were 20% insole length and 35% toe width. The insole designs were exported to INESCOP CAM software,

which generated milling paths and enabled the manufacture of the insoles in the CNC Router milling machine. The EVA insoles were made of 40Shore A hardness material. The rubber insoles were made off-site using additive manufacturing (AM) techniques, and were made of thermal rubber, also with a 40Shore A hardness. As the subject of this study is the effect of pressure reduction on callused skin, no further analysis of insole design and manufacture are presented; the only criteria for inclusion in the final data analysis was a reduction in peak pressure beneath the region of interest, i.e. the callused area.

7.4.3. Data collection

Biophysical skin measurements

The primary outcome of this study was callus lesion regression over a 12 week period. Baseline data included skin measurements and shod plantar pressure data. Skin biophysical properties data was collected by one investigator and plantar pressure data was collected by two investigators. The skin measures included skin hydration (Corneometer®), skin distensibility (Cutometer®) and skin surface topography parameters (Visioscan®), as in previous chapters. The skin sites measured included the centre and edge of the callus, skin adjacent to the callus, and two normal weight bearing skin sites including (1) a plantar metatarsal head free from callus, and (2) skin overlying the base of the fifth metatarsal. The skin sites were marked with a ball point pen. Both control sites were identified through palpation and marked. The distance of the radii of the measurement probes was marked over the centre of each skin measurement site to allow accurate probe placement (Figure 7.1).

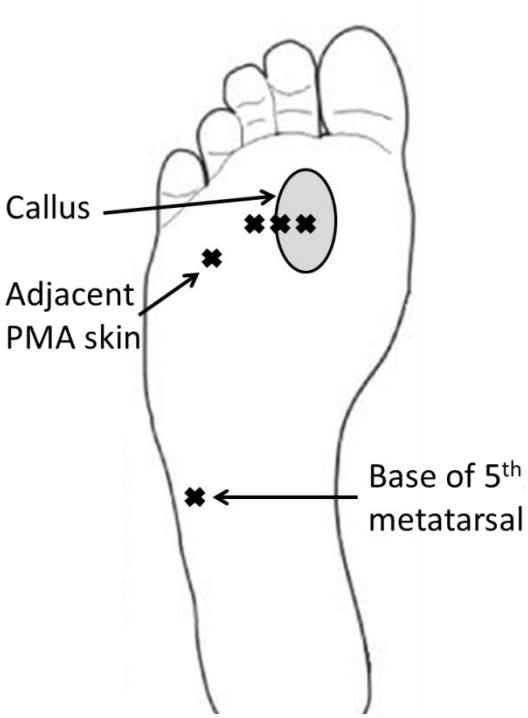


Figure 7.1 - The measurement sites on the callus include the centre, edge, and skin directly adjacent to the callus; and skin overlying the PMA and base of the 5th metatarsal (adapted from Hashmi and Malone-Lee, 2007 p.253).

Measurements were conducted as in previous chapters: 10 measurements were taken per skin site using the Corneometer® and one image was taken per site for the Visioscan®. For the Cutometer®, 500mbar of negative pressure was applied for 30 seconds, then the skin was allowed to relax for 30 seconds.

Pressure data

The pressure parameter used in this study was peak pressure. It was deemed that using other parameters such as PTI would not be beneficial because peak pressures are most likely the main contributor to callus production in healthy feet (based on the literature), and thus almost all of the pressure data relating to callus has focussed on this parameter. Also, a recent systematic review (Bus and Waaijman, 2013) has shown that reporting PTI is of limited benefit due to the fact that the peak pressure causes the greatest amount of damage to the skin, and PTI strongly correlates with peak pressure data.

The plantar pressure data was collected in the participants' own shoes using Novel Pedar-X in-shoe pressure sensors (Novel, Germany) both without and with the orthotic insoles in situ. These measures were completed at each appointment. Pedar insoles have a matrix of 99 sensors (which vary in size according to the size of insole). The in-shoe

pressure measurements were obtained using the participants' own shoes. The participants were not required to wear the same footwear at each visit, because it was likely that, upon becoming accustomed to the insoles, they might use them in different shoes, and change preference as to which shoes to use the insoles with. This would give a more realistic 'real-life' pressure profile. For that reason, they were asked to bring the shoes they wore the insoles with most often. During data collection, the participants were asked to walk along an eight metre walkway five times at a self-selected walking speed, which was chosen as it would give the most realistic pressure profile for each participant. Timing gates were used to record speed to ensure consistency in the walking trials. The pressure data was analysed using InShoe Pressure Analyser© version 1.0 (2012), a pressure analysis programme written in the Foot and Ankle Research department at the University of Salford. The code separates the walking trial into blocks of steps. The first and last three steps were then removed as they represent gait initiation and end. The code then takes the peaks from all the sensors in each mask and then means them, so there is a mean peak pressure for each step, and then it gives a mean peak pressure in each mask for the entire walking trial. The masks include the first metatarsal head, second to fourth metatarsal heads, fifth metatarsal head, hallux and heel.

The peak pressure increase for callused subjects compared with controls is, across the literature, on average in the region of 35% (Potter and Potter, 2000b, Abouaesha et al., 2001, Pataky et al., 2002, Duffin et al., 2003, Menz et al., 2007). One orthotic study conducted in adolescents with plantar callus found a mean pressure reduction of 17.7% using orthotic insoles (Duffin et al., 2003) which is a fairly typical pressure reduction using contoured insoles. It is worth noting that while pressure may decrease as a result of the orthotic, as the skin of the callus becomes more compliant, this might further reduce pressure. In this study, participants were included if they experienced a reduction in plantar pressure under an area of callus due to the insoles, and this site was used for all data collection and analysis. In cases where the participant did not bring suitable shoes for the insoles, a stock trainer was used for data collection.

7.4.4. Statistical analysis

Means, 95% confidence intervals and percentage differences were calculated for hydration, distensibility, topography for all skin sites; and for peak pressure data beneath the region of interest. To assess whether these differences were statistically significant, the repeated measures Analysis of Variance (ANOVA) with post-hoc Bonferroni adjustment,

or Friedman's ANOVA with post-hoc Wilcoxon Signed-Ranks Test were used for parametric and non-parametric data distributions respectively. SDC (calculated from SEM) was calculated to help contextualise changes in skin biophysical data. Statistical analysis was undertaken on SPSS 20.0 and Microsoft Office Excel 2010.

7.5. Results

7.5.1. Participant and pressure profile

Thirty five healthy adults were enrolled into the study. Out of individuals who completed the study, pressure reductions were achieved in 15 with a total of 26 calluses (Table 7.2). Their data was then analysed. This sample of participants was 66.7% female and had a mean age of 53.6 (± 15.5). Out of the 26 calluses, 13 were consistent with Merriman's grade 1 'no specific callus plaque, but diffuse or pinch callus, or present in narrow bands' and 13 were consistent with grade 2 'circumscribed, punctuate oval or circular, well-defined thickening of keratinized tissue' (Springett and Merriman, 1995, p.207). On average, participants wore insoles for six days per week and 8.2 hours per day between baseline and week 6, and for 5.7 days per week and 7.5 hours per day between weeks 6 and 12. At baseline, the pressure was reduced at the region of interest by 18%; at six weeks the reduction was 15.2%; and at week 12, the reduction was 13.6%. The most common sites of maximum pressure increase were at the midfoot and fifth metatarsal head. The room conditions were monitored throughout the trial but not controlled. The mean room temperature and humidity at baseline were 21^{o}c and 42.8% respectively; at week 6 they were 21.8^{o}c and 47.4% respectively; and at week 12 they were 21.7^{o}c and 53.6% respectively.

