Non-invasive methods of quantifying the composition of the plantar epidermis: The interpretation of data in health, ageing and disease.

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Contents

List of tables

List of figures

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For Sweeney.

List of Abbreviations

AU	Arbitrary Units
CORN	Corneometer® CM825
CRS	Confocal Raman Spectroscopy
DASI	Dry skin/ichthyosis area and severity index
DERM	DermaStat®
EEMCO	European Group on Efficacy Measurement of Cosmetics and other Topical Products
EPSRC	The Engineering and Physical Sciences Research Council
FSkHQ	Foot Skin Health Questionnaire
IQR	Interquartile Range
MGC	Merriman's Grades of Callus
MMD	MoistureMeter D®
MMSC	MoistureMeter SC™
NHS	National Health Service
OCCS	Overall Clinical Cutaneous Score
OCS	Overall Cutaneous Score
ODSS	Overall Dry Skin Score
PPK	Plantar Palmar Keratoderma
RH	Relative Humidity
RiverD	River Diagnostics, International B.V. Rotterdam, The Netherlands
SC	Stratum Corneum
SD	Standard Deviation
SRRC	Specified Symptom Sum Score System: grading of scaling, roughness, redness, and cracks
TDC	Tissue Dielectric Constant
XAS	Xerosis Assessment Scale

Abstract

Background

Epidermal composition influences the physical behaviour of the skin and its ability to withstand trauma. This is important on the plantar foot due to the high compression, shear, and torsion forces it is exposed to during weightbearing. Plantar skin pathology is common and can lead to pain and disability, particularly in people of advanced age, or those with diabetes. Despite this, little information is available on plantar epidermis composition.

Method

Within this PhD, two studies are undertaken: Study 1: 'An investigation into the hydration of the foot skin and associated skin characteristics' in which three commercially available devices (Corneometer® CM825, MoistureMeter D® and MoistureMeter SC™) are used to quantify plantar epidermal hydration alongside measures of skin hardness, elasticity, surface texture, and perceived skin health. Study 2: 'An evaluation of the biochemical composition of the foot skin using CRS' in which in-vivo Confocal Raman Spectroscopy and four commercially available hydration measurement devices (Corneometer® CM825, MoistureMeter SC™, MoistureMeter D® and DermaStat®) are used to measure the composition of the plantar epidermis with age, diabetes, and following emollient application.

Results

Study 1 provides insight into the relationship between plantar epidermis hydration and physical behaviour, surface features, and perceived health of the skin, when quantified using a variety of commercially available device.

Study 2 generates a novel data set detailing the plantar epidermis composition, and uses these data to demonstrate how age, diabetes, and emollient application impact plantar epidermis composition, and how this is represented by commercially available hydration measurement devices.

Conclusion

The first data set examining the plantar epidermis composition using in-vivo Confocal Raman Spectroscopy is presented. These investigations culminate in a set of recommendations for the use of commercially available hydration measurement devices on the plantar epidermis and the formulation of emollients for plantar skin of people of advanced age and with diabetes.

Coronavirus Impact Statement

The author would like to advise the reader of the impact of the Coronavirus pandemic on their research activity, primarily Study 1: 'An investigation into the use of the Corneometer® CM825, MoistureMeter SC and MoistureMeter D® on the foot skin: A pilot study'. This study was conducted from December 2021 to March 2022.

Due to the closure of the University of Salford campus, data-collection for this study was unable to start as originally planned for late-summer of 2021. When facilities were reopened, research activity was permitted only when stringent guidelines were followed, and only post graduate students and teaching staff were allowed to work on-campus. This resulted in further delays in the study starting (due to the additional protocol requirements to comply with guidance) and limited participant numbers being available to the researcher.

If the design and implementation of this study had not been impacted by the Coronavirus pandemic, the author would have commenced data-collection at an earlier date and recruited a larger number of participants. This would have generated a larger volume of data that was suitable for more rigorous statistical analyses than those presented in this thesis, therefore enhancing confidence in the findings of this aspect of the work.

Data-collection began despite the restrictions in-place at the time, with the knowledge that this would result in a reduced participant number, as it was not clear when restrictions would be lifted, and the progression of the PhD work was considered priority. Further delay would have negatively impacted the conduction of studies 2-4.

Chapter 1: Introduction

1.1. Introduction to the thesis

This thesis is the product of a PhD Studentship sponsored by Scholl footcare which was completed between October 2019 and September 2023 at The University of Salford, within the EPSRC Centre for Doctoral Training in Prosthetics and Orthotics. Through the course of this PhD, the sphere of research focus has been moulded by the needs of Scholl and the opportunities available to the author through partnerships with other academic institutions involved in the doctoral school.

The original project brief provided by Scholl stated the sponsored PhD Studentship must concern the soft tissue characteristics of the foot and broadly support the research and development processes of the company. Initial work followed this broad scope, exploring the features of soft tissue that can be quantified and reviewing the literature where these had been observed on the foot. This is reflected in the design of the first literature review (See Section 2.2.) and resulted in the author developing a broad understanding of the landscape of research available on the foot.

With this knowledge, and the additional insight of a 'gap analysis' provided by Scholl detailing areas for advancement within their research and development processes, the scope of this project was narrowed to investigate the hydration of the foot skin. The remainder of the initial literature review reflects this journey (See Section 2.3.).

This review concludes with a demonstration of the need to investigate the use of commercially available hydration measurement devices on the plantar skin due to its unique anatomical structure. This need drives the first two studies undertaken as part of this project 'An investigation into the use of the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® on the foot skin: A pilot study' and 'An investigation into the hydration of the foot skin and associated skin characteristics' which are presented in Chapters 5 and 6.

During this first literature review process, however, the author also identified an alternative method for quantifying skin content in-vivo which has not previously been applied to the foot skin: Confocal Raman Spectroscopy. This technology represented an opportunity to resolve some of the uncertainties surrounding the use of commercially available hydration measurement devices on the plantar foot, as well as exploring other skin components not

previously captured in-vivo on plantar skin. To maximise the benefit obtained from the use of this device, the researcher opted to expand the scope of the project to encompass participants of demographic groups known to have varied levels of foot skin risk: young people, older people, and people with diabetes. Sections 2.4. and 2.5. present the data available on skin change resulting from age and diabetes status, and the limited instances in which Confocal Raman Spectroscopy has been used to investigate these. Section 2.5. concludes in recommendations for use of Confocal Raman Spectroscopy on the foot and generates hypotheses detailing anticipated change between groups.

Experimental work using Confocal Raman Spectroscopy is presented across two chapters, Chapter 7 details the methodology and outcomes relating to the investigation 'An evaluation of the biochemical composition of the foot skin using CRS', and Chapter 8 presents an alternative analysis of these data for the purposes of 'Using CRS to investigate the validity of commercially available hydration measurement devices on the plantar skin'.

Due to the large number of devices used within this project, and the key importance of their design to interpretation of data, an instrumentation chapter (Chapter 4) follows the literature reviews. Within this section, all devices used with the later studies are presented and instances in which they have previously been applied to the foot are reviewed. Practical aspects of their use and cost are also presented to provide a wider understanding of their potential for application within a clinical or commercial setting.

Despite their distinct objectives (Chapter 3), each of the four experimental chapters within this thesis contributes to a wider discussion on the application of technology to plantar skin characterisation, and how these data should be interpreted.

This thesis culminates in a set of recommendations for the use of commercially available hydration measurement devices for the assessment of the foot skin in a clinical and commercial setting. A series of recommendations for future research relating to the foot skin are also given (Chapter 9).

Throughout this PhD project, a balance has been sought between the requirement for this work to support the research and development processes of Scholl, and to represent a novel contribution to foot health and care science. In some instances, the momentum and novelty of work has been prioritised over the pilot study design, or the transferability of resulting data to a commercial setting. This is demonstrated in the accelerated creation and piloting of the Foot Skin Health Questionnaire (see Section 4.6.), and also in the selection of the participants used for an emollient trial in 'An evaluation of the biochemical composition of the foot skin using CRS'. These sacrifices are recognised, discussed, and considered justifiable in the context of this work.

This careful consideration of the context in which project outcomes will be applied has led to the generation of data with wide-reaching implications. This is in addition to the demonstrable novelty of the data collected as part of this work: most evidently from the aspects of this work in which Confocal Raman Spectroscopy is applied to the foot skin, but also more subtly, the application of plantar skin structure and measurement mechanism of commercially available hydration measurement devices to the interpretation of the data they provide.

Within the next section, the underpinning knowledge required to understand and interpret the ideas discussed in first literature review (see Section 2.3.) are provided. Further background information is provided in Section 2.4. in response to the broadening of the project scope.

A full demonstration of the structure of this thesis is given in Figure 1.

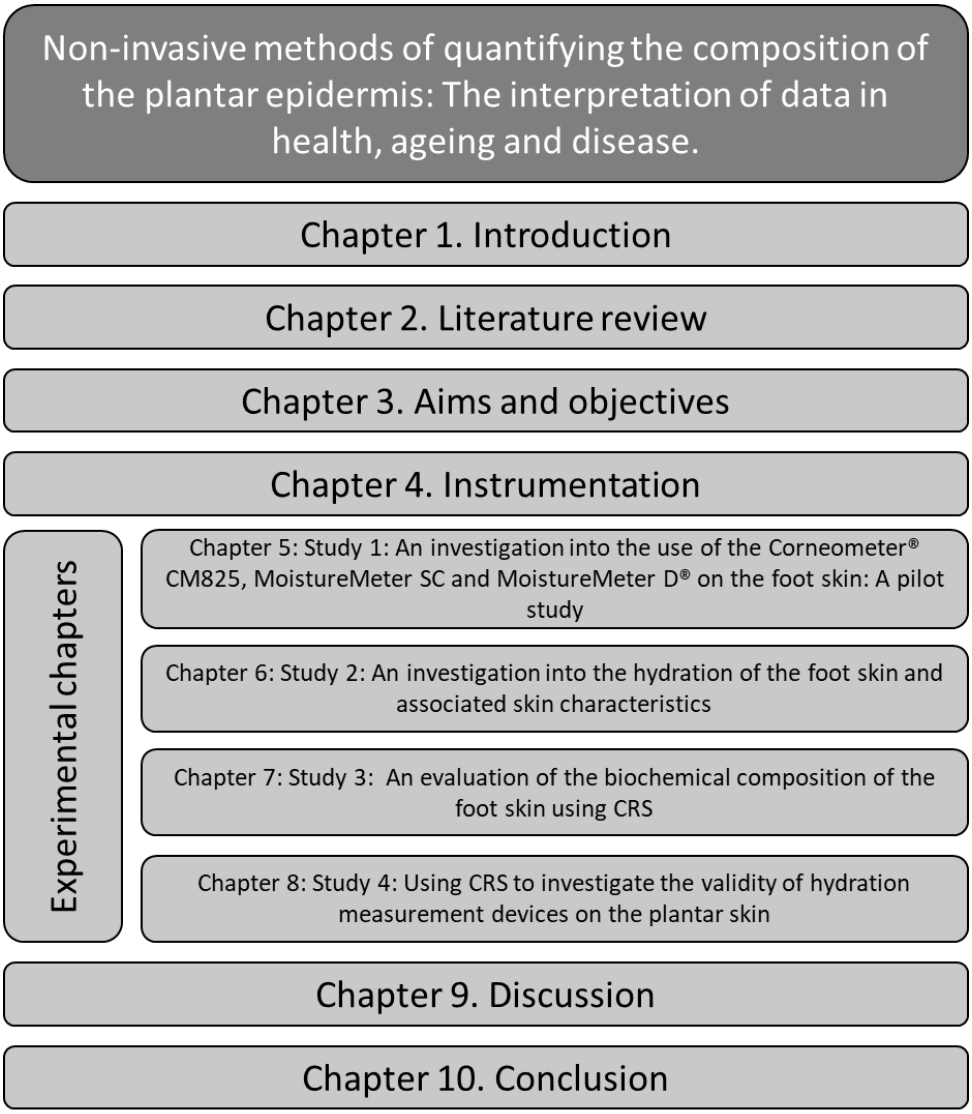


Figure 1. Thesis structure.

1.2. Introduction to the study area

1.2.1. The skin

As the primary interface between the body and the external environment, the skin is an organ of vital importance. The physical barrier provided by the skin protects the internal structures from mechanical trauma, harmful radiation, excessive water loss, and damage by hazardous substances and pathogens (Norris, 2012). The large surface area aids in regulating body temperature, excretion of waste products and prompts immunologic responses to chemical and biological hazards (Norris, 2012). Plantar skin has the same multifarious role, with the additional requirement to withstand high compression, shear, and torsion loads during ambulation (De Clercq et al., 1994; Hosein & Lord, 2000; Keller et al., 1996). The ability of the skin to form and retain a responsive, sensitive, and secure physical and chemical barrier is facilitated by its complex anatomy and physiology. The next section describes the structure and function of plantar and non-plantar skin.

1.2.2. Skin structure and physiology

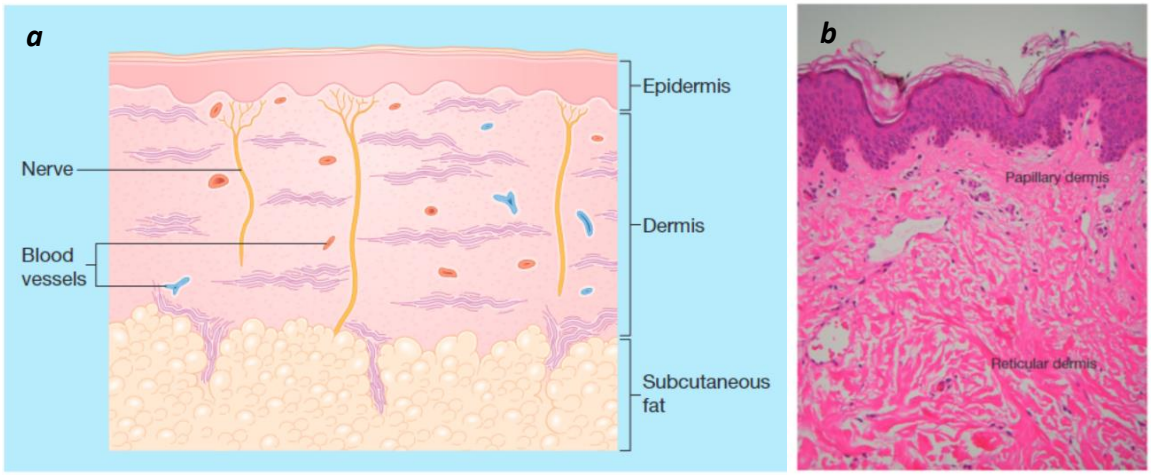


Figure 2. a: Diagram demonstrating the structure of the dermis and epidermis. b: Histology section of the skin showing the structure of the papillary and reticular dermis. Both images extracted from 'Lookingbill and Marks' principles of dermatology' Sixth Edition (Marks & Miller, 2019). Used with permission.

The skin is a layered structure, the deepest of which is the dermis, a layer of biologically active connective tissue that lies superficial to the subcutaneous fat and inferiorly to the epidermis (See Figure 2 *a*). The dermis is split into two components (reticular dermis and papillary dermis), which are primarily composed of elastin, collagen and extra fibrillar matrix, but

display distinct mechanical characteristics due to the different arrangement and density of these fibres (See Figure 2 *b*) (Norris, 2012).

The reticular dermis is the lower, thicker layer of the dermis. It contains most dermal elastic fibres and a dense irregular network of thick collagen fibres, providing a strong but elastic structure that cushions the structures below (Langton et al., 2017). The skin appendages are contained within this layer (hair follicles, sebaceous glands, apocrine and eccrine sweat glands) and the blood vessels travel superiorly into the papillary dermis (Norris, 2012).

The papillary dermis is a less dense structure with finer and more loosely arranged collagen fibres (Wang et al., 2015). This layer contains the superficial plexus of nerves and vasculature that sustains the overlying epidermis, the junction at which these two structures meet is called the dermo-epidermal junction (DEJ) or the 'basement membrane' (Norris, 2012). This membrane is characterised by its undulating surface, formed through the 'rete ridges' (See Figure 3.) projecting downwards from the epidermis into the dermis (Boyle et al., 2019; Langton et al., 2017). This increased surface area is said to aid cohesion between the epidermis and dermis, preventing the separation of the layers due to shear forces (Marks & Miller, 2019).

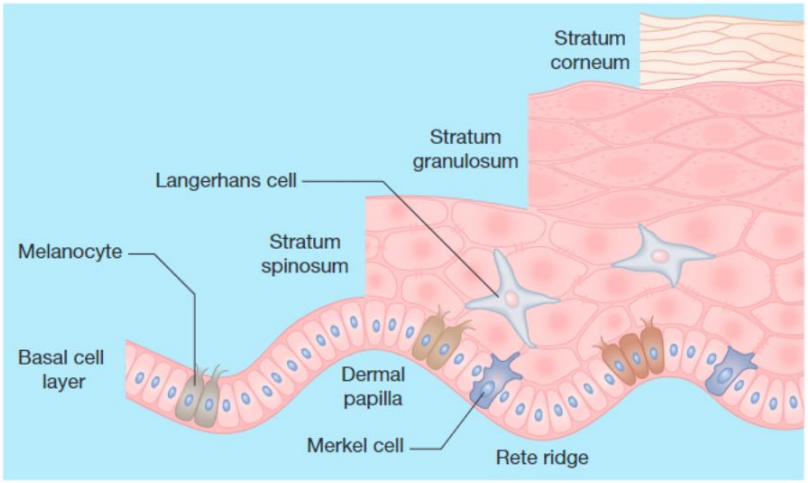


Figure 3. Diagram representing the layers of the epidermis. Image extracted from 'Lookingbill and Marks' principles of dermatology' Sixth Edition (Marks & Miller, 2019). Used with permission.

The epidermis comprises five layers of keratinocytes at different stages of maturation. The innermost layer of the epidermis, the stratum basale, comprises columnar basal cells that are strongly adhered to their surrounding cells (including the DEJ) by desmosomes and

hemidesmosomes. These basal cells produce undifferentiated daughter cells called keratinocytes (Norris, 2012).

Keratinocytes are the primary 'building blocks' of the epidermis. These migrate upwards through the skin due to the continual production of new cells at the basal layer, and the changes that these undergo as this process occurs define the features of the other layers within the epidermis (Gokul & Shetty, 2012), these are detailed below:

The stratum spinosum lies directly above the basal layer. At this location, the keratinocytes produce a fibrous protein called keratin, a major component of the uppermost barrier layer of the epidermis (Gokul & Shetty, 2012). This protein forms intercellular bridges (composed of desmosomes) that adhere adjoining cells together, which appear 'spiny' on observation with a microscope, hence the name stratum spinosum (Marks & Miller, 2019; Presland & Dale, 2000).

The process of keratinisation continues as the cells migrate upwards into the stratum granulosum layer, in which the cells become more flattened and digestive enzymes destroy the nucleus (Gokul & Shetty, 2012). This process results in the formation of keratohyalin granules and lamellar granules, each containing materials essential to the next stage of cell differentiation. Keratohyalin granules contain two proteins: profillagrin, the precursor to filaggrin, which binds keratin filaments, and involucrin, which contributes to developing the 'cell envelope'. Lamellar granules contain polysaccharides, glycoproteins, and lipids, which are extruded into the intercellular spaces (Marks & Miller, 2019; Verdier-Sévrain & Bonté, 2007).

The uppermost layer of the skin, and the primary skin barrier, is the stratum corneum (SC) (Marks & Miller, 2019). Transitioning into SC from the stratum granulosum represents a distinct change for the keratinocytes; they are no longer viable, nucleated cells but entirely flattened, keratin-filled cell envelopes (Menon et al., 2012). These cells are renamed corneocytes in this layer due to their hard, cornified cell envelope. These are stacked tightly in a lamellar fashion, between 10-25 layers deep on non-plantar/palmar skin and are surrounded by an extracellular lipid matrix – often referred to as the 'mortar' between the corneocyte 'bricks' (Menon et al., 2012).

As the corneocytes are pushed upwards through the SC, the corneodesmosomes that bind them to neighbouring cells are degraded. At the top surface of the SC, there are so few remaining corneodesmosomes, the uppermost corneocytes readily detach (Elias, 2005). The corneocytes shedding from the skin surface is known as desquamation. In a healthy individual, a keratinocyte passes from the basal layer through the epidermal layers and is shed from the SC in around 28 days (Marks & Miller, 2019).

1.2.3. The Stratum Corneum

Historically, the SC has been considered a layer of biologically inactive tissue produced as a by-product of keratinisation and cell-death within the epidermis (Del Rosso & Levin, 2011). However, in recent years this perception has changed as evidence has emerged of the more complex role the SC holds, as not just a barrier but a reactive, self-maintaining structure (Menon et al., 2012).

The physical structure of the SC poses a major barrier for materials such as strong surfactants, chemical irritants, and microorganisms, entering the skin and causing harm (Elias, 2005). Within the SC the corneocytes have a strong cornified cell envelope, which is resistant to ingress of such materials, and the cells are stacked atop each other with cell edges overlapping, a formation often likened to bricks in a wall. This creates a long winding path through the cells which, as well as an extracellular lipid matrix between the cells, reduces the penetration of compounds into the skin (See Figure 4) (Elias, 1991; Menon et al., 2012).

In addition to limiting ingress of harmful materials into the SC, this structure also prevents the passive evaporation of body water out through the skin, which is known as trans epidermal water loss (TEWL) (Menon et al., 2012). TEWL is commonly used as an indicator of skin integrity (Akdeniz et al., 2018), as it is raised when the SC barrier is damaged, such as following chemical or physical injury (Gardien et al., 2016) or in skin disease (Montero-Vilchez et al., 2021).

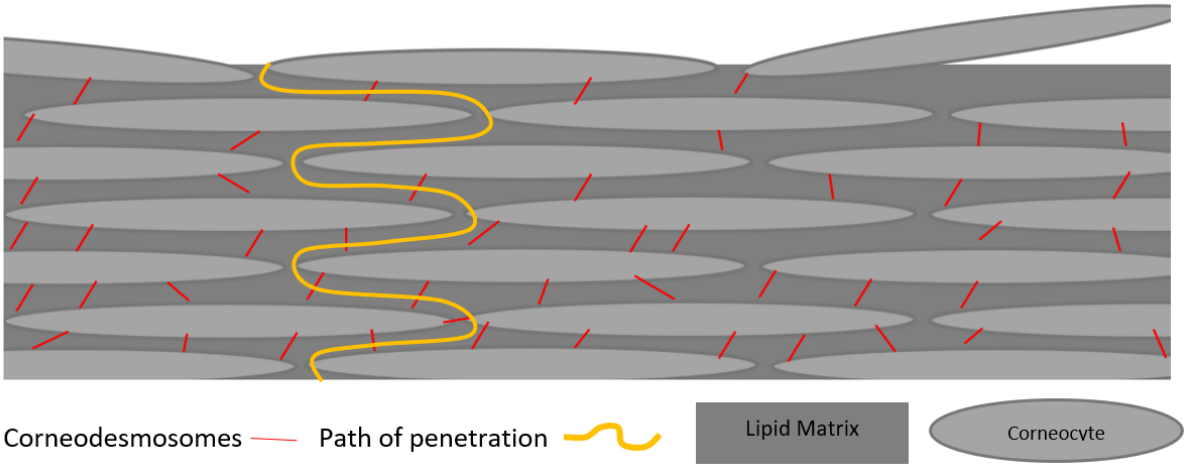


Figure 4. Diagrammatic representation of the 'Bricks and Mortar' structure of the SC.

The SC barrier also extends to an immune function, inducing an immune response following activation of receptor cells and representing a hostile environment for potentially hazardous microbes due to the antimicrobial peptides present in the sweat, sebum and some SC lipids, and the constant renewal of the uppermost cells (Elias, 2005). Further, the SC represents an antioxidant barrier to prevent damage from reactive oxygen species and a photoprotection barrier to damaging UV light (Elias, 2005)

The ability of the SC to self-repair is rapid and efficient. For example, when TEWL is increased due to disruption to the SC barrier via chemical or physical assault, available lipids from lamellar bodies are released immediately. Within 2-3 hours, precursor lipid production in lamellar bodies is increased to replenish SC lipids, reducing TEWL (Del Rosso & Levin, 2011). Further, the SC can control its water content and water-holding ability by synthesising Natural Moisturising Factors (NMF). These are a mixture of free amino acids, pyrrolidone carboxylic acid and urocanic acid mixed with simple sugars and electrolytes that are produced following the degradation of filaggrin within the granular layer (Del Rosso & Levin, 2011; Elias, 1991).