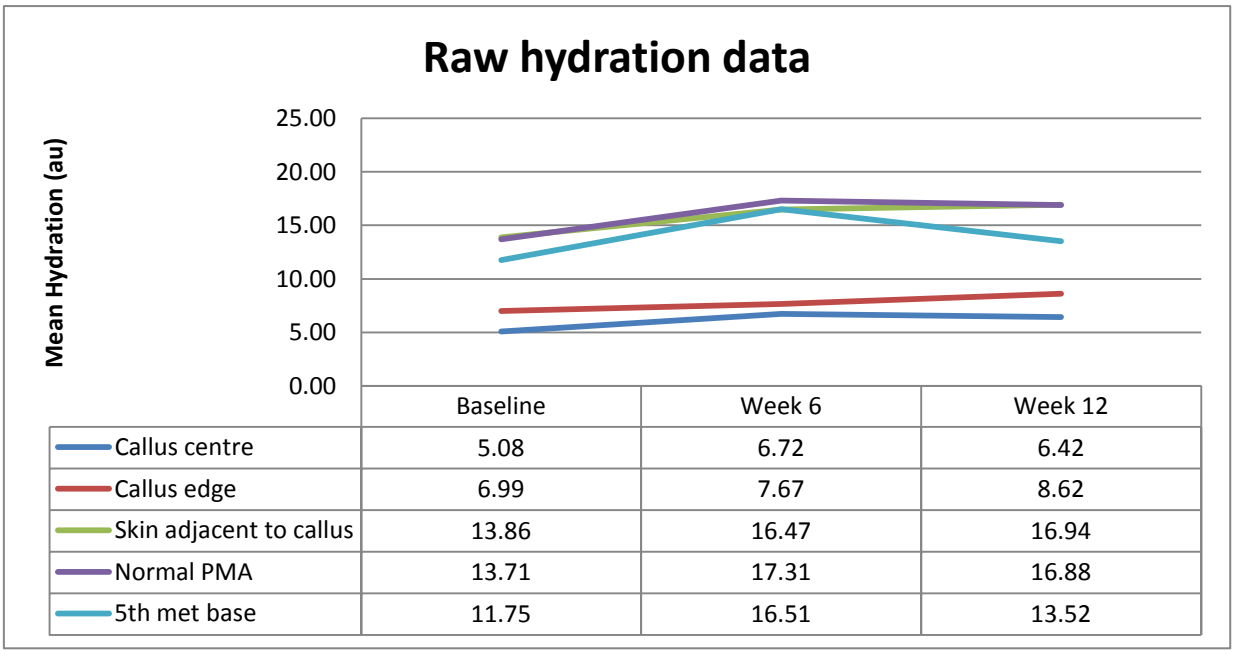
Table 7.2 Pressure reduction group participant profile

	Mean (SD)
Age	53.6 (± 15.5)
Height (cm)	165.5 (± 11.7)
Weight (kg)	72.5 (± 15.4)
BMI	26.5 (± 5)

7.5.2. Skin hydration data

Figure 7.2 shows the raw data at each time point for skin hydration, Table 7.3 shows the percentage change at each skin site per time point and Table 7.4 shows the percentage change between callus and control sites at each time points. The SDC throughout the trial is shown for each skin site in Table 7.5. None of the skin sites deviated significantly in

their values between any of the time points. At baseline, there was a significant difference between the callus centre and PMA and base of fifth metatarsal skin sites (63.0% and 56.8% difference respectively; p = 0.002 and 0.000 respectively). The differences remained significant throughout the study period and were 62% and 52.5% different respectively at week 12 (p = 0.004 and 0.001 respectively). Similarly the skin of the callus edge was significantly different to the PMA and base of fifth metatarsal at baseline (49% and 40.1% respectively). This difference remained significant for the rest of the trial, and differences of 48.9% and 36.3% (p = 0.027 and 0.006 respectively) were recorded at week 12. Skin adjacent to callus was not significantly different to skin overlying the PMA or base of fifth metatarsal at any point in the trial. The SDC data over the trial ranged from 4.99au (callus centre) to 14.12au (skin adjacent to callus) over the study period.



	Baseline	Week 6	Week 12
Callus centre	5.08	6.72	6.42
Callus edge	6.99	7.67	8.62
Skin adjacent to callus	13.86	16.47	16.94
Normal PMA	13.71	17.31	16.88
5th met base	11.75	16.51	13.52

Figure 7.2 – Raw hydration data

Table 7.3 Percentage change between time points at each skin site

	Callus centre	Callus edge	Skin adjacent to callus	Normal PMA	Normal 5th met base
Baseline – Week 6 (p value)	32.3 (0.178)	9.7 (1.0)	18.8 (0.419)	26.3 (0.370)	40.5 (0.005)
Baseline – Week 12 (p value)	26.5 (0.065)	23.3 (0.493)	22.2 (0.219)	23.2 (0.422)	15.1 (0.158)
Week 6 – Week 12 (p value)	-4.4 (1.0)	12.4 (0.583)	2.9 (1.0)	-2.5 (1.0)	-18.1 (0.334)

Table 7.4 Percentage change between callus and control sites at each time point

	Callus and PMA (p value)	Callus and 5th met (p value)	Edge and PMA (p value)	Edge and 5th met (p value)	Adjacent and PMA (p value)	Adjacent and 5th met (p value)
Baseline	-63.0 (0.002)	-56.8 (0.000)	-49 (0.029)	-40.1 (0.006)	1.15 (1.0)	18 (1.0)
Week 6	-61.2 (0.003)	-59.3 (0.000)	-55.7 (0.015)	-53.6 (0.000)	-4.9 (1.0)	-0.3 (1.0)
Week 12	-62 (0.004)	-52.5 (0.001)	-48.9 (0.027)	-36.3 (0.006)	0.3 (1.0)	25.3(1.0)

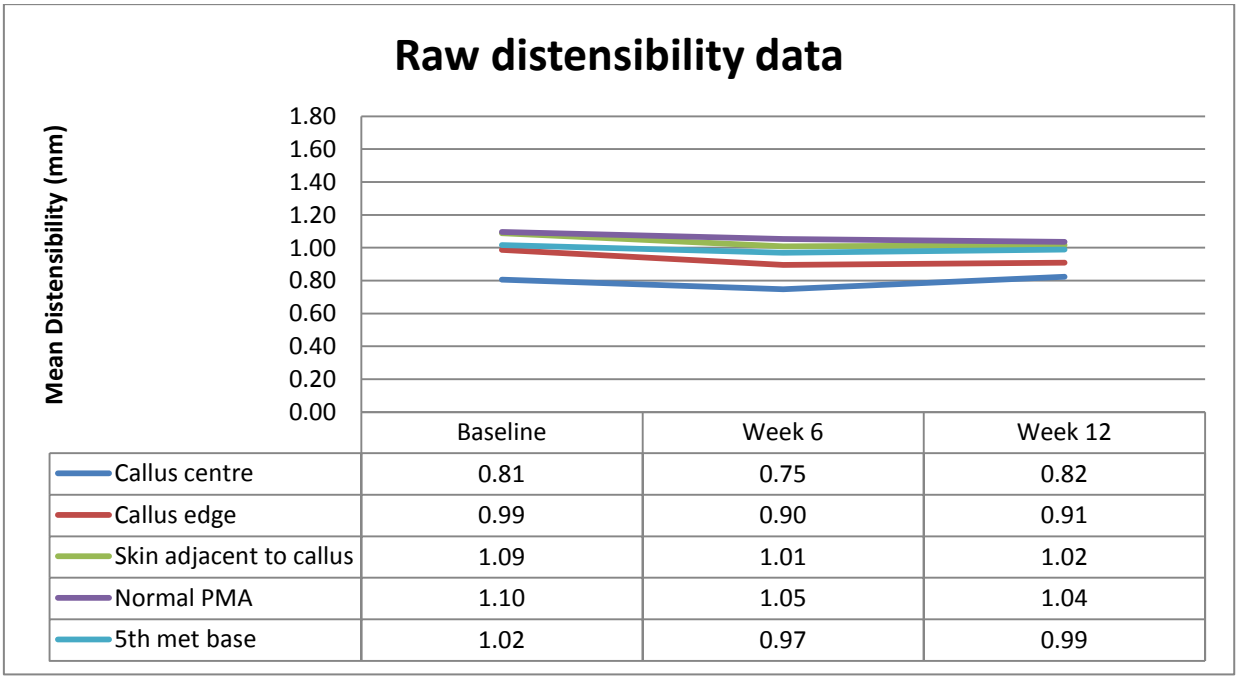
Table 7.5 Smallest detectable change for hydration data