1.2.4. Plantar skin

The structure and function of the plantar skin are very similar to that of the non-plantar skin, with a few exceptions that increase its ability to withstand high levels of pressure and shear.

1.2.4.1 Morphological differences

The rete ridges in the skin are most pronounced on plantar and palmar surfaces where the tissues are most exposed to friction (Maceo, 2009). This can be seen in Figure 5 in the undulating border between the viable epidermis and dermis in the plantar skin. This increased interdigitation has historically been thought to reduce the risk of separation of the dermis and epidermis resulting from shear forces (Marks & Miller, 2019). However, Boyle et al (2016) found through computational modelling, that this structure does not have a primary role in reducing the risk of skin injury. Instead, the authors suggest that this interdigitation may support the greater nutritional demand of plantar skin or support tactile sensation (Boyle et al., 2019).

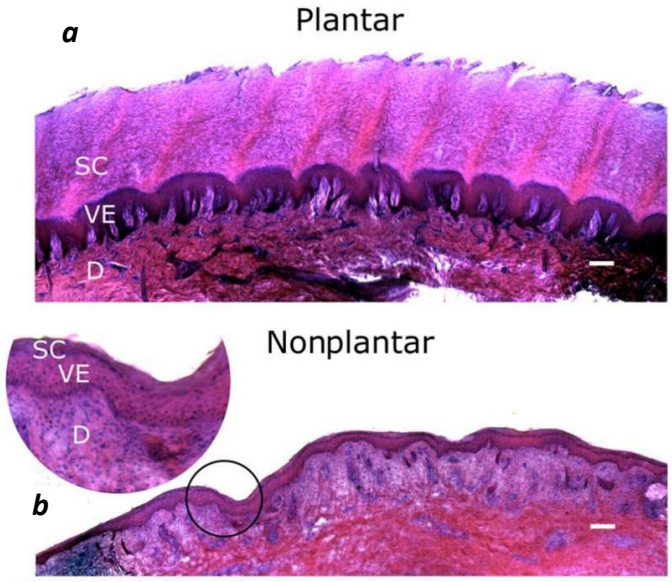


Figure 5. Histological sections of skin a. plantar b. non-plantar from 'Morphology and composition play distinct and complementary roles in the tolerance of plantar skin to mechanical load' (Boyle et al., 2019). Used with permission.[*]

Due to the interdigitation between the epidermis and dermis at the DEJ, ridges are formed at the border between the viable epidermis and SC (See Figure 4). These ridges continue through the SC and are visible as the dermatoglyphs at the skin surface (See Figure 6). These structures are topped with eccrine sweat glands (in contrast to non-plantar skin,

[*] SC = stratum corneum, VE = viable epidermis, D = dermis.

where sweat glands are present within the skin furrows) that are innervated during periods of emotional arousal and exercise (Maceo, 2009). This structure and pattern of perspiration increase friction, aiding grip when in contact with a surface (Adelman et al., 1975; Havenith et al., 2008; Maceo, 2009).

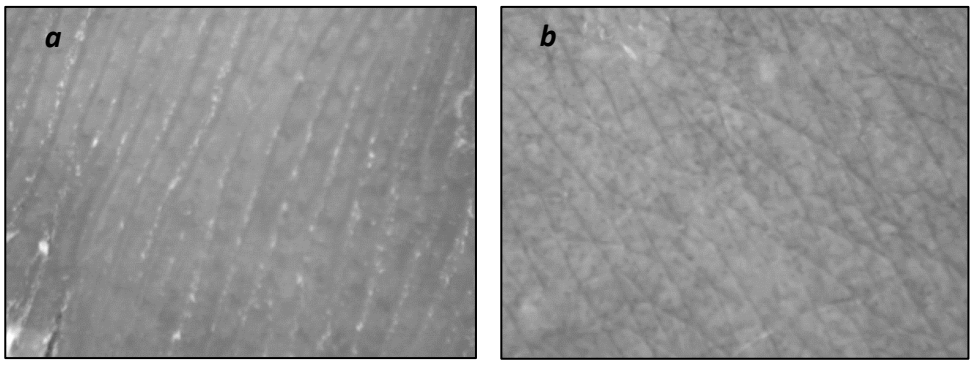


Figure 6. Images of skin taken by Visioscan® VC98 (Courage and Khazaka, Koln, Germany) UV-A light camera (unpublished images from the author) at the (a) heel skin, and (b) ventral forearm skin.

As is also visible in Figure 5, the plantar SC is much thicker than non-plantar SC. Vela-Romera (2019) evaluated the thickness of friction ridged skin and found that the average (± standard deviation) plantar SC thickness was 487.7 (± 160.2) µm, whereas the dorsal foot skin was 35.9 (± 27.8) µm, this difference is statistically significant (p-value = 0.0062). The palmar SC was also found to be significantly thicker than dorsal hand SC (p-value = 0.018), at 148.5 (± 36.4) µm compared to 33.1 (± 1.6) µm, respectively. Although Boyle et al (2019) did not publish any data, the plantar SC data provided by Vela-Romera et al (2019) align with Boyles's findings that plantar SC is 16 times thicker than non-plantar SC. Through computational modelling, Boyle et al (2019) established that this increased thickness reduces stress in underlying tissues when under mechanical load.

1.2.4.2. Keratin

In the stratum spinosum and stratum granulosum, the keratinocytes generate large volumes of keratin. Three specialised keratins (proteins) are generated on the plantar palmar skin that are not found within non-plantar skin: K9, K6 and K16; these are thought to provide improved integrity and flexibility within the epidermis (Boyle et al., 2019; Dun Jack Fu, 2014; Swensson et al., 1998).

Irregularity in the expression of these keratins is associated with the development of palmoplantar keratoderma (PPK). In this condition, the palms and plantar epidermis become extremely thickened, leading to stiffness, pain and fissure formation when unmanaged (Guerra et al., 2018). Mutations in the K6 and K9 genes have been found in individuals with focal and epidermolytic PPK (Reis et al., 1994; Shimomura et al., 2010; Wilson et al., 2010), and K9 and K16-null mice (modified via a knockout model) develop PPK-like lesions on the foot pads (Dun Jack Fu, 2014; Lessard & Coulombe, 2012).

1.2.4.3. Desmoglein

Boyle et al (2019) also identified that plantar skin contains larger volumes of Desmoglein 1 than non-plantar skin. Desmoglein 1 is a major component of the desmosomes that adhere the epidermal cells together, indicating that these are larger or more numerous in the plantar skin. This is thought to contribute to plantar skin resistance to mechanical trauma (Boyle et al., 2019).

Finally, although little discussed in contemporary research, an additional layer of the skin has been described on the plantar and palmar sites between the stratum granulosum and the SC: the stratum lucidum (See Figure 7). This is a layer of 4-6 entirely flattened, dead corneocytes containing high volumes of keratin and a thick plasma membrane. This is named after its clear appearance and is described as contributing more physical strength to the skin (Tortora & Derrickson, 2009).

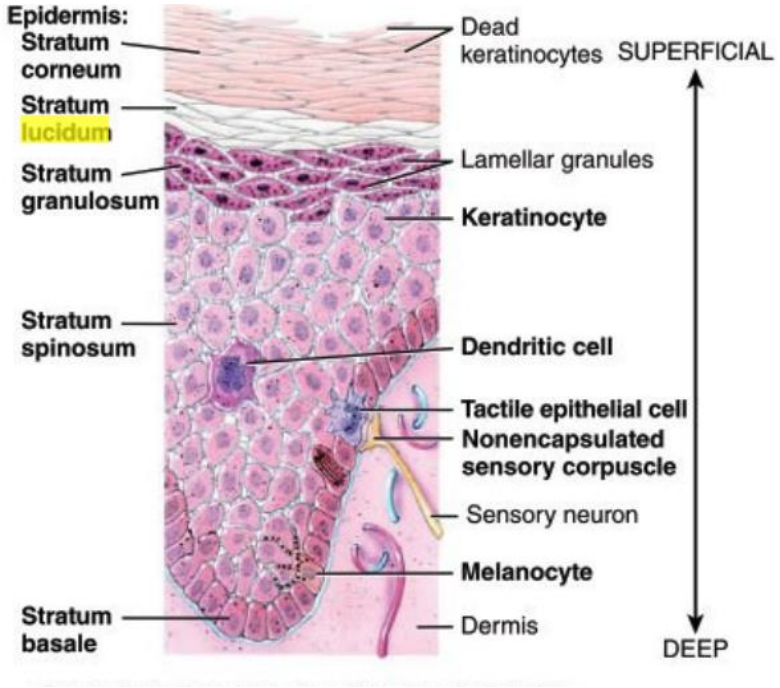


Figure 7. Diagram showing the location and structure of the stratum lucidum (highlighted yellow). Image extracted from 'Principles of anatomy and physiology: Vol.1, Organization, support and movement, and control systems of the human' body (Tortora & Derrickson, 2009). Used with permission.

1.2.4.4. Response to stress

Plantar skin not only has altered morphology and composition compared to other body locations but also behaves differently in response to excessive mechanical forces. In response to recurrent compression and shear forces, the keratinocytes of the SC hyper proliferate, leading to incomplete differentiation as they migrate upwards through the skin layers at an accelerated pace. This increases intercellular cohesion, producing a thickened, hard SC layer to protect underlying tissues from trauma, commonly known as callus (Kim et al., 2010; Rubin, 1949; Thomas et al., 1985).

1.2.5. Plantar skin pathology

Despite the adaptations to plantar skin described above, the foot is a common location for skin pathology to arise that can cause significant discomfort, pain, and even pose a risk to life in some cases (Armstrong et al., 2020; Farndon et al., 2006).

1.2.5.1. Xerosis (or anhidrosis)

Xerosis is the medical term for dry skin. The clinical presentation of xerotic skin is dull appearance with a flaky and rough texture, often causing discomfort and itching (Voegeli, 2007). Severe xerosis can cause fissures and cracks in the skin, which can cause discomfort

and provide a portal of entry for microorganisms, which can result in opportunistic bacterial infection (Oe et al., 2012). For individuals with compromised immunity system, these local infections represent an increased risk of sepsis (Angus & van der Poll, 2013; Danai et al., 2006).

Xerosis can develop because of internal or external influences. Seasonal reductions in temperature and humidity negatively impact skin integrity (xerosis occurrence increases in winter) (Black et al., 2000), as does an abrupt change in environmental conditions (Katagiri et al., 2003), and exposure to indoor climate-controlled environments with low humidity (Sato et al., 2002). Dissolution of SC lipids due to surfactant exposure can also trigger xerosis (Nielsen et al., 2000; Okuda et al., 2002).

Psychological stress has been shown to increase skin barrier dysfunction (Choi et al., 2005; Denda et al., 2000), and some atopic individuals are genetically prone to xerosis (Paul et al., 2011; Proksch et al., 2003). Despite the uncertainty surrounding the skin barrier function and epidermal hydration with age (explored in Section 2.4.), advanced age is a risk-factor for xerosis (Paul et al., 2011).

Xerosis is a common foot skin complaint observed in podiatric practice, however there is limited data available on the aetiology of this condition. Baird et al (2003) suggested that foot skin xerosis is common due to the reliance of the skin on water provided through perspiration. Reduction of peripheral sweating is often found in older people and people with diabetes due to peripheral autonomic neuropathy (Schroder & Weis, 2014). However, foot skin xerosis is not exclusive to older people or people with diabetes.

Most of the research into foot skin hydration to date has focused on assessing emollient efficacy using a visual scoring system as an outcome measure or a hydration measurement device. Unfortunately, this has led to a paucity of data available on healthy foot skin. Without an understanding of healthy skin hydration and the variability in healthy foot skin hydration, it is not possible to identify a target skin hydration that represents the resolution of xerosis or identify meaningful change in foot skin hydration. There is a need to generate such a dataset to support the evaluation of emollient efficacy on the foot.

1.2.5.2. Hyperkeratosis

The propensity of the foot skin to hyper proliferate in response to mechanical stress (Kim et al., 2010; Rubin, 1949; Thomas et al., 1985) can lead to pathological hyperkeratotic lesions developing, commonly known as corns and callus.

A corn is an area of hard, dry, circumscribed hyperkeratosis that forms in a conical shape, pointing inwards into the skin (Freeman, 2002; Hashmi, Nester, et al., 2015). Callus is similarly hard and dry, but is more diffuse than a corn and has no defined central core (Freeman, 2002). These lesions can develop due to various intrinsic and extrinsic factors, such as ill-fitting footwear, dehydrated skin, and high plantar pressures (Collier & Brodbeck, 1993).

Corns and calluses can cause significant pain, deformity and disability (Helfand, 2003) and are a risk-factor for ulceration, particularly for people with diabetes (Murray et al., 1996; Pavicic & Korting, 2006). For older people (who are more likely to develop corns and callus (Burzykowski et al., 2003)) foot pain can be particularly deleterious, leading to reduced health related quality of life, functional impairment and an increased risk of falling (Menz & Lord, 2001; Mickle et al., 2010, 2011).

The prevalence of corns and callus in the population is reported with much variability (16-68%), in-part, due to the historical collection of data from cohorts with varied foot-health risk (Wright, 2015). For example, in one study, 68% of individuals were found to have corns and calluses, although the population surveyed were all over 80 years of age (White & Mulley, 1989).

1.2.5.3. Diabetic Foot Ulcers

The number of people living with diabetes in the UK is increasing, with almost 3.5 million people living with diabetes in England in 2022 (National Cardiovascular Intelligence Network, 2022). These individuals have a 10-25% lifetime incidence of developing a diabetic foot ulcer (DFU), a chronic wound resulting from complications of diabetes (Yazdanpanah et al., 2018).

Developing a DFU has huge implications for an individual: representing five-year mortality rate similar to several cancers (Armstrong et al., 2020) and a large risk of minor or major lower limb amputation (National Cardiovascular Intelligence Network, 2022). The costs associated with diabetic ulceration and amputation also have a huge impact on the UK National Health Service, estimated to represent almost 1% of the NHS overall budget in 2014-15, with 90% of this expenditure directly related to ulceration care (Kerr et al., 2019).

An individual is more at risk of developing a DFU if they have certain complications of diabetes, such as peripheral neuropathy and peripheral arterial disease, amongst others (Boulton, 2004; Yazdanpanah et al., 2018). Neuropathy not only inhibits an individual's ability to detect when their foot is injured, but leads to deformity that increases underfoot pressures which can lead to callus formation (Boulton, 2014), which is a predictor of ulceration (Murray et al., 1996).

Therefore, maintaining plantar skin integrity is highly important to reducing DFU and amputation risk.

1.2.6. Emollients

One well researched intervention that can modify pathological epidermis to near its healthy integrity is the topical application of emollients (Voegeli, 2007). Topical emollients are available in a variety of delivery mechanisms (for example, creams, lotions, gels and sprays), which all perform the same function: delivery of water to the SC and / or prevention of TEWL (Voegeli, 2007). The first of these is primarily achieved through the delivery of water and humectant materials into the SC, such as propylene glycol, urea and glycerol, to improve the skins water-binding ability (Parker et al., 2017). The second of these involves the deposition of an occlusive lipid film on the skin surface to prevent water evaporating from within the SC (Lodén, 2005). Emollient use will be discussed in further detail in section 2.4.

In some cases, emollients may be prescribed by an individual's healthcare provider (Amakye et al., 2022), however, skin conditions such as xerosis are widely self-identified and treated by individuals without seeking medical advice (Schofield et al., 2009). Of the 54% of people who experience a skin condition in a given 12-month period, 69% of these

will choose to administer self-care, often through the use of over the counter (OTC) skin treatments (Schofield et al., 2009). In 2007, OTC sales of skin treatments in the UK reached £413.9 million, representing 18% of all OTC sales (Schofield et al., 2009).

1.3. The role of water in the SC

Before the in-depth exploration of the three papers where healthy foot-skin hydration has been investigated, it is pertinent to review why the water content of the SC, in particular, is so essential to maintaining a healthy skin barrier.

1.3.1. Delivery and retention

Water is delivered to the SC through diffusion from underlying soft tissues and via the excretion of sweat from the sweat glands penetrating the skin surface (Blank, 1952). The SC retains this water through three main mechanisms:

- The complex arrangement of corneocytes (See Figure 3) creates an indirect and complex pathway for internal water to reach the surface of the SC, therefore reducing TEWL (A. Rawlings et al., 1994).
- The intercellular lipid membrane in the SC surrounding the corneocytes comprises epidermal lipids that impede water transit through this area, further reducing TEWL (Elias, 1991).
- Finally, the NMFs within the corneocytes act as a natural humectant (a substance that draws water into the skin from the air or surrounding tissues) (Del Rosso & Levin, 2011) as they comprise a mixture of small hygroscopic compounds, binding the water within these cells (Verdier-Sévrain & Bonté, 2007).

1.3.2. Role in enzymatic processes and implication of dehydration

Water is required for the degradation of filaggrin. When this process is impeded due to low SC hydration, NMFs cannot be produced (Scott & Harding, 1986), which reduces the ability of the skin to retain water.

Low environmental humidity also inhibits the action of enzymes that degrade the desmosomes. This results in excess corneocytes remaining adhered to the skin surface, clumping together to create scales and flakes, creating a rough, dry surface (Leyden & Rawlings, 2019; Verdier-Sévrain & Bonté, 2007) that has an impaired ability to absorb and retain water (Tagami et al., 1982).

Rawlings and Matts (2005), explain in detail the cyclical nature of xerosis development and propagation. Reduced SC hydration is caused by abruptly changing environmental conditions, low temperature, and humidity, contact with surfactant, genetic factors, or ageing. This leads to increased fragility or brittleness and impaired SC barrier which, in turn, allows NMFs to be washed from the SC, further reducing the ability of the SC to retain water. This insult to the SC physiology leads to inflammation, causing hyperproliferation of keratinocytes that are poorly differentiated, reducing enzymatic activity that inhibits desquamation, leading to a thickened, dry SC.

1.3.3 The impact on the mechanical characteristics of the skin

During walking, the plantar skin is exposed to high compression, torsion, and shear forces (Hosein & Lord, 2000; Jasiewicz et al., 2019; McKay et al., 2017; Vette et al., 2019).

The foot skin needs to be supple and elastic enough to withstand these forces, and allow the underlying soft tissues to deform to absorb and redistribute these forces (Yum et al., 2019). A hard, inflexible skin surface (e.g. callused plantar skin) has the propensity to split and form fissures or develop areas of high pressure under the foot (Oe et al., 2012).

The foot has a high incidence of pathology that is related to mechanical trauma – primarily blister formation, and development of hyperkeratosis and tissue breakdown due to high-pressures (Collier & Brodbeck, 1993; Hashmi, Nester, et al., 2015; Kirkham et al., 2014; Murray et al., 1996). As is to be expected due to the role of water in the physiological processes within the SC, skin hydration directly impacts the mechanical characteristics of the skin.

1.3.3.1. Low hydration reduces foot skin elasticity and increases hardness.

The elasticity of the foot skin has previously been examined using the Cutometer® 575 (Courage and Khazaka, Cologne, Germany) and was found to positively correlate to skin hydration (measured using the Corneometer® CM825, Courage and Khazaka) at 6 out of 9 skin sites tested on the plantar foot (centre and surround of callus plaques and heel fissures, xerotic heel skin, and the base of the 5th metatarsal) (Hashmi, Nester, et al., 2015). However, the physiologically healthy plantar skin tested in this study showed a relatively low correlation (5th metatarsal base correlation (r-value = 0.25, p-value = 0.01)) or non-significant correlation (plantar metatarsal area correlation (r-value = 0.13, p-value unreported)). Therefore, this relationship between plantar skin elasticity and hydration has been primarily demonstrated at sites of pathology (Hashmi, Nester, et al., 2015). The authors suggest this may be due to the increased variability of the elasticity and hydration measures taken from the physiologically healthy skin than from the pathological lesions examined during this study, which also indicates that pathological lesions are less variable between individuals than healthy plantar skin (Hashmi, Nester, et al., 2015).

It would be beneficial to undertake a larger study to examine this phenomenon further. A larger dataset from the foot skin would reduce the confounding effect of the variability within the healthy data, creating more opportunities to explore the relationship between skin elasticity and hydration in non-pathological areas.

Foot skin hardness has also been linked to SC hydration. Schmidt et al (2018) found that artificially hydrated skin was less hard than non-hydrated skin. In this study the skin was hydrated via immersion in water for 45 minutes (Schmidt, Germano et al. 2018). This limits the clinical application of these findings to circumstances where the foot skin is entirely occluded or submerged in water for extended periods. A comparison between skin hardness and hydration data from healthy and xerotic foot skin would generate data that is directly applicable to the development and testing of emollients intended to modify skin behaviour through increasing hydration.

1.3.3.2. Skin hydration impacts frictional behaviour.

Tribological studies have identified significant differences in frictional behaviour on body areas other than the foot with different skin hydration levels. The friction coefficient (a ratio of the friction force between two surfaces and the forces pushing them together) of

skin on the forearm was found to increase by 43% in females and 26% in males when skin hydration was 'normally moist' (>40 AU) compared to 'very dry' (<30 AU) skin (Gerhardt et al., 2008). However, in this study skin hydration was manually adjusted through application of fluids, limiting its comparability to physiological skin hydration (Gerhardt et al., 2008).

The association between hydration and incidence of friction blister formation has been investigated on the foot. Kirkham et al (2014) conducted a study in which the skin of the heel was subject to cyclic shear forces following immersion in water for 5 minutes to artificially raise skin hydration (Kirkham et al., 2014). The study used a temperature change of 3°C, using infrared thermography, as indicative of imminent blister formation (Hashmi et al, 2013) The contralateral foot (side randomly allocated) was subject to the same loading protocol, without water immersion (Kirkham et al., 2014). This study found that the hydrated skin took only 6 minutes to reach the study endpoint compared to 9 minutes for non-hydrated. Skin hydration was measured using the Corneometer® CM825 (Kirkham et al., 2014).

This study demonstrates that increased foot skin hydration is linked to friction blister formation, but it offers no insight into how reduced foot skin hydration influences frictional behaviour of the skin. Xerotic skin has increased surface texture (Hashmi, Nester, et al., 2015). An assessment of the frictional behaviour of the skin at its surface (not its propensity to form friction blisters), alongside skin hydration and surface texture would provide data more applicable to the development and testing of emollients.

1.3.4. Factors influencing skin hydration.

A number of intrinsic and extrinsic factors can influence skin hydration. As well as the natural variation in skin hydration between anatomical sites (Bogerd et al., 2011; Lechner et al., 2019; Mayrovitz, McClymont, et al., 2013), the water content of an individuals' skin can vary with age (Cho et al., 2019; Egawa & Tagami, 2008b), increase with systemic pathologies (Mayrovitz, McClymont, et al., 2013), and reduce with sleep deprivation (Jang et al., 2020). External factors such as environmental temperature and humidity (Katagiri et al., 2003; Nam et al., 2015), properties of contact materials (Bogerd et al., 2011) and

cleansing routines (Caspers et al., 2003) are also known to influence the water content of the tissue.

1.4. Conclusion

When collecting data on skin hydration, it is essential to consider all extrinsic and intrinsic factors that may influence the data collected in relation to study design. A table demonstrating these factors, generated by Du Plessis et al (2013), has been reproduced below for future reference (Table1).

Table 1. Demonstration of factors influencing skin hydration, replicated with permission from 'International guidelines for the in vivo assessment of skin properties in non-clinical settings: Part 2. transepidermal water loss and skin hydration' (Du Plessis et al., 2013).