	Callus centre	Callus edge	Skin adjacent to callus	Normal PMA	Normal 5th met base
Mean difference	0.91	1.05	3.42	1.06	1.44
SD of differences	2.54	3.94	7.20	5.60	4.27
SEM	1.80	2.79	5.09	3.96	3.02
SDC	4.99	7.73	14.12	10.97	8.37

7.5.3. Skin distensibility data

Figure 7.3 shows the raw data at each time point for skin distensibility, Table 7.6 shows the percentage change at each skin site per time point and Table 7.7 shows the percentage change between callus and control sites at each time points. The SDC throughout the trial is shown for each skin site in Table 7.8. There were no significant changes at callus centre or edge site between time points, but there was a significant change at the skin adjacent to callus between baseline and week 6 (7.3% difference; $p = 0.04$). At baseline, skin at the centre of the callus was 26.5% less distensible than skin of the PMA ($p = 0.005$). This remained significantly different throughout the trial and the difference was 20.4% at week 12. The skin at the callus centre was 20.7% different to skin overlying the base of the fifth metatarsal at baseline, but this did not reach significance ($p = 0.081$). The difference increased by week 6, reaching significance (22.9%; $p = 0.019$) and fell slightly by week

12, but remained significant (16.6%; p = 0.041). There were no significant differences between skin of the callus edge and skin adjacent to callus, and the control sites at any time point. The SDC data over the trial ranged from 0.029mm (normal PMA skin) to 0.63mm (skin overlying the callus edge).



	Baseline	Week 6	Week 12
Callus centre	0.81	0.75	0.82
Callus edge	0.99	0.90	0.91
Skin adjacent to callus	1.09	1.01	1.02
Normal PMA	1.10	1.05	1.04
5th met base	1.02	0.97	0.99

Figure 7.3 – Raw distensibility data

Table 7.6 Percentage change between time points at each skin site

	Callus centre	Callus edge	Skin adjacent to callus	Normal PMA	Normal 5th met base
Baseline – Week 6 (p value)	-7.3 (0.089)	-9.2 (0.406)	-7.3 (0.040)	-3.9 (0.576)	-4.6 (0.523)
Baseline – Week 12 (p value)	2.3 (1.0)	-7.8 (0.376)	-6.5 (0.106)	-5.5 (0.133)	-2.7 (0.697)
Week 6 – Week 12 (p value)	10.3 (0.055)	1.6 (1.0)	0.9 (1.0)	-1.61 (0.518)	2.0 (1.0)

Table 7.7 Percentage change between callus and control sites at each time point

	Callus and PMA (p value)	Callus and 5th met (p value)	Edge and PMA (p value)	Edge and 5th met (p value)	Adjacent and PMA (p value)	Adjacent and 5th met (p value)
Baseline	-26.5 (0.005)	-20.7 (0.081)	-10.0 (0.298)	-2.9 (1.0)	-0.8 (1.0)	7.1 (1.0)
Week 6	-29.0 (0.006)	-22.9 (0.019)	-16.0 (0.465)	-7.6 (1.0)	-4.25 (1.0)	4.0 (1.0)
Week 12	-20.4 (0.026)	-16.6 (0.041)	-12.2 (0.984)	-8.0 (1.0)	-1.8 (1.0)	2.9 (1.0)

Table 7.8 Smallest detectable change for distensibility data

	Callus centre	Callus edge	Skin adjacent to callus	Normal PMA	Normal 5th met base
Mean difference	0.12	0.17	0.12	0.07	0.08
SD of differences	0.19	0.32	0.16	0.15	0.16
SEM	0.13	0.23	0.11	0.11	0.11
SDC	0.37	0.63	0.31	0.29	0.31

7.5.4. Skin homogeneity data

Figure 7.4 shows the raw data at each time point for skin hydration, Table 7.9 shows the percentage change at each skin site per time point and Table 7.10 shows the percentage change between callus and control sites at each time points. The SDC throughout the trial is shown for each skin site in Table 7.11. Statistically significant increases in homogeneity values were apparent between baseline and week 6 for all skin sites, but then plateaued between weeks 6 and 12. Significant differences were also apparent between baseline and week 12 for callus centre (5.7%; p = 0.000) and callus edge (5.2%; p = 0.000). There was no significant change between week 6 and week 12 for any callus site. At baseline, skin of the callus centre was significantly different to skin overlying the PMA and fifth metatarsal base (8.9% and 4.9% respectively; p = 0.000 and 0.021 respectively). These differences remained significant throughout the trial, with differences of 6.4% and 5.6% (p = 0.000 and 0.007 respectively) recorded for these sites respectively at week 12. Skin overlying the callus edge was significantly different to skin overlying the PMA at baseline (6.1%; p = 0.003), and remained significant throughout the study period, ending on 4.1% difference at week 12 (p = 0.003). Skin of the callus edge was not significantly different to skin overlying the fifth metatarsal base at baseline (2%), however at week six, the difference did reach significance (3.5%; p = 0.039). By week 12, the difference was not significant, falling to 2.2%. The SDC data over the trial ranged from 0.16au (normal PMA skin) to 0.55au (callus centre skin) over the study period.

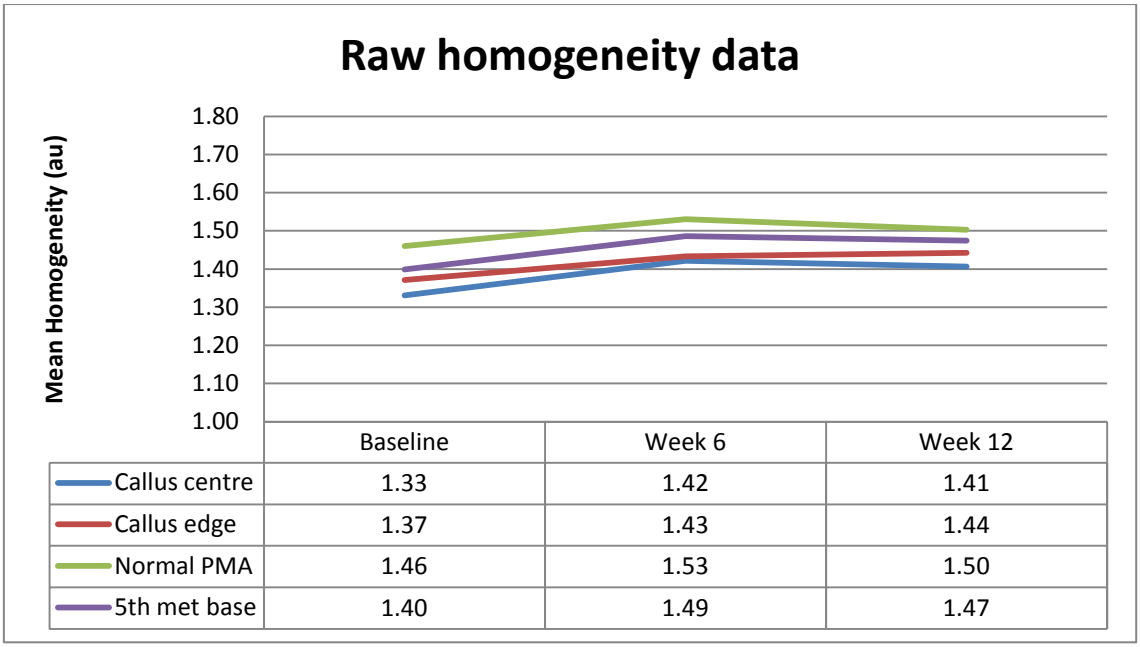


Figure 7.4 – Raw homogeneity data

Table 7.9 Percentage change between time points at each skin site

	Callus centre	Callus edge	Normal PMA	Normal 5th met base
Baseline – Week 6 (p value)	6.8 (0.001)	4.6 (0.028)	4.8 (0.002)	6.2 (0.011)
Baseline – Week 12 (p value)	5.7 (0.000)	5.2 (0.000)	2.9 (0.056)	5.4 (0.002)
Week 6 – Week 12 (p value)	-1.0 (1.0)	0.6 (1.0)	-1.8 (0.057)	-0.8 (1.0)

Table 7.10 Percentage change between callus and control sites at each time point

	Callus and PMA (p value)	Callus and 5th met (p value)	Edge and PMA (p value)	Edge and 5th met (p value)
Baseline	-9.0 (0.000)	-4.9 (0.021)	-6.1 (0.003)	-2.0 (1.0)
Week 6	-7.2 (0.002)	-4.4 (0.010)	-6.4 (0.001)	-3.5 (0.039)
Week 12	-6.4 (0.000)	-4.6 (0.007)	-4.1 (0.003)	-2.2 (0.566)

Table 7.11 Smallest detectable change for homogeneity data

	Callus centre	Callus edge	Normal PMA	Normal 5th met base
Mean difference	0.03	0.07	0.06	0.08
SD of differences	0.28	0.09	0.08	0.12
SEM	0.20	0.07	0.06	0.09
SDC	0.55	0.18	0.16	0.24

7.5.5. Skin variance data

Figure 7.5 shows the raw data at each time point for skin hydration, Table 7.12 shows the percentage change at each skin site per time point and Table 7.13 shows the percentage change between callus and control sites at each time points. The SDC throughout the trial

is shown for each skin site in Table 7.14. The variance values decreased from baseline to week 6 (changes at each skin site were significant except at the callus edge), but plateaued between weeks 6 and 12. At baseline, skin of the callus centre was significantly more variable to skin overlying the PMA and fifth metatarsal base (38.9%; p = 0.001). These differences remained significant throughout the trial, with a difference of 33.4% (p = 0.000) recorded at the end of the trial. The callus centre was not significantly different to the skin overlying the base of the fifth metatarsal (15.4%; p = 0.066), but this difference increased slightly, becoming significant at week 6 (22.1%; p = 0.004) and remaining significantly different at week 12 (19.4%; p = 0.017). Skin overlying the callus edge was significantly different to skin overlying the PMA at baseline (24.0%; p = 0.004). This difference remained significant throughout the trial, ending on 21.4% difference (p = 0.002) at week 12. The skin of the callus edge was not significantly different to skin overlying the fifth metatarsal base at any point during the study. The SDC data over the trial ranged from 0.16au (normal PMA) to 3.47au (callus edge) over the study period.

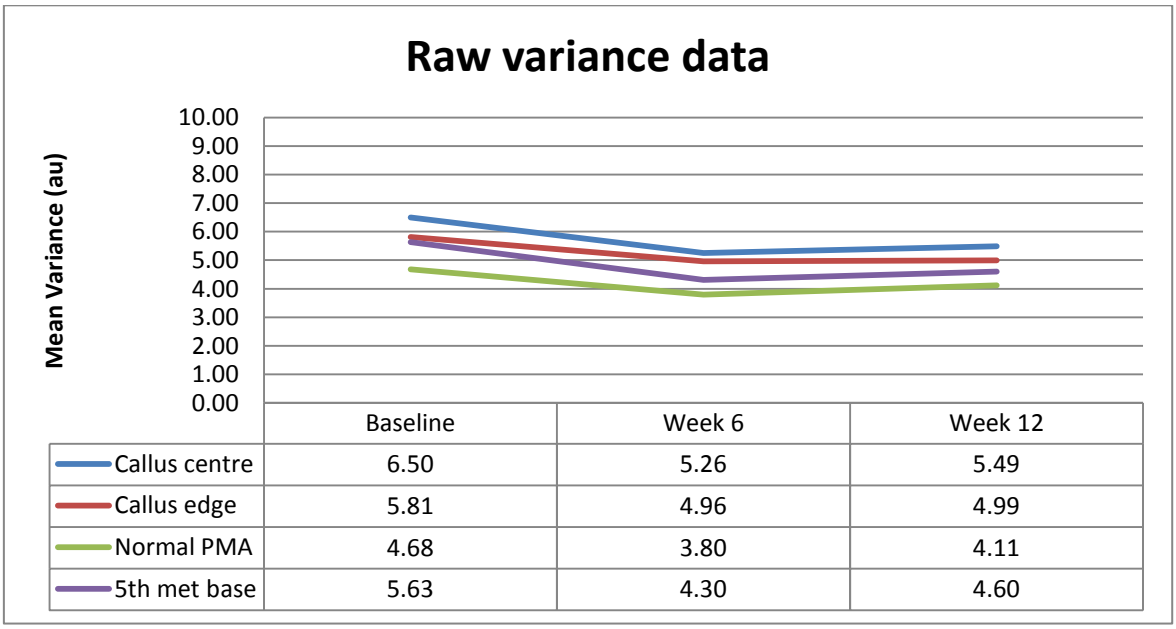


Figure 7.5 – Raw variance data

Table 7.12 Percentage change between time points at each skin site

	Callus centre	Callus edge	Normal PMA	Normal 5th met base
Baseline – Week 6 (p value)	-19.08 (0.001)	-14.51 (0.099)	-18.89 (0.001)	-23.51 (0.002)
Baseline – Week 12 (p value)	-15.47 (0.002)	-14.0 (0.005)	-12.11 (0.031)	-18.27 (0.001)
Week 6 – Week 12 (p value)	4.46 (1.0)	0.6 (1.0)	8.36 (0.065)	6.84 (1.0)

Table 7.13 Percentage change between callus and control sites at each time point

	Callus and PMA (p value)	Callus and 5th met (p value)	Edge and PMA (p value)	Edge and 5th met (p value)
Baseline	38.84 (0.000)	15.43 (0.066)	24.03 (0.004)	3.2 (1.0)
Week 6	38.41 (0.001)	22.1 (0.004)	30.74 (0.001)	15.33 (0.063)
Week 12	33.43 (0.000)	19.38 (0.017)	21.37 (0.002)	8.6 (0.762)

Table 7.14 Smallest detectable change for variance data

	Callus centre	Callus edge	Normal PMA	Normal 5th met base
Mean difference	1.38	-0.07	-0.06	-0.08
SD of differences	1.77	0.09	0.08	0.12
SEM	1.25	0.07	0.06	0.09
SDC	3.47	0.18	0.16	0.24

7.6 Discussion

The data from this study suggests over the population of this study (n = 15) that pressure reduction of the scale achieved by the insoles, and over a 12 week period, does not lead to a change in callused skin properties that is different than changes in normal plantar skin sites. If it is assumed that callus and loading are related, there are several possible reasons why the data might show this. Firstly, it could be that the skin of the foot has become accustomed to the level of load it receives on a daily basis and has become 'programmed' to form callus through biochemical changes including keratins, proteins involved in adhesion and differentiation, as highlighted by Kim et al. (2010) and discussed in Chapter 2. It might be that reducing the load on the skin is not enough to reverse this process once the biochemical triggers have become well established. If this is indeed the case, it can be suggested that callus is indeed a chronic skin lesion.

The above reason however assumes that the level of pressure reduction was sufficient, but in reality it might be the case that a larger reduction in pressure is a requirement to reverse the hyperkeratotic process. Whole foot and regional data from previous studies suggests that peak plantar pressures at sites of calluses is high compared to controls, ranging from 5% to 145% greater peak plantar pressures (Potter and Potter, 2000b, Abouaesha et al., 2001, Pataky et al., 2002, Duffin et al., 2003, Menz et al., 2007). As these studies collected data from both specific regions and the whole foot, it can only give an example of what pressure increases might be occurring specifically as a result of

callus but does highlight the possibility that in order for pressure reduction to cause a callus-reversing effect, the reduction in pressure has to be large. In Chapter 6 (skin loading pilot study), it was shown that increases in peak pressure (ranging from 13.6% to 28%) over a prolonged period did not lead to any changes in plantar skin properties. In this study, the magnitude of the change in peak pressure changes was similar. The mean reduction in pressure at the first appointment was 18%, 15.2% at week 6 and 13.6% at week 12. Whilst these reductions are in line with those in the literature, and thus the orthosis design was appropriate, perhaps a more aggressive approach to pressure reduction might be necessary to drive some form of skin change. In diabetes, there are several methods of offloading with the most notable examples including rocker shoes in prevention and total contact casting as a treatment for plantar ulceration, both of which can substantially reduce pressures by as much as 39% and 70% respectively (Burns and Begg, 2011, Chapman et al., 2013). While the populations in these studies may have had very high peak pressures to begin with, making pressure reduction easier to achieve, it is likely that offloading techniques like these would reduce pressures beneath callused skin. These would allow the greatest chance of skin change, but are largely impractical and perhaps unethical to use in healthy individuals given the aesthetic and mobility issues associated with them. In this study, the pressure reduction data included cases where the actual regional reduction was small. An alternative approach would have been to set a minimum pressure reduction required for inclusion in the study.