	Influence	References
Endogenous factors		
Age	Yes	(Barel, 2006; Darlenski et al., 2009; Farinelli, 2006)
Gender	No	(Barel, 2006; Darlenski et al., 2009; Jacobi et al., 2005)
Ethnicity	Yes Controversial	(Berardesca & Maibach, 2003; Berardesca & Maibach, 1988a, 1988b) (Darlenski et al., 2009; Fluhr et al., 2008; Rawlings et al., 2008)
Anatomical position	Yes	(Barel, 2006; Berardesca, 1997; Black et al., 2000; Darlenski et al., 2009; Farinelli, 2006; Kleesz et al., 2012)
Skin temperature	Yes	(Darlenski et al., 2009)
Sweating	Yes	(Darlenski et al., 2009; Goh, 2006)
Circadian rhythm	Yes Controversial No	(Le Fur et al., 2001) (Darlenski et al., 2009) (Yosipovitch et al., 1998)
Skin health	Yes	(Proksch et al., 2008)
Exogenous factors		
Skin washing and wet work	Yes	(Kezic & Nielsen, 2009; Voegeli, 2008)
Solvents/surfactants	Yes	(Kezic & Nielsen, 2009)
Occlusion	Yes Controversial	(Kezic & Nielsen, 2009; Zhai & Maibach, 2002) (Nielsen et al., 2007)
Skin damage	Yes	(Wetzky et al., 2009)
Smoking	Yes	(Wolf et al., 1992)
Environment and measurement factors		
Air convection/movement	Yes	(Darlenski et al., 2009)

Ambient temperature	Yes	(Darlenski et al., 2009)
Relative humidity	Yes	(Barel, 2006; Black et al., 2000; Darlenski et al., 2009; Goh, 2006)
Season	Yes Controversial	(Black et al., 2000; Darlenski et al., 2009; Huixia et al., 2011) (Darlenski et al., 2009)

Participant demographics that are of consequence to skin hydration must be recorded and controlled where necessary: i.e., by restricting participants to a narrow age-range, or excluding participants with systemic diseases that are not expressly being examined within the study. Exposure of skin to chemicals (such as surfactants) must be controlled or recorded to prevent unintended influence on skin hydration, and environmental conditions must be maintained within a pre-established temperature and humidity range suitable for the hydration measurement device being used, which is usually published by the manufacturer (see Chapter 4).

Within this section, the importance of skin hydration for maintaining an effective skin barrier has been described, as well as the commonality of foot skin pathology and the deleterious effect this can have an individual and the wider healthcare system. What has been scarcely reported, however, are instances in which the plantar SC hydration has been investigated. This is not an oversight, but a reflection of a data gap – plantar SC hydration has not been widely investigated, despite being integral to skin barrier integrity and linked to common pathology. In order to understand the aetiology of, and effective treatment for, plantar xerosis, the physiological hydration of the plantar SC must be examined.

In Section 2.3., all instances in which the plantar SC hydration has been measured previously are compiled and reviewed.

Chapter 2: Literature Review

2.1. Introduction

Throughout this project, the focus of the work has been defined in response to gaps identified within the literature and assessing the need from the industry sponsor. The design of the initial literature search detailed in Section 2.2 was guided by initial brief from the industry sponsor, Scholl, that this PhD project must relate to the soft tissue characteristics of the foot.

Following review of the materials retrieved during this search, and discussion with Scholl, an area of study was defined as the focus of the literature review presented in Section 2.3: The hydration of the foot skin.

The findings of this literature review necessitate further exploration of plantar SC hydration using an alternative technology: Confocal Raman Spectroscopy (CRS). The wide capability of this technology prompts the expansion of this work to include measurement of other skin compounds related to hydration and exploration of the composition of foot skin in individuals with endogenous aetiologies leading to altered skin structure, function and therefore hydration (for example older people and people with diabetes). These are described in Section 2.3. followed by a review of the instances in which CRS has been used to measure skin composition within populations of interest, Section 2.4.

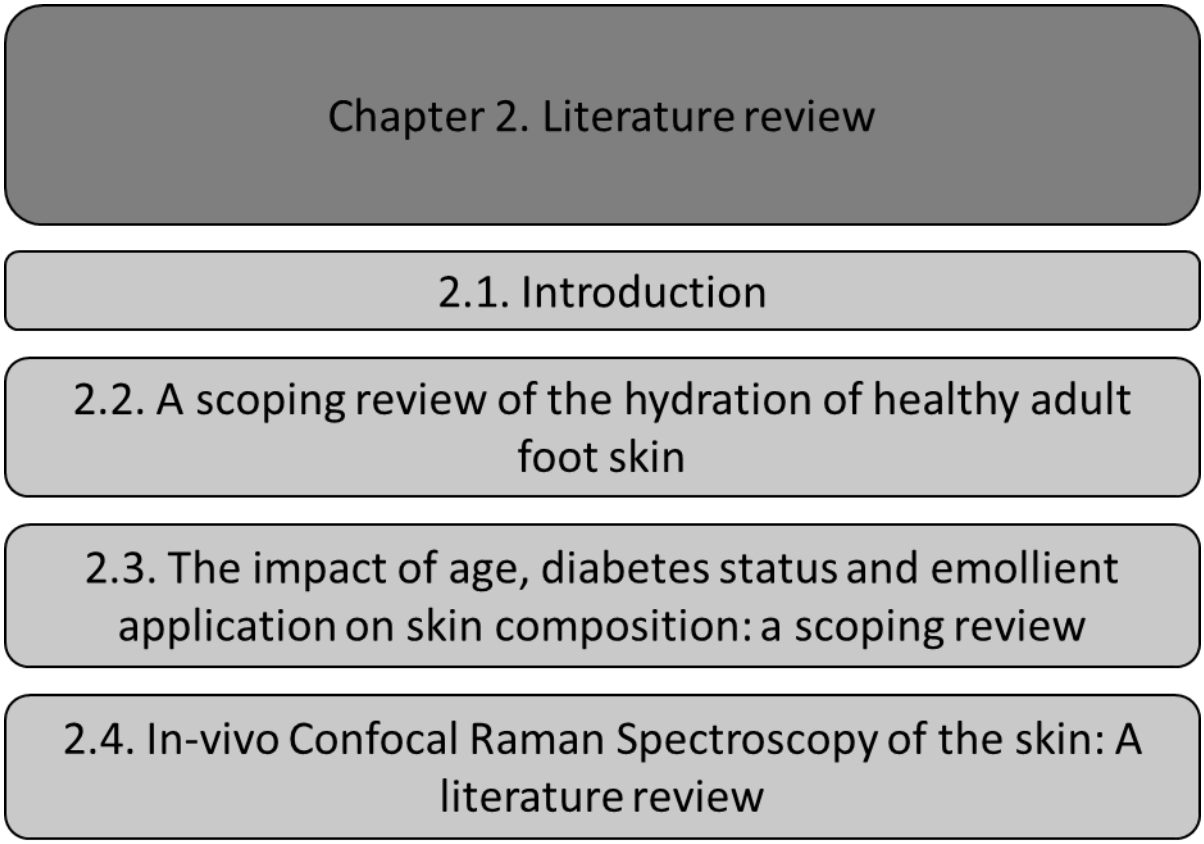


Figure 8. Literature review structure.

2.2. A scoping review of the hydration of healthy adult foot skin

2.2.1. Introduction

The overarching aim of this PhD project is to generate data on the characteristics of the soft tissues of the foot that assists the research and development processes of Scholl and represents a novel and worthwhile contribution to Podiatric knowledge.

A large range of characteristics can be attributed to soft tissues, such as elasticity, hardness, viscoelasticity, and hydration. No single characteristic was identified by the research team or colleagues at Scholl as a particular area of focus, generating a broad research question guiding this literature search:

What data are available on the soft tissue characteristics of the healthy adult foot?

A scoping method has been used for this review due to the broad range of literature included in the review and no focused research question having been established at the time of literature search (Peters et al., 2021).

2.2.2. Search strategy

The broad nature of this literature search restricts application of commonly used searching strategies such as PICO (patient or problem, intervention or exposure, comparison intervention or exposure , clinical outcome of interest) (Eriksen & Frandsen, 2018), SPIDER (sample, phenomenon of interest, design, evaluation, research type) and SPICE (setting, perspective, intervention, comparison, evaluation) (Eriksen & Frandsen, 2018), as no definitive populations, interventions or outcomes were being tested within this review.

Alternately, the PCC (population, concept, and context) framework has been used. The PCC is recommended for use by JBI (previously the Joanne Briggs Institute) to assist formation of an appropriate research question and inclusion criteria for scoping reviews (Peters et al., 2021). No specific population is selected for inclusion within this review. Concept and context are defined as any instances in which the soft tissues of the foot (context) have been quantified (concept).

2.2.3. Search approach and literatures screening

A literature search was conducted across four major health-literature databases in May 2020

(CINAHL, Cochrane Library, ProQuest Health and Medicine, ScienceDirect) to identify original research using the following keywords: **characterise, measure, study, skin, soft tissue, foot, plantar, sole and heel pad.**

Boolean words, tailored syntax and searching mechanisms were utilised within each database search engine to optimise the relevance of results (e.g., ‘:ti, ab, kw’ to capture a phrase only if it is present within the title, abstract or keywords) (See Table 2). The full process used to identify, screen, and ensure the inclusion of relevant literature can be found in Figure 9.

Table 2. Literature Searching Process.

Database	Date	Search Terms and Syntax
CINAHL	05/05/20	Characterize OR Measure OR Study AND Skin OR Soft Tissue AND Foot OR Plantar OR Sole OR Heel pad
Cochrane Library	07/05/20	((skin):ti,ab,kw OR (soft tissue):ti,ab,kw) AND ((foot) OR (sole) OR (plantar)) AND ((characterize):ti,ab,kw OR (measure):ti,ab,kw OR (study):ti,ab,kw)
ProQuest Health and Medicine	08/05/20	(ab(Characterize) OR ab(Measure) OR ab(Study)) AND (ab(skin) OR ab(soft tissue)) AND (noft(foot) OR noft(plantar) OR noft(sole) OR noft(heel pad))
ScienceDirect	13/05/20	(characterize OR measure OR study) AND (skin OR soft tissue) AND (foot OR plantar OR sole OR heel pad)

Within the first screening stage, exclusion criteria were: literature published within a language other than English, or where the characteristics discussed are not captured on the foot. Due to the keywords used within the literature search, some irrelevant studies were identified – e.g., where 'sole' was used in reference to a species of fish or where 'feet' was used in reference to the characteristic 'crow's feet' wrinkle within cosmetological research. By eradicating studies relating to these subjects within this initial screening, fewer studies required more in-depth examination during the second screening stage.

Within the second screening stage, exclusion criteria were: literature not on the soft tissue characteristics of the foot. 387 papers remained at the end of this screening process.

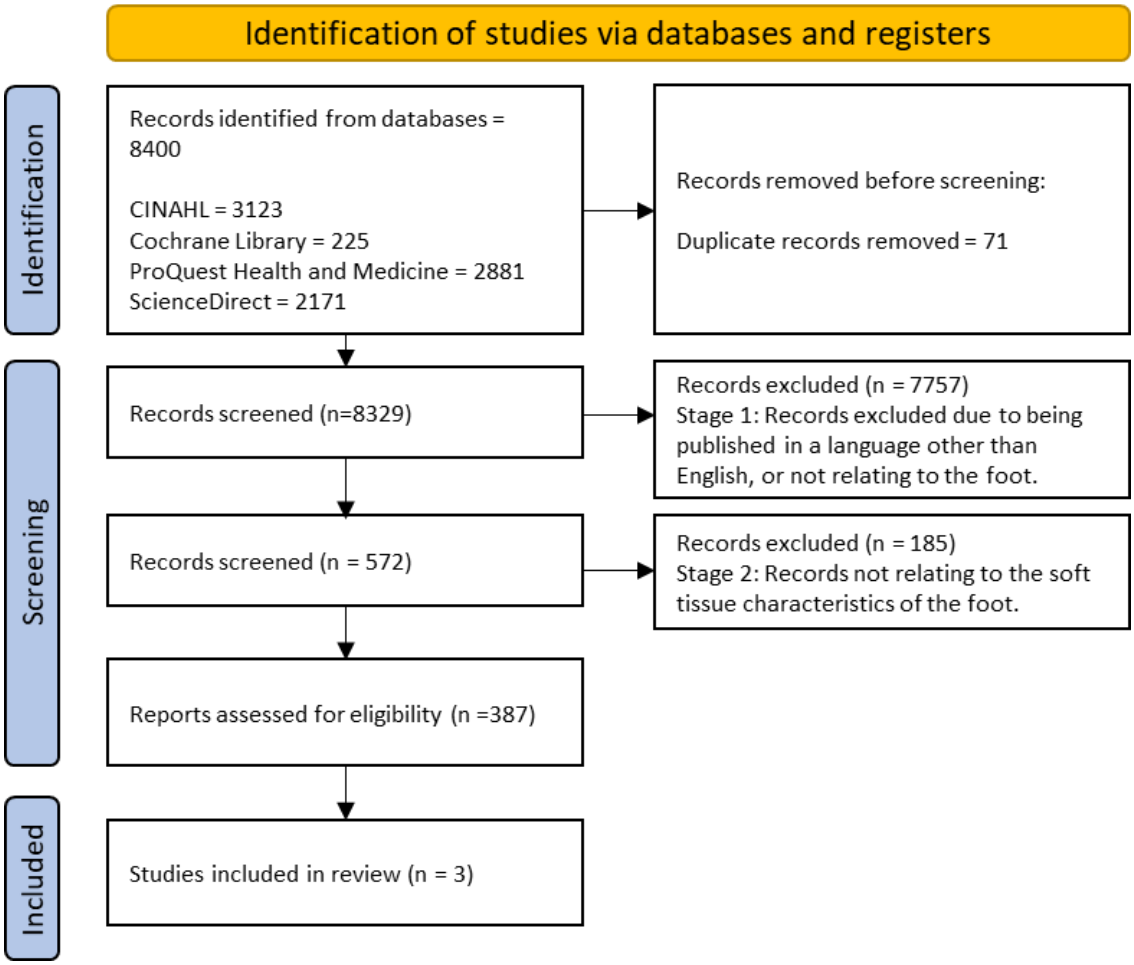


Figure 9. A record of the literature search and screening undertaken. PRISMA diagram format (McKenzie et al., 2021).