One issue affecting plantar pressure and skin in ‘real world’ is activity level. One may assume that the activity levels remain consistent throughout the study, but in reality these could and are likely to vary dramatically. This would not necessarily affect the reduction in pressure caused by the insole, but will increase cumulative loading on the skin to the point that it is actually being loaded more than before enrolment into the study. This could lead to an increased rate of hyperkeratosis and thus have affected skin data, cancelling out any effect of reductions in peak plantar pressure. Monitoring subjects using an activity monitor, or asking them to fill out questionnaires relating to physical activity levels would give information about how much loading the foot might experience (as undertaken in the previous chapter). However, the benefit of this would be minimal as in reality there is no way of controlling how much an individual’s foot is loaded outside the laboratory setting, so it has to be accepted that the foot is never loaded in a homogenous manner over time. It could only assist in explaining the data.

Similarly, while pressure data at each appointment was collected in a stock shoe and in participants' own shoes, there is no way of knowing what pressures are like in each pair of shoes the individual wears without testing them all of them – this is unrealistic due to the amount of time it would take to conduct all of the measurements. There is no control over which shoes individuals wear outside of the study, so again it must be accepted that different shoes with the insoles may distribute pressures differently beneath the feet, and potentially increase them in some cases. This could also have had an impact on skin data.

Another possible issue with this study is that it was conducted over a 12 week period. As this study was to an extent exploratory, there is no gold standard duration of insole wear which will lead to an effect on the skin and it is likely individuals will respond differently due to anatomy, wear time, skin properties and other factors. In this study, 12 weeks was deemed appropriate because it would allow approximately three cycles of skin cell turnover and thus theoretically enough time for some skin change to occur. Certainly, previous studies which have manipulated skin loading conditions have noted marked changes within 35 days (Rubin, 1949, Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998). However, these studies were applying loads to the skin, not reducing them, so it could be that the skin's response to reductions in pressure is a process that takes longer to appear. It would have been interesting had any of the aforementioned authors studied how quickly the skin, having been loaded, recovered. This may have given a more realistic idea of how long to allow for skin changes.

The previous studies which have reported callus regression from insole wear (Colagiuri et al., 1995, Duffin et al., 2003) were undertaken over a period of 12 months which would allow a greater time for any effect of pressure reduction on callused skin to manifest. However, the participants in these studies had diabetes which means that a longer timeframe might be needed due to possible vascular impairment to the lower limb, and as these studies were conducted in hospital diabetes clinics, they would have had access to a large number of potential participants. In this study, 12 weeks was deemed appropriate due to the fact that keeping participants in a longitudinal study can be difficult, and given the low volume of potential participants, it was deemed important to maximise the chances of keeping them in the study. There are also possible pitfalls with extended insole use which are only just starting to emerge, such as an effect on soft tissue thickness in the foot (Sweeney et al., 2014), plantar somatosensory sensations (Vie et al., 2015), and possible changes in foot shape and function (D'Août et al., 2009).

The previous insole studies which reported callus reduction (Colagiuri et al., 1995, Duffin et al., 2003) did not report pressure data at follow-up time points, nor did they measure skin properties. While it can be suggested that pressure reduction may have been a factor in reducing callus, other factors may also have contributed to their findings. This study found that hydration and roughness over all the skin measurement sites improved slightly over the duration of the study. This is unlikely to be a product of pressure reduction because pressure reduction did not occur at each skin site. The most common areas of pressure increase included the midfoot and surrounding metatarsal heads. It is more likely that the skin improved as a result of either increasing temperature of the environment (the study was conducted between winter and summer, 2015) which caused an increase in heat inside the shoe leading to greater sweat production – studies have shown that skin hydration and mechanincal properties at non-plantar sites can be affected by seasonal variation (Cravello and Ferri, 2008); or a result of the material used for the insole itself causing an increase in heat and causing some degree of skin occlusion which has been shown to increase hydration (Zhai and Maibach, 2002). Some participants in this study mentioned that their feet felt ‘warmer’ and ‘clammier’ with the insole inside the shoe and this is likely to be due to the material. The materials used in this study were EVA and rubber. While there is literature investigating insole materials (reviewed earlier), they look at pressure reduction properties and not the effect of the materials on skin. This might be an interesting area of future study – general skin improvement using insoles could have clinical significance and might be helpful in individuals where pressure reduction is not a necessity.

One hypothetical reason why the callused skin did not regress significantly could be a result of the pressure reduction itself. Perhaps the offload of the callus was such that the most superficial layers of dead keratin, as is found in thick plaques in callused skin, were retained. Walking could be a mechanism for shedding the superficial keratin sheets through rubbing at the foot-shoe interface, but since the callused skin did not regress, it might mean that pressure reduction decreased the amount of skin shedding that may have otherwise have occurred had the pressure remained the same. There is no way of studying this other than through biopsies taken before and after the study and histologically analysing the superficial keratin layers of the stratum corneum. However, taking biopsies would be painful and thus may affect gait (as callus occurs at weight bearing areas). It would thus be unethical to do so on any more than one occasion and would likely severely impact upon recruitment.

It might be that the severity of the calluses used in this study was such that pressure reduction was unable to reverse the process of hyperkeratosis, at least in this timeframe. Due to the relatively low volume of recruitment, individuals with calluses of all grades were enrolled into the study. If recruitment was more successful, it might have been possible to enrol only individuals with relatively light calluses which may have shown a response within the timeframe, compared to pressure reduction than thicker calluses which may require longer.

A potential area of weakness with the study methodology is that detailed activity data was only collected at baseline, as part of a questionnaire collecting participant background information. It may have been useful to detail whether or not activity levels increased over the duration of the study. If pressure reductions were achieved but the total accumulative load experienced under the region of interest increased, this could counteract the potential benefit of the pressure reduction causing increased total pressure on the skin. While it is unlikely that each participant would have dramatically increased the volume of exercise taken over the study period, it cannot be discounted as a potential factor affecting the results, particularly as weather conditions improved throughout the study (which took place from winter to summer). This methodological error is one that must be taken into account in future research studies where accumulative loads could affect data.

Other potential areas affecting the data lie with study recruitment and participant compliance. This study formed part of a larger project looking at insole pressure reduction properties and durability over time and cases selected for analysis in this study were drawn from a larger collection of data. Due to the low recruitment volume, the researcher was forced to be less selective in cases included in the final data analysis in terms of pressure reduction and compliance. While the mean pressure reduction and compliance was relatively good across the dataset, in a few cases they were small. Also, it must be stated that in a study like this, compliance stated by the participant does not necessarily reflect reality. There is always the possibility that participants can over-estimate the amount of time wearing the insoles, or due to fear of affecting the project, give false answers. While participants were encouraged to be honest in their responses and were reassured that there would be no repercussions regarding poor compliance, there is still the possibility of feedback lacking accuracy. There is no way this can be avoided other than participant reassurance and regular contact with them, both of which were done in this study.

The final area which warrants discussion in this chapter is the normal variation of the skin between data collection points. As found in Chapters 3 and 6, the SDC data

suggested that in order for a change in properties to be considered ‘real’, it would have to be relatively large. If small skin changes in the callused regions did occur as a result of pressure reduction, there is a risk that these changes may have been less easy to identify given that normal variation in skin properties occurred over time. However, to minimise this effect, numerous skin sites were chosen with two control sites. Variation could affect the measurements longitudinally, but when performing comparisons between skin sites at each time point, any skin effect caused by the pressure reduction would likely show in the data as a change in callus skin properties in relation to the other skin sites. A very obvious change could not be explained by natural variation, and this type of skin testing has proved successful in studies on callus and heel fissure treatments, studies that are currently awaiting publication (Hashmi et al., 2015). This study showed that increases in hydration of up to 152%, and distensibility of up to 100% were possible in callused skin sites, before and after podiatry treatment. This magnitude of skin change is far larger than can be attributed to normal skin variation, and these changes were obvious in relation to the control site. Likewise, other studies on pedal skin, mentioned in previous chapters, have noted large increases in hydration post-treatment. Increases in hydration of up to 57.3% were reported by Garrigue et al. (2011) after 28 days, and 20.6% increase in hydration after 14 days were reported by Papanas et al. (2011). It is clear that pronounced skin changes would clearly show in the datasets, and there was no evidence of any callus skin reduction occurring in this study.

While the results of this study provide only a starting point from which further research can be developed, it might be worth noting that using insoles as a treatment modality for existing calluses would likely be improved when used in conjunction with other treatments aimed to remove the actual skin, such as debridement. It is possible that debridement of the lesion, which may provide some degree of pressure relief (Pitei et al., 1999, Pataky et al., 2002), followed by insole wear might have resulted in a slowing of callus regrowth by removing the mechanical stimulus and a later study could investigate this. It might be that using insoles as a preventative measure for plantar callus regrowth in conjunction with better established primary treatments might be more effective than using insoles alone.

7.7 Conclusion

As with previous sections of this thesis, this chapter demonstrates the author's ability to design, implement and evaluate research in autonomously. Like Chapter 6, reasons for a nil-response of the skin to changes in loading conditions are evaluated and discussed, and learnings which can be applied to further research have been made explicit. To summarise, the results from this chapter suggest that pressure reduction may not be enough to reverse the skin changes associated with callus. The results should be treated with caution as there are several factors which may have influenced them. The pressure reduction magnitude and/ or the duration of insole wear may have been insufficient; the possibility that pressure reduction may have slowed desquamation; and the callus grades used in the study are all factors which should be taken into account. These results may be useful in clinical practice when insoles are considered for callus treatment. Pressure reduction may have more benefit as a preventative measure as opposed to a primary treatment so combining insole prescription with debridement could be considered until more evidence is available supporting pressure reduction as a means of callus reduction.

Chapter 8: Overall discussion and conclusion

8.1 Summary of findings

There were two main aims to this PhD: (1) to investigate the biophysical properties of callused skin; and (2) to quantify the skin's response to changes in external loads specifically, whether callus results from increased pressure and whether callus regresses from pressure relief. To accomplish these aims, six investigations were necessary: (1) a review of the literature surrounding callus and skin measurement; (2) an investigation into reliability of skin measurement devices; (3) an investigation into the biophysical characteristics of normal and callused plantar skin; (4) development of a novel device designed to load plantar skin; (5) the effect of loading normal plantar skin in individuals prone to plantar callus; and (6) the effect of pressure reduction beneath plantar callus.

The literature review found that callus is a common clinical and foot health problem. There is anecdotal evidence that one of the causative factors is increased plantar vertical pressure and shear pressure acting on the skin, and there is a small body of literature which has focussed on non-pedal (Rubin, 1949, Goldblum and Piper, 1954) and animal (Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998, Sanders et al., 2002) skin sites which have shown how the skin adapts to external loads. These studies show that skin thickening can occur in a short space of time (within 35 days) given the right conditions. However, these studies are cited in the callus literature, but cannot be applied to the foot because the skin sites used are completely different anatomically and functionally to plantar skin, and are not designed for bearing loads. Plantar pressure studies have shown that peak pressures tend to be significantly higher beneath callused skin sites compared with control subjects (Potter and Potter, 2000b, Pataky et al., 2002, Duffin et al., 2003). However, conflicting evidence exists about whether or not the plantar pressure is the precursor to, or a result of callused skin (Pitei et al., 1999, Potter and Potter, 2000b, Woodburn et al., 2000, Pataky et al., 2002). Individuals with callus have been reported to also present with factors such as limited joint mobility, deformities and decreased tissue thickness which may result in increased pressures (Bevans and Bowker, 1999, Abouaesha et al., 2001, Menz et al., 2007), but no studies have attempted to investigate specifically whether a link between plantar pressure and callus exists, therefore this thesis aimed to address this. In addition to identifying the gap in the literature between callus and pressures, the review also identified that there are a range of potential measurement modalities that could be used in plantar skin testing, some of which could be beneficial in

callus profiling. Several instruments were highlighted which could be of potential use in the reliability study and the skin characterisation study.

The reliability study presented in Chapter 3 investigated several devices highlighted in the literature review which could be beneficial in testing callus. The Corneometer® (hydration), Cutometer® (distensibility – a surrogate measure of stiffness) and Visioscan® (roughness) were chosen for investigation. Eight volunteers with plantar forefoot callus were tested over two days by two investigators. Skin tests were undertaken on callused and non-callused sites. It was found that generally the inter-rater and inter-day reliability and agreement across each of the instruments was good but there were some discrepancies which were discussed. The reliability literature available for these devices is scant, but those studies which do exist generally agree with the results in this study. The ICC values for the Cutometer® on callused skin were similar to the values reported by Fong et al. (1997) and Draaijers et al. (2004) on scar tissue for inter and intra-rater reliability tests. Visioscan® data showed that the values reported in this study were slightly lower than those reported by Kottner et al. (2013) but these can be attributed to differences in methodology. As the instruments used in this study were able to identify differences between each skin site, these methods of skin profiling were deemed suitable for use on plantar skin, so long as control sites are also used as a reference, particularly when collecting longitudinal data. This study adds to both the literature in biophysical measurements and in foot research by identifying devices that can reliably measure plantar skin – this has only previously been done with one device (Hashmi and Malone-Lee, 2007). This will allow clinicians and researchers to measure plantar skin with these devices, with the knowledge that they can provide reliable measurements.

The skin characterisation study presented in Chapter 4 investigated the biophysical profile of callused and non-callused plantar skin. Forty six individuals with 61 calluses were recruited onto the study and data was collected at one appointment by one investigator. Measurements included hydration, stiffness and roughness parameters. The results showed that callus is significantly less hydrated, less distensible (more stiff) and less homogenous and more variable in appearance (rougher) than skin adjacent to the lesions and normal plantar skin of the PMA and base of the fifth metatarsal. There were also some differences noted between control sites. The parameters of hydration and distensibility were moderately and significantly correlated on callused sites.

This study adds to the general literature of skin biophysical characterisation, of which there are only a few studies (Ryu et al., 2008, Krueger et al., 2011, Luebberding et

al., 2014). While they report differences between regions, only two tested these for significance (Ryu et al., 2008), the others focussing more on age related changes. One very recent study by Nam et al. (2015) adds to this literature. The authors investigated seasonal variations at different skin sites. Using the Corneometer®, they identified that skin of 'crow's feet' was significantly more hydrated than skin of forearm and cheek ($p < 0.05$) throughout the trial. Using the Cutometer®, they observed significant differences in mechanical parameters between the forearm and the other skin sites ($p < 0.05$) throughout the study period. Skin scaliness (using Visioscan®) was significantly greater at the forehead compared to other skin sites ($p < 0.05$).

At the time of writing, no studies have attempted to characterise plantar skin using a range of parameters, and the study in Chapter 4 adds to this gap in the literature. These results in this study can be linked to the findings of previous immunohistochemistry papers focussing on callus. The increase in stiffness may be due to an increase in the expression of keratins and proteins involved in cohesion and differentiation which would increase the rate of keratinisation process (Kim et al., 2010) leading to a build-up of hard skin. The increased rate of proliferation may result in poor differentiation (Thomas et al., 1985) leading to intercellular lipids becoming fragile (Harding et al., 2003), impairing the skin barrier and leading to a reduction in hydration (Wickett and Visscher, 2006, Baroni et al., 2012). The rougher appearance of callused skin may be a result of altered desquamation in the stratum corneum, reported by Thomas et al. (1985). The results of this chapter make explicit the biophysical differences between normal and callused skin which can be used as a benchmark for further research studies which might look to 'change' callused skin properties through treatment.