2.2.4. Study selection process

This initial search generated a large volume of resources with variable content.

At this time, an opportunity was identified to narrow the scope of the project. The materials remaining following the second screening were reviewed closely by the author, to familiarise themselves with which soft-tissue characteristics of the foot have been explored extensively and those that remain unexplored. This insight was combined with a 'Gap analysis' provided by Scholl Wellness Co to identify an area of study that would benefit Scholl and represent a novel and worthwhile contribution to Podiatric knowledge:

Scholl has a large range of products designed to hydrate the foot skin. These range from products intended for use on healthy foot skin (to 'maintain' hydration), to products intended to remedy moderate-severe xerosis or reduce hyperkeratosis. Within the gap

analysis provided by Scholl, several areas of focus were identified that aligned to this group of products. These are summarised below.

1. *Development of standards to predict the performance of emollients.* For example, according to their ingredients, by relating then to existing products with known efficacy or by identifying data that can be collected outside of a clinical trial setting that could contribute to emollient efficacy testing.
2. *Product claims support for emollients*. i.e., what data can be generated to support claims used in marketing, for example skin being perceived as smooth or soft by the consumer.
3. *Knowledge generation for the stages of heel fissure resolution*. For example, how do the biophysical characteristics of skin align with different severity of heel fissures? How does fissure resolution relate to foot skin hydration and lipid content?

For this reason, the research team met with the Research and Development team at Scholl to discuss their wider testing protocols for foot-skin emollients. Issues were described in the efficacy testing of emollients that were contributing to the areas of focus listed in the gap analysis and it was established that the effectiveness of their foot emollients was being assessed using a commercially available hydration measurement device (the Corneometer® CM825). The success of the product was being judged according to the same parameters as an emollient on other body areas. i.e., the expectation was that an effective emollient on the foot would generate the same magnitude of change from before treatment to after treatment that an emollient used elsewhere on the body would achieve.

From this discussion, and review of the materials captured during the literature search, two questions were raised:

Are commercially available hydration measurement devices suitable for use on the foot skin?

While reviewing the materials captured through the literature search, the author came across numerous instances where the foot skin hydration had been quantified using commercially available hydration measurement devices. However, no instances in which

these devices had been validated for use on the foot, in particular, the plantar surface of the foot.

Despite visible improvements in skin texture following emollient application, data collected using such a device by Scholl did not indicate a significant change in foot skin hydration. It is possible that the unique structure of the plantar skin makes these inappropriate for use on this area.

How should emollient efficacy be assessed on the foot skin?

If no published data are available, it is not possible to establish a benchmark for emollient effectiveness – either in the form of a 'target' hydration that indicates when a xerotic foot skin surface is restored to 'healthy' hydration levels or to identify what constitutes a clinically important change in foot skin hydration.

For these reasons, the scope of this review has been narrowed to include instances in which healthy foot skin hydration has been measured using commercially available hydration devices. The data collected and the suitability of the measurement devices used are discussed in the following section (Section 2.3).

2.3. Discussion of literature

Three papers have been identified that report data on the hydration of the foot skin in a healthy population, captured using several different commercially available hydration measurement devices. The mechanism these devices use to measure the water content of the skin is key to this project and leads to the discussion of another technology that changes the direction of this project considerably.

However, these papers provide the limited data currently available on the hydration of the foot skin and represent an opportunity to evaluate the methods used so far to measure foot skin content. Therefore, critique of these studies will provide insights into developing further research in this area.

For ease of reference, a summary of the study design for the papers discussed in this section is presented in Table 3.

Table 3. Summary of study design of papers relevant to project.

Paper title (authors)	Measurement device used, details, output.	Measurement locations	Environmental conditions (Acclimatisation period)	Participants and further details
The evaluation of three treatments for plantar callus: A three-armed randomised, comparative trial using biophysical outcome measures. (Hashmi et al., 2016)	Corneometer® CM825 (Courage-Khazaka, Cologne, Germany) a capacitance-based measure of skin hydration that captures data at <15 µm (Courage and Khazaka electronics GmbH, 2010). Output = AU.	Plantar aspect of the fifth metatarsal base (control site) All other sites have pathological lesions.	Average room temperature: 23.6 ± 1.0°C Average relative humidity: 53.1 ± 8.4% (15 minutes)	Group 1: n=21, 86% female, age range 24-68, median age 52. Group 2: n=20, 85% female, age range 23-73, median age 54. Group 3: n=20, 80% n=female, age range 24-68, median age 43.5. Exclusion criteria: Skin infections, dermatitis, psoriasis, unhealed skin wounds, ulcers or blisters. Systemic diseases (peripheral vascular disease, rheumatoid arthritis, diabetes, any foot or ankle musculoskeletal disorder). There is no use of foot products in the 48 hours before the screening appointment.
The effect of hydration on the risk of friction blister formation on the heel of the foot. (Kirkham et al., 2014)	Corneometer® CM825 co a capacitance-based measure of skin hydration that captures data at <15 µm (Courage and Khazaka electronics GmbH, 2010). Output = AU.	Posterior aspect of the heel Medial malleolus (control site)	Unreported (15 minutes)	20 (10 female) people aged >18 (age range = 20-43; median age = 23.5) Exclusion criteria: Self-reported skin disorders, diseases affecting vascular and neurological symptoms, systemic diseases, and musculoskeletal disorders of the foot and ankle. No use of anti-inflammatory medication, pain killing medication, steroids, immune-suppressant medication for 48 hrs before testing, and no use of topical applicants to the foot skin before data collection.
Biophysical measures of skin tissue water: variations within and among anatomical sites and correlations between measures. (Mayrovitz, Bernal, et al., 2013)	MoistureMeter D® (Delfin Technologies Ltd, Kuopio, Finland): 2.5 mm probe, 1.5 mm probe, 0.5 mm probe. MoistureMeter SC™ (Delfin Technologies Ltd.) Tissue Dielectric Constant (TDC) based measures of tissue water, spanning various depths (EvaluLab, 2018). Output = AU.	Forehead, Cheek Forearm anterior Forearm dorsum, Hand palm (thenar) Hand palm (centre), Thumb pulp Hand dorsum (web) Hand dorsum (mid) Medial gaiter Anterior gaiter (shin) Lateral gaiter Medial peri-malleolus Foot dorsum (1–2 toe) Foot dorsum (4–5 toe) Great toe dorsum Great toe plantar	Start temp: 24.4 ± 1.4°C End Temp: 24.9 ± 1.2°C. Relative humidity at start: 34.9 ± 4.7% Relative humidity at end: 35.1 ± 4.6%. (10 minutes)	32 females aged >18 (age range = 19-77; mean ± SD age = 33.0 ± 13.9) No implanted wires or electronic medical devices, no evidence of abnormal skin condition or open wounds in the vicinity of any measurement sites. No skin cream or lotion application on the day of data collection.

2.3.1. Paper 1: (Kirkham et al., 2014)

Device used: The Corneometer® CM825 (Courage and Khazaka, Colne, Germany).

This investigation aimed to examine the relationship between skin hydration and the risk of friction blister formation on the posterior of the heel. The protocol used within this study has been discussed in detail in Section 2.3.3.

Hydration was measured using the Corneometer® CM825. This device uses the capacitance method to measure skin hydration at a depth of 15 µm (see Chapter 4 for a detailed description of the device).

Hydration data were collected at a test site (posterior heel) and a control site (inferior medial malleolus) (See Figure 10) on each side of the body for each participant. This was undertaken after a body site was randomly selected for experimentation for each participant (hence the data being labelled as hydrated and non-hydrated) but before submersion in water and cyclic shear force application.

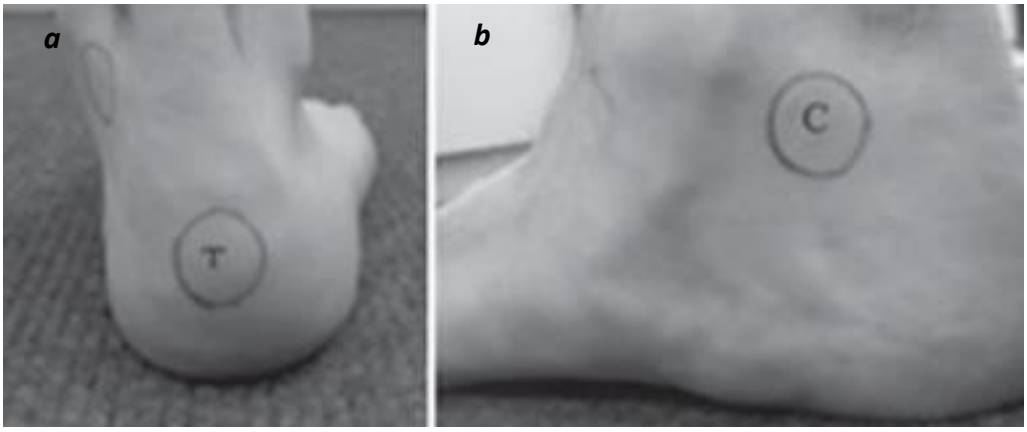


Figure 10. Measurement location diagram. a: T=Test site b: C=Control site from 'The effect of hydration on the risk of friction blister formation on the heel of the foot' (Kirkham et al., 2014). Used with permission.

Despite only having a small number of participants (two groups n = 10) and two measurement locations, the data collected in this study provides valuable insight into how the water content of the SC can vary significantly between skin sites that are close in proximity, and between individuals. The median values at the test site were slightly lower than those observed at the control site, as were the minimum and maximum hydration values observed at these locations (Control Site (median and range): 24.47 AU, 14.82-95.86

AU and 24.09 AU, 16.62-99.30 AU; Test Site (median and range): 21.73 AU, 12.66-60.56 AU and 23.60 AU, 10.00-78.54 AU (See Table 4).

The interquartile range (IQR) for the non-hydrated control site was lower than the test site on the non-hydrated foot (Control site IQR: 17.26, Test site IQR: 28.60), suggesting that the hydration measures at this location were more consistent between individuals than the test site. However, this is not the case on the hydrated foot (Control site IQR: 19.77, Test site IQR: 17.54).

Table 4. Baseline hydration values. Test site = Posterior Heel, Control Site = Inferior Medial Malleolus (Kirkham et al., 2014).

	Hydrated foot (n=10)		Non-Hydrated foot (n=10)	
	Test Site	Control Site	Test site	Control site
Skin surface hydration (AU)				
Median	23.60	24.09	21.73	24.47
Minimum	10.00	16.62	12.66	14.82
Maximum	78.54	99.30	60.56	95.86
Interquartile range	19.77	17.54	28.60	17.26

If this difference were consistent between both data sets, this might be explained by morphological differences at the skin sites. The posterior heel area is typically exposed to more compression and shear than the inferior malleolar area during shod walking, which could lead to SC thickening (Kim et al., 2010; Rubin, 1949; Thomas et al., 1985). It is also possible that the posterior heel skin has some similar features to the thick plantar skin due to its proximity to the plantar heel. Uemura et al (2016) investigated the point of delineation between plantar and non-plantar skin on the inferior malleolar skin using the skin characteristics associated with plantar skin (increased SC thickness, K9 expression, reduced elastic fibres) to identify the edge of the plantar surface, however this was not applied to the posterior heel. In palmoplantar keratoderma, plantar skin thickening can extend onto the posterior heel, also indicating that the morphology of the plantar tissue may extend beyond the plantar surface (Guerra et al., 2018; Sakiyama & Kubo, 2016).