With Chapter 4 providing data on differences between callused and normal skin sites, further work could then be done to establish whether a relationship between pressure and callus exists. Previous work investigating skin adaptation to external loads has work has been undertaken in humans (Rubin, 1949, Goldblum and Piper, 1954, Pinkus, 1952, Brophy and Lobitz, 1959) and animals (Mackenzie, 1974a, Mackenzie, 1974b, Goldstein and Sanders, 1998, Sanders et al., 2002) but never for investigating plantar skin properties. Therefore, Chapter 5 presents work undertaken to develop a device to apply loads to plantar skin in a safe and repeatable manner for use in a later skin loading study. The device in question needed to be powerful enough to deliver large forces to the foot in excess of what would normally be experienced during gait. The device built comprised of a horizontal and vertical actuator to allow device movement in these directions, and a

horizontal and vertical load cell to measure the forces in these directions. Tests using static weights confirmed that the load cells were accurate and consistent but when a horizontal force is added, there are some small discrepancies in vertical readings. These are discussed in the chapter but were not deemed a problem given that the work for this PhD was to focus on the delivery of vertical pressure. A computer was built for the device with a number of loading cycles built in to instruct the device as to which movements were needed. For the purposes of this thesis, the 'compression' programme was deemed the most important and was therefore tested on plywood and medium density EVA. The results showed a linear relationship between air pressure delivered to the actuator and the resulting force applied. Furthermore, the device was capable of producing very repeatable loads during each compression cycle and was therefore deemed suitable for use on plantar skin.

Chapter 6 focussed on using the loading device on plantar skin in a small pilot test over a minimum period of six weeks. Preliminary testing aimed to fine tune protocol considerations including foot positioning and force fine tuning on plantar skin which showed that compression forces could be applied within 10% of the target load. Five individuals (one of whom dropped out) with plantar callus were recruited onto the study. The skin loading site in all cases was a callus-free area either at the second or fourth metatarsal head. To establish a target load, in-shoe pressure measurements were obtained using the Pedar X measurement system and the target load was calculated. During the study activity monitoring data using ActivPAL™ and barefoot pressures measured using Novel EMed® were also collected for calculating a loading profile for each participant to conceptualise the load applied with the device. Each individual attended three appointments per week for a minimum of six weeks and the target load was set at 2-3 times normal pressure for the loading site but adjusted based on participant feedback for comfort. Skin data as described previously was collected at weekly intervals, then at one appointment four weeks after the skin loading was concluded. The data showed that no real skin change occurred as a result of the skin loading. Since the percentage weekly load applied to the participants' feet using the device was relatively large, it suggests that a longer duration of study or the introduction of shear force in subjects recruited from narrower criteria might be necessary to elicit a skin change toward that of callus. Safety was also discussed and suggestions such as recruiting individuals who exercise regularly, who may have plantar skin accustomed to larger loads, or beginning with a relatively small load and slowly increasing this over a period of weeks to allow the foot to become

accustomed to it, were made. Other factors such as the day-to-day variability of the skin's properties and the limitations measuring longitudinal changes in skin were also discussed and it was concluded that while variation of the skin could arguably make changes at the load site difficult to see, the use of control sites essentially minimised the risk of this affecting the results by providing data for comparison.

The final study in this thesis, Chapter 7, focussed on a 'real world' scenario looking at pressure reduction beneath callused skin using insoles. Each participant was given a pair of insoles to wear for a period of 12 weeks. 15 individuals with pressure reductions at 26 callused sites were included in the final analysis. Skin data using the same devices used in the other chapters were collected at baseline, six weeks and 12 weeks into the study. Plantar pressures were monitored in participants' own shoes and stock shoes using Novel Pedar X at these same appointments. The results suggested that plantar pressure reduction was not enough to cause a callus skin change. The reasons for this are multifactorial. Potentially, the pressure reduction was inadequate. At week 1, mean peak pressure reduction was 18%, at week 6, 15.2%, and at week 12, 13.6%. While variable, probably as a result of different footwear and insoles 'wearing in', these reductions are relatively high. It may be that the duration of insole wear was insufficient, the duration of the study was inadequate or the magnitude of pressure reduction itself was not high enough to trigger a change in callus properties. Normal variations in biophysical properties were observed across skin sites, but it was concluded that the use of control sites essentially minimised any negative effect on identifying callused skin changes. Other areas such as reduced desquamation as a result of the pressure reduction, the nature of the calluses themselves, and activity levels were also highlighted potential factors affecting the results.

Previous studies (Colagiuri et al., 1995, Duffin et al., 2003) have reported reductions in calluses as a result of insole wear, but the study presented in this chapter takes this further by specifically testing the skin to determine whether, over a 12 week period, pressure reduction leads to a change in callus skin properties. This chapter also reports pressures at each data time point which was not done in either of the previous papers but is important in ensuring that the intervention is working as intended.

8.2 Implications for clinicians and researchers

The research conducted for this PhD thesis has created knowledge of plantar callus from both a physiological and biomechanical standpoint. No previous work has been conducted that has both validated and then used a range of instruments to measure plantar skin biophysical properties. This is a small but necessary breakthrough in plantar skin research. While it has been postulated that callus and normal skin differ in terms of hydration, stiffness and roughness, measuring skin in-vivo without having to surgically remove it is beneficial in both single data collection appointments and particularly for longitudinal studies. It has confirmed the major differences between normal and callused skin. These methods of skin measurement could be far reaching in clinical research and practice, but despite the reliability of these methods, the normal variation of the skin can still affect the data, therefore the use of control sites which show consistent differences from the treatment site is essential. The reliability study from Chapter 3 can be used to inform other authors wishing to undertake plantar skin research as to the value of potential devices. As in other studies highlighted in the literature review on non-plantar skin, these devices could be used to characterise a whole range of skin disorders which may be beneficial in research and practice. For instance, skin measurement could be used to evaluate treatments such as therapeutic insoles for skin problems, as has been conducted in the final study of this thesis, or other modalities for different conditions. Differences between skin sites at baseline and after treatment could give an indication as to whether the treatment was successful, or if not, why it was not successful. Some of the work undertaken for this PhD was part of a larger project leading to papers published in instrument reliability (Hashmi et al., 2015b), skin characterisation (Hashmi et al., 2015a), and the efficacy of clinical and commercially available treatments for callus and heel fissures (Hashmi et al., 2015, papers under review) and the devices were able to successfully detect differences before and after treatment leading to more information about the value of the treatments. While this was in a research setting, these devices could theoretically be used in clinical practice also, in order to track the progress of podiatric treatments for xerotic skin disorders.

The skin loading study in this PhD (Chapter 6) is the first to try and develop a callus-like skin response mechanically in the laboratory setting. It is also the first to contextualise load applied in the laboratory as a percentage of weekly load experienced under the foot, calculated using activity monitoring data. While unsuccessful in defining a

link between callus and pressure, it provides readers with more information about callus, and potentially how complex a condition it is. The final study showed no skin change in callus as a result of pressure reduction. This study was the first to use skin measurements and pressure reduction measures to track callused skin during an insole trial. While several factors discussed previously might have affected the results, the study highlights the possibility that simply removing a mechanical stimulus is not enough to reverse the callused skin response. These two biomechanical studies could potentially benefit clinicians by challenging the 'traditional' beliefs of callus and pressure. While there is almost certainly a link between the two to some extent, it may be naïve to consider that pressure alone is enough to cause callus, and conversely, removing the mechanical stimulus and thus offloading the callus, will be enough to reverse the hyperkeratotic process. Perhaps the biochemical process is somewhat irreversible. However, there may potentially be a benefit if pressure reduction is used in conjunction with a primary treatment such as scalpel debridement. This may be an interesting avenue of research if one were to compare callus regrowth in debridement alone and debridement plus pressure reduction.

8.3 Recommendations for further research

The reliability study presented in Chapter 3 found that while reliability was good across the instruments, some variation was evident, particularly between days. Inter-rater and inter-day reliability was appropriate to test due to the intended use of the measurement devices. However measuring intra-rater reliability, involving separate measures at the same skin sites for one investigator during a single testing session, might have shown improved reliability scores, and would also have shed further light on the reliability and agreement measures of the devices. Comparing inter-day and intra-rater reliability, for example, would allow one to assess how much more variation might occur between days than within a single session, and in doing so, be able to better explain data from future studies.

The skin biophysical characterisation study presented in Chapter 4 showed significant differences between callus and non-callus skin sites in hydration, distensibility, homogeneity, and variance parameters. The main limitation associated with this study was the fact that no method was employed to visualise a cross-sectional area of the skin, which could have then been linked to biophysical properties. Researchers wishing to add to this

work may wish to seek a method of visualising a cross-sectional area of skin, whether this is by biopsy or by imaging, as it would be beneficial in linking biophysical properties to actual measures of anatomy. Similarly, cross-sectional anatomical measures would be beneficial in other types of study, such as the skin loading, and pressure reduction methods employed in this thesis. However, as previously stated, biopsies would cause discomfort and would thus affect participant recruitment so employing imaging techniques, such as OCT, would be the most beneficial.

The second limitation associated with this study was that 80% of the sample was female, which could have affected data, given that male and female skin may differ in properties (Luebberding et al., 2013, Luebberding et al., 2014). Researchers attempting similar work may want to address this by ensuring participants in the study are gender-matched. This would allow researchers a more accurate profile of normal and callused skin, and would also allow comparisons in biophysical plantar skin properties between genders, which might lead to other interesting skin studies.

Future researchers may also wish to include a control group of age- and gender-matched individuals without callus for comparison with the callus group. Comparison of general skin quality may be beneficial and allow inferences about whether individuals prone to callus display certain skin properties compared to those without. This could benefit future research and clinical practice by identifying individuals who might be at risk of callus development, based on their skin properties.

The study which assessed the skin's response to vertical pressure application (Chapter 6) found no effect of increased pressure on skin properties. Several factors which could have affected the data include dose of load (both magnitude and duration over which it was applied in a single session), number of sessions per week, and the direction in which the load was applied. It is unlikely that increasing the magnitude of pressure applied to the foot would be possible, and might be unsafe, and it is equally unlikely that participants would commit to more than three load application sessions per week. The most effective study design would be to change the direction of pressure applied to the foot, instead of applying vertical pressure, applying it horizontally and test the skin's response to shear pressure. Non-plantar human and animal skin studies have shown a response to shear pressure (Rubin, 1949, Mackenzie, 1974b, Mackenzie, 1974a) so this is a pertinent area to investigate.

Several device modifications would be needed (Appendix 1) if using the existing device, to better control the pressures applied and improve the horizontal movement of the

device. Researchers may wish to test the effect of shear with the contact pad rubbing over the skin (often referred to as 'friction') with a low vertical pressure, and with a higher vertical pressure where there is more resistance preventing the contact pad sliding over the skin. A series of case studies looking at the effects of these in different participants who have been carefully selected (such as over 40 years old, female and with some evidence of plantar callus) would allow comparison between these two shear components. A study such as this would add to the research in this thesis and might show different results, and improve understanding of callus.

The study presented in Chapter 7, which looked at the effect of reducing pressure beneath plantar calluses, found no skin response over a 12 week period. The factors which might explain this nil-response are the duration of the study and magnitude of pressure reduction. These are areas which can be addressed in further studies. In addition to increasing the duration of the study which might enable a greater chance of detecting skin change, methods of ensuring pressure reduction is substantial would also be beneficial. Researchers may wish to further reduce pressure, either by changing the geometry of the insole, such as increasing the arch height, or by changing the material to one which has been shown to be effective in pressure reduction, such as poron with plastazote (Tong and Ng, 2010), which has also been shown to retain its pressure reduction qualities after increased wear (Rogers et al., 2006). Also, using a larger sample size will enable researchers to be more selective in data that they analyse. It would be beneficial in this type of study to set a threshold for pressure reduction, e.g. a minimum of 10% pressure reduction beneath callused skin, which would mean that all data analysed are from calluses which have experienced a substantial reduction in pressure compared to a shoe-only condition.

One other area which is worth considering is the use of a control group. The pressure reduction study in this thesis identified that while no specific skin response to callus was detected, the overall quality of the skin improved slightly. This could have been due to seasonal variation or the effect of the insole on the skin, causing some degree of occlusion. Using a control group, a group of individuals with callus who receive no pressure reduction measures, would allow researchers to test the effects of the pressure reduction, by comparing callused skin properties between groups; and the effects of the insole itself on the skin, by comparing all skin sites between groups. In addition, if all skin sites improved between the insole group and the control group, this might suggest seasonal

variation is a key factor in skin change. This type of study would thus have direct clinical consequences.

8.4 Conclusion to Ph. D. Thesis

This thesis presents several findings which include the biophysical properties of normal and callused skin, the nil-response of normal plantar skin to vertical pressure application, and the nil-response of callused skin to pressure reduction measures. Some of these findings may challenge the supposed link between pressure and callus, but cannot be generalised to the whole population – further research is needed to better understand the role that loading has in callus production. While none of the research conducted for this thesis has aimed to change practice – an area which may be hard to influence due to routines, cognition, attitudes and motivation (Grol and Grimshaw, 2003) – some of the research presented here might be enough to allow clinicians to think about the way they approach plantar callus and possibly influence existing knowledge of their pathophysiology. The work conducted for this PhD thesis has provided a good starting platform from which more research into the physiology and biomechanics of plantar callus can now be conducted.

The learnings from conducting this PhD have been substantial. Chapter 2 greatly improved knowledge and understanding of plantar skin physiology and biomechanics, and allowed an understanding of the main research problems surrounding plantar callus. Chapter 3 allowed the author to gain experience in research design, implementation and evaluation, and specifically biophysical plantar skin measurement methodology and statistical techniques. Understanding device limitations was critical in evaluating future data and making informed decisions. Chapter 4 allowed the author to further the body of knowledge in plantar skin physiology by conducting original research using the measurement techniques and learnings from Chapter 3. Chapters 5 and 6 involved design, implementation and evaluation of an experimental research project using new techniques, looking at the effect of increasing peak pressure under the plantar metatarsal area. It allowed the author to make informed decisions on methodology and a thorough evaluation of results using previous learnings. Chapter 7 demonstrated the author's ability to conduct a research project investigating plantar skin biophysical properties and biomechanics, this time from a different perspective – looking at skin properties after reducing peak pressure beneath callused skin. Again, the author was required to thoroughly analyse and evaluate

the results to explain a nil-response. This PhD has allowed the author to fulfil the criteria outlined in the Descriptor For A Higher Education Qualification At Level 8: Doctoral Degree (QAA, 2008, p.23) and has contributed to many transferable skills which can be used to fulfil a career in research and other academic professions.

Appendix 1: Considerations for loading device improvements for further research projects using shear pressures

Improve shear movement

Preliminary tests with the foot loading device showed that shear movement was very difficult to achieve, due to the fact that a dragging effect occurred. This occurred even on smooth surfaces. Several possible areas to consider include structure and function. The device components are made of aluminium. Replacing the components with larger, more robust components made of a stronger material, such as titanium, might help to reduce device compliance. This would also allow more accurate compression load cell measurements during the movement as device compliance would not have as much of an effect on the load cell.

If dragging still occurs, the researcher might wish to consider the precision of device positioning relative to the foot. The horizontal movement might be affected by indentation of the device contact pad being too deep in the material. A system which enables fine-tuning of the position of the device in the vertical direction and also takes account of the material's elasticity could be considered. An adaptation which allows measurement of the indentation depth would allow optimal control of device positioning and may reduce any dragging effect.

If these adaptations do not improve the quality of the shear movement, this may indicate that the actuator is not powerful enough to deliver shear at the intended compression. If reducing compression to minimum levels (and thus measuring rubbing effects) is not desirable, the researcher might wish to consider fitting a more powerful shear actuator to the device. Any work done on human feet after such an adaptation should be approached with care to avoid injury by using too high a shear pressure for the foot to tolerate.

Improvements in the accuracy of pressure delivery

In its current state, the air pressure to the device's actuators is controlled manually using pressure regulator gauges. To improve the design to allow more precise control of pressure delivery, the researcher might wish to employ a closed feedback loop system. Output signals from the load cells could be delivered to a conditioning unit. The conditioning unit would in turn send signals to an electronic pressure control system which would

automatically adjust the pressure, based on feedback from the load cells, to ensure that the correct pressure to the foot is being delivered. This method would allow excellent control of the pressures delivered, but might be expensive. An alternative method of fine tuning pressure delivery would be to use a pressure transducer which converts the air pressure reading into a digital display, rather than using a mechanical pressure regulator gauge. This would allow for more accurate manual adjustment of pressure settings.

Improvements in controlling loading cycles

The device is controlled by a box with a series of pre-programmed options for loading cycles. For greater usability, further researchers might wish to employ a system whereby they could build their own loading sequences using a simple computer language.

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