Within this study, this difference in IQR between the test and control sites is not reflected within both groups (Kirkham et al., 2014). Data collection from a larger cohort would be required to establish whether this is an accurate reflection of the typical distribution of hydration data from the posterior heel and inferior medial malleolar site. Kirkham et al

(2014) do not describe having conducted a power calculation to ascertain an appropriate sample-size for this work.

Finally, of interest within this study is the use of the contralateral foot as a control: by confirming that there was no difference between feet as baseline (p-value = 0.452), the authors used paired inferential statistics for their analysis (Kirkham et al., 2014). This is a positive aspect of protocol design and could be useful in future studies measuring foot skin hydration.

2.3.2. Paper 2: (Hashmi et al., 2016)

Device used: The Corneometer® CM825.

This study aimed to investigate the efficacy of three different treatments for callus: application of potassium hydroxide (KOH, 40%), trichloroacetic acid (TCA) or routine podiatry care (sharp debridement). The researchers recorded several biophysical parameters of the treated skin (an area of plantar callus) and a control site (the plantar surface at the base of the 5th metatarsal on the same foot) (Hashmi, Nester, et al., 2015). Data collected from the control site are the only data collected from healthy plantar foot skin in the study and will be directly compared with data from other studies in this review.

Due to the different randomly allocated interventions investigated in this study, participants were split into three groups and data were reported for each. Despite the similarity in demographic data between the groups (See Table 5), there are some differences in hydration at the control and test sites at baseline (no p-value provided) (See Table 6). Hydration change from baseline was therefore used to assess treatment efficacy (Hashmi et al., 2016).

It is not understood whether this variation in baseline hydration is the result of undetermined variation in characteristics between participant groups. If other data were available for healthy plantar SC hydration it would be possible to discuss the magnitude of these differences in reference to the typical variation between individuals. Due to the relatively small participant numbers (n = 21, 20, 20) data is readily influenced by a minor variation in individual values.

Table 5. Baseline characteristics of participants within three intervention groups (Hashmi et al., 2016).

Variable	Type of intervention		
	Podiatry (n=21)	Potassium hydroxide (n=20)	Trichloroacetic acid (n=20)
Age (years)			
Median	52	54	43.5
Minimum, maximum	24, 68	23, 73	24, 68
Interquartile range	24.5	26.75	19
Height (m)			
Median	1.57	1.65	1.47
Minimum, maximum	1.42, 1.98	1.47, 1.88	1.49, 1.88
Interquartile range	0.23	0.11	0.18
Sex (% female)	86	85	80

Table 6. Baseline data for three intervention groups (Hashmi et al., 2016).

	Skin sites					
	Podiatry (n=21)		KOH (n=20)		TCA (n=20)	
	Callus	5th metatarsal base	Callus	5th metatarsal base	Callus	5th metatarsal base
Skin surface hydration (AU)						
Median	2.83	8.67	1.96	9.05	3.45	10.35
Minimum, maximum	0.00, 15.55	3.03, 22.0	0.20, 16.07	3.5, 50.41	0.48, 13.24	0.36, 28.37
Interquartile range	3.46	7.98	1.96	8.10	3.07	10.02

The hydration at the control site in this study (plantar surface of the 5th metatarsal (median (AU): 8.67, 9.05, 10.35) is lower than that measured by Kirkham et al (2014), who reported on the medial peri-malleolus (median (AU): 24.09, 24.47), and the posterior heel (median (AU): 23.6, 21.73). Similarly, the IQR for these data are also much lower (plantar 5th metatarsal site IQR: 7.98, 8.10, 10.02; medial peri-malleolus IQR: 17.54, 17.26; posterior heel IQR: 19.77, 28.60). This supports possible variations in morphology at these skin sites, which could have implications on the data collected the Corneometer® CM825 due to its shallow measurement depth.

The SC is the epidermal layer with the lowest water content, and is much thicker on the plantar skin than on the non-plantar skin (Boyle et al., 2019). The mean thickness of plantar SC is (mean ± SD) 487.7 ± 160.2 µm (Vela-Romera et al., 2019), where the same population's dorsal foot SC (the most equivalent location to the non-plantar sites discussed here) thickness is 35.9 ± 27.8 µm. The measurement depth of the Corneometer® CM825, as described by the manufacturer, is 15 µm (Courage & Khazaka electronics GmbH, 2010). This means that the Corneometer® CM825 collects data from a relatively superficial depth. Therefore, measures taken on thick SC will only capture hydration within the superficial portion of the SC, whereas from non-plantar skin (relative thin SC) it may capture hydration

beyond the SC, i.e., deeper epidermal or even dermis layers. These data suggest that the hydration of the plantar SC at this measurement depth is lower, and more consistent between individuals, than the hydration of the same measurement depth within non-plantar SC.

This theory is strengthened by the lower SC hydration measures from callus tissue (Callus median (AU): 2.83, 1.96, 3.45, plantar 5th metatarsal head median (AU): 8.67, 9.05, 10.35). These findings support that the more superficially the SC is measured, the lower the hydration values collected, or that the most superficial layers of the SC are very dry.

However, there are some other considerations to be made of the sampling techniques used by the authors and how they could impact upon data. Kirkham et al (2014) tested the feet of 20 participants and analysed these data separately. However, Hashmi et al (2016) tested the plantar skin on both feet of the participants where they presented with bilateral callosities and included these data within the same analysis. Therefore, data collected from one individual may appear twice within the analysis. If hydration levels are more homogenous within an individual than between individuals that could artificially reduce the variability within the data (Rogiers et al., 1990), which has implications on the validity of the data collected (Menz, 2004). Future research should consider the sampling techniques used to ensure data collected is representative of a whole population and not disproportionately influenced by the values derived from one individual.

Rogiers et al (1990) investigated the standardized conditions required to collect accurate hydration data. These recommendations will be considered in the context of the investigations conducted by Hashmi *et al*. (2016) and Kirkham *et al.* (2014).

2.3.3. Considerations of method design for skin hydration studies

2.3.3.1. Participant demographics

The generalisability of the data collected within these studies is restricted due to the relatively small participant numbers (Hashmi et al, 2016 n= 61: Kirkham et al, 2014 n=20). This issue is exacerbated by the breakdown of participants into multiple smaller groups for analysis. Hashmi et al (2016), describe the use of a power-calculation to determine their participant number, however, this was designed to allow for the detection of a difference

in xerotic skin hydration following an intervention, not for the detection of differences in physiologically health skin between groups.

Additionally, there are some inconsistencies between the population demographics between these studies that may have an influence on skin hydration. In the study by Hashmi et al (2016), the age range of participants was significantly broader and had a higher median value (Median – 48 years, range - 23-78 years) than the age range in the study by Kirkham *et al* (2014) (Median: 23 years., Range: 20-43 years). This may partly be attributed to the larger sample size within the study conducted by Hashmi et al (2016), however, more likely stems from the population from which they recruited: Kirkham *et al* (2014) recruited staff and students from a university, whereas Hashmi *et al* recruited using flyers within a university, as well as via an advertisement in a local newspaper.

Increased age is associated with reduced skin hydration (Barel, 2006; Darlenski et al., 2009; Farinelli, 2006). These discrepancies create uncertainty in the comparison of data between these studies. It is possible that the higher and less variable hydration measures collected in the latter study were a result of a younger participant group, rather than a representation of differing hydration levels between skin sites (Du Plessis et al., 2013).

Kirkham et al (2014) also had a much lower proportion of female participants (50%) than Hashmi et al (2016) (80%, 85% and 86%). Although the influence of sex on foot skin hydration has never been explicitly explored, sex has not been reported to have a significant influence on skin hydration elsewhere on the body (See Table 1).

2.3.3.2. Study design

It has previously been demonstrated that environmental temperature and humidity may influence hydration measurements taken from the human skin surface (Nam et al., 2015). Although Kirkham et al (2014) report that the temperature and hydration of the testing environment were monitored, the values collected were not reported. Hashmi et al *(2016),* however, reported that the average room temperature and relative humidity recorded during data collection were 23.6 ± 1.0 °C and 53.1 ± 8.4 %, respectively; this demonstrates relative consistency in environmental factors between measurements. Reporting environmental conditions is essential as it enables the reader to assess whether the

requirements for the use of the hydration measurement device specified by the manufacturer were met.

Both of these studies reported using an acclimatisation period of 15 minutes, which, although advised within some literature (Berardesca et al., 2018), represents half of the time recommended elsewhere (Rogiers et al., 1990; Serup et al., 2006). Using a short acclimatisation period may introduce variability within the data (due to instability in the skin hydration following activity- i.e., due to perspiration). This would be limited through an extended acclimatisation period (Rogiers et al., 1990).

The length of time between cleansing of the skin and hydration testing should be a minimum of 5 hours. Rogiers et al (1990) found that the skin hydration was less variable 5 and 7 hours after cleansing than at 2-hours post-cleansing, however this was only established on the ventral forearm. Equivalent data from the palm, forehead, and wrist was too variable at all three time-points to determine the impact of cleansing on hydration (Rogiers et al., 1990). No information is presented on the time that passed since skin cleansing by Hashmi et al (2016) or Kirkham et al (2014).

Rogiers et al (1990) also demonstrated that application of an emollient can influence skin hydration, although the impact of this is dependent on skin type (as determined by a cosmetician) and emollient formulation (Rogiers et al., 1990). Although participants were required to refrain from using topically applied products on the feet before the data collection period for the work conducted by Hashmi et al (2016) and Kirkham et al (2014), only one study specified the length of time required for this (Kirkham et al., 2014). As the effect of these products on the hydration of the skin is heavily dependent upon their formulation, it is not possible to determine whether this time period was sufficient within this instance (Rogiers et al., 1990).

Although it is possible to highlight some limitations of the studies discussed above, the data collected through these investigations are of great value. These studies represent the only source of data on the hydration of the SC on healthy foot skin that is collected using the same device under similar circumstances, allowing for comparison between locations and discussion of variables that require consideration when measuring hydration.

In the next section, other devices are discussed that quantify the water content of the SC using a slightly different mechanisms to the Corneometer® CM825.

2.3.4. Paper 3: (Mayrovitz, Bernal, et al., 2013)

Device used: The MoistureMeter SC™ (Delfin Technologies, Kuopio, Finland) and MoistureMeter D® (Delfin Technologies, Kuopio, Finland)

Two devices were used to measure the water content of the skin and soft tissues at 17 locations across the body (See Table 7); five of which were located on the foot and five on the hand. Of the foot skin sites, one of these was the plantar surface (plantar hallux), three locations were on the dorsum of the foot (dorsal hallux, 1-2 toe dorsum, 4-5 toe dorsum) and one on the hindfoot (medial peri-malleolus) (See Figure 11).

Table 7. Measures of tissue hydration from skin sites using the MoistureMeter SC™ and MoistureMeter D® amended from Mayrovitz et al (2012).

	Hydration measurement device and units			
Measurement site	MoistureMeter SC™ (AU)	MoistureMeter D® 0.5 mm probe (TDC)	MoistureMeter D® 1.5 mm probe (TDC)	MoistureMeter D® 2.5 mm probe (TDC)
Great toe plantar (mean ± SD[†])	30.1 ± 17.8	31.6 ± 5.0	33.9 ± 3.9	38.1 ± 3.9
Great toe dorsum (mean ± SD)	24.0 ± 14.9	33.0 ± 5.5	33.6 ± 4.0	34.0 ± 4.3
Foot dorsum (1-2 toe) (mean ± SD)	15.8 ± 14.4	27.9 ± 4.1	28.2 ± 3.2	28.2 ± 3.5
Foot dorsum (4-5 toe) (mean ± SD)	13.6 ± 11.6	27.7 ± 3.6	26.7 ± 2.8	26.9 ± 3.0
Medial peri-malleolus(mean ± SD)	10.0 ± 8.9	27.1 ± 4.6	26.7 ± 3.6	26.6 ± 33.5

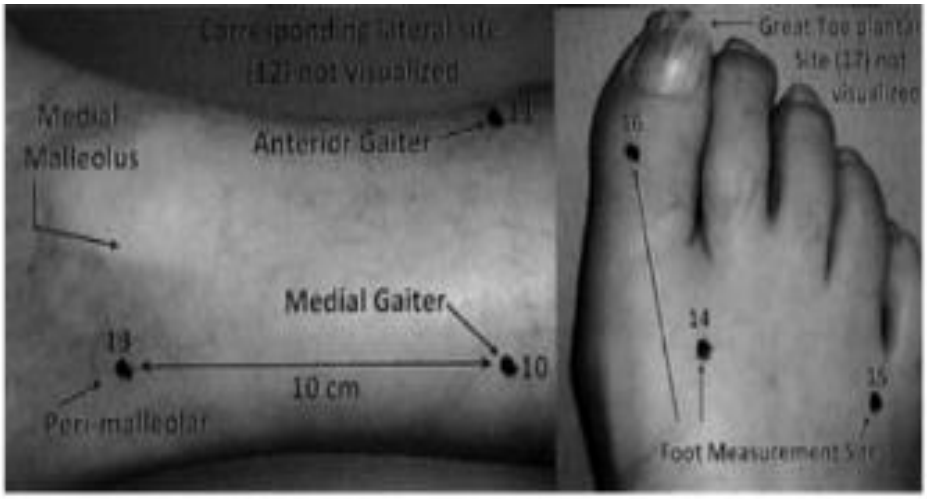


Figure 11. Skin sites used for testing within 'Biophysical measures of skin tissue water: variations within and among anatomical sites and correlations between measures' (Mayrovitz, Bernal, et al., 2013).

[†] SD = Standard deviation

2.3.4.1. MoistureMeter SC™ Data

The MoistureMeter SC™ uses capacitance to measure the water content of the skin, although provides a different output to the Corneometer® CM825, described by the manufacturers as 'effective hydration' of the SC, as opposed to the water content of tissue as a specific depth or a range of depths as is purported by other devices (Delfin Technologies, 2016). This vague descriptor is justified by Alanen et al (2004), who details the measurement mechanism of this device, before summarising "the MoistureMeter measures the 'effective hydration', taking into account the water content of the dry layer and its thickness" (direct quote (Alanen et al., 2004)). The author, however, does not indicate how the thickness of the dry layer is quantified. For simplicity, for the remainder of this document, the output of the MoistureMeter SC™ will be referred to as 'hydration'. Technical details on the specification of this device can be found in section 4.2.

The hydration of the foot skin measured in this study varies between skin sites. The highest value was reported on the plantar hallux (mean ± SD: 30.1 ± 17.8 AU), followed by the dorsal hallux (24.0 ± 14.9 AU), the dorsal foot proximal to toes 1-2 (15.8 ± 14.4 AU), the dorsal foot proximal to toes 4-5 (13.6 ± 11.6 AU), and the medial peri-malleolus (10.0 ± 8.9 AU) (Table 7).

Although data collected using the MoistureMeter SC™ device are not directly comparable to those collected using the Corneometer® CM825 (Alanen et al in 2004), the existence of common measurement sites between this study and those previously discussed facilitates their comparison (Hashmi et al., 2016; Kirkham et al., 2014):

When measured using the Corneometer® CM825, the plantar skin underlying the 5th metatarsal head displays much lower hydration values than the skin inferior to the medial malleolus (5th metatarsal head (AU): 8.67, 9.05 and 10.35, inferior medial malleolus (AU): 24.09 and 24.47) (Hashmi et al., 2016; Kirkham et al., 2014). However, when measured using the MoistureMeter SC™, the plantar skin (plantar hallux) has much higher hydration values than the skin inferior to the medial malleolus (plantar hallux (AU): 30.1, medial malleolus (AU): 10) (Mayrovitz, Bernal, et al., 2013).

This could be interpreted in several ways. Firstly, it could be assumed from these data that the plantar skin hydration varies significantly across the surface of the foot (Hashmi,

Nester, et al., 2015). Due to the limited research available on the structure of the plantar skin across its breadth, this cannot be excluded. However, it is also possible that this pattern may have developed because of the protocols used for measuring skin hydration, or the measurement mechanisms of the Corneometer® CM825 and the MoistureMeter SC™. These will be discussed further below, following interpretation of the data collected within this study using a third commercially available hydration measurement device, the MoistureMeter D®

2.3.4.2. MoistureMeter D® Data

The MoistureMeter D® uses Tissue Dielectric Constant (TDC) to measure the water content of tissues at a deeper level than the MoistureMeter SC™, penetrating to 0.5 mm, 1.5 mm, 2.5 mm and 4 mm depending on probe selection. This is a well-established technique that uses the dielectric properties of human tissues to indicate their water content, that is widely used to monitor tissue water (Mayrovitz, McClymont, et al., 2013; Miettinen et al., 2004; Nuutinen et al., 2004).

Mayrovitz et al. (2012) demonstrate that the hydration profile of the plantar and palmar surfaces differs significantly from those found at other body areas.

Historically, TDC values collected by other researchers on the forearm have been found to decrease monotonically with increasing depth; this is thought to result from the transition from the water-rich dermis to the lipid-based subcutaneous fat layer (Mayrovitz & Luis, 2010). Across the 17 body sites examined by Mayrovitz et al. (2012), however, this pattern was only observed in 6 locations (the forehead, two forearm sites, and three sites on the calf) and 8 locations did not show any significant change in TDC with increased tissue depth (cheek, two dorsal hand sites, three dorsal foot sites and the thumb pulp). Interestingly, 3 locations displayed an inverse trend, i.e. the water content increased with increased tissue depth – the plantar hallux and two palmar sites (Mayrovitz, Bernal, et al., 2013).

The authors propose that this difference may be due to the relatively small amount of subcutaneous fat and the high volume of eccrine sweat glands on plantar and palmar tissues (Mayrovitz, Bernal, et al., 2013). However, when the specialised features of the epidermis within these locations are considered, it is possible that other, unexplored,

factors could contribute to this distinction. These factors are discussed in detail within section 2.3.7.

2.3.4.3. Comparisons between data obtained using the MoistureMeter D® and MoistureMeter SC™

As the MoistureMeter D® and MoistureMeter SC™ data were collected simultaneously at the same anatomical locations (Mayrovitz, Bernal, et al., 2013), analysis was conducted to investigate the relationship between hydration of the superficial epidermal layers (using the MoistureMeter SC™) and the underlying tissues (MoistureMeter D®).

A positive correlation was identified between the MoistureMeter D® and MoistureMeter SC™ data, most evident from the plantar and palmar skin data. When all locational data were combined, the positive correlation became less significant as the MoistureMeter D® measurement depth increased (correlation coefficient: 0.5 mm: r-value =0.604, 1.5 mm: r-value =0.568, 2.5 mm: r-value =0.424). Although when this same pattern was displayed by the data analysed within locations, the plantar and palmar tissues displayed significantly higher correlation coefficients (see Table 7) between MoistureMeter SC™ values and the shallowest MoistureMeter D® measures.

The greatest MoistureMeter SC™- MoistureMeter D® correlation across all locations was found at the thumb pulp location (r-value = 0.803 at 0.5 mm depth). Although the remainder of the data on the 0.5 mm SC-TDC correlation coefficients are unfortunately unreported, it is noted that the strength of the correlation coefficient between the MoistureMeter SC™ and MoistureMeter D® data at different measurement depths varies only slightly. Equivalent data are presented for the 1.5 mm MMD data (See Table 8). This shows the highest correlation coefficient at the plantar hallux (r-value = 0.786), followed by the palm (r-value = 0.779) and thumb pulp (r-value = 0.773). This demonstrates that the MoistureMeter SC™- MoistureMeter D® correlation is strongest at the plantar and palmar sites.

Table 8. Correlation coefficient (r-value) between data collected using MoistureMeter SC™ and MoistureMeter D® 1.5 mm probe by Mayrovitz et al 2012.

#	Measurement site	r-value	p-value
17	Great toe plantar	0.786	0.001
6	Hand palm (centre)	0.779	0.001
7	Thumb pulp	0.773	0.001
13	Medial peri-malleolus	0.724	0.001
5	Hand palm (thenar)	0.669	0.001
16	Great toe dorsum	0.588	0.001
2	Cheek (middle)	0.545	0.001
15	Foot dorsum (4-5 toe)	0.529	0.002
14	Foot dorsum (1-2 toe)	0.439	0.009
9	Hand dorsum (mid)	0.415	0.018
1	Forehead (middle)	0.384	0.031
3	Forearm anterior	0.374	0.035
8	Hand dorsum (web)	0.371	0.037
4	Forearm dorsum	0.358	0.044
11	Anterior gaiter (shin)	0.322	0.072
12	Lateral gaiter	0.257	0.155
10	Medial gaiter	0.063	0.524

These data represent a phenomenon not previously observed that is unique to the plantar and palmar skin, that the hydration of the skin at these locations correlates with the deeper measures of tissue hydration at these sites, in an inverse pattern to other body areas, and to a much higher degree (Mayrovitz, Bernal, et al., 2013). This could be due to the unique structure of the skin at these sites and the measurement mechanism employed by the MoistureMeter SC™ and MoistureMeter D®, which are discussed in section 2.3.6.1.

2.3.4.4. Experimental design

Due to the researchers' interest in lymphoedema monitoring with TDC, all 32 participants in this study were female (Mayrovitz, Bernal, et al., 2013). Although this represents a significantly different population to the two studies previously discussed, this is of little consequence as when comparisons are made between age - matched men and women no significant differences can be found in SC hydration, albeit this has only been confirmed on the head and forearm using the Corneometer® CM820 (Rogiers et al., 1990).

The participants within this study were of a similar age range (19-77 years) to those tested by Hashmi et al (2016) (23-73 years), however had a lower average age ((Mayrovitz, Bernal,

et al., 2013): mean 33 years, (Hashmi et al (2016): median 48 years). This is in contrast to the age of participants of the Kirkham et al (2014) work who had a much smaller age range and a lower average age (age range: 20-43, median age 25.5). This difference in population age demographics further reduces comparisons that can be drawn between this study and previous equivalent works, beyond the different tools used.

In this study, measurements were taken from each body site sequentially, starting from the forehead and working down to the plantar hallux location. This was repeated for each measure, first, with the deepest measuring MoistureMeter D® probe, then the shallowest, before the MoistureMeter SC™ measurements and TEWL measures were taken. Although the time between sequential measures at each location was regulated using this method, the lengthy data collection period (between 42 and 67 minutes) would have resulted in some skin sites being acclimatised to the laboratory environment and body position for a longer period before the first measurement was taken, which could potentially influence the validity of measurements (Nam et al., 2015). This issue is not reflected in the work conducted by Hashmi et al (2016) and Kirkham et al (2014) due to the smaller number of locations they were collecting data from.

Finally, study participants were required to cease applying topical products for the day of the study (Mayrovitz, Bernal, et al., 2013). In contrast, other studies measuring baseline hydration levels (chiefly for the evaluation of emollient effectiveness) have requested abstaining from product use from between two days up to two weeks (Baalham et al., 2011; Federici et al., 2012; Garrigue et al., 2011; Hashmi et al., 2016). Although there is a possibility that this may have influenced the hydration measures, as mentioned previously, it is not possible to determine the impact of this due to high variability in the effect of topically applied products (Rogiers et al., 1990).

2.3.5. Review of findings

These research studies provide insights on the hydration of the foot skin, and the devices used to measure skin hydration.

Firstly, these data demonstrate that skin hydration varied across the surface of the foot. This has been established both through the use of the Corneometer® CM825 by Hashmi et

al (2016) and Kirkham et al (2014), and through the use of the MoistureMeter SC™ (2013). The Corneometer® CM825 data indicated the plantar skin is less-hydrated than the non-plantar skin (plantar surface of the 5th metatarsal (median (AU): 8.67, 9.05, 10.35), medial peri-malleolus (median (AU): 24.09, 24.47), posterior heel (median (AU): 23.6, 21.73) (Hashmi et al., 2016; Kirkham et al., 2014), whereas the MoistureMeter SC™ reports higher values on the plantar skin than non-plantar skin (plantar hallux (mean ± SD): 30.1 ± 17.8, dorsal hallux: 24.0 ± 14.9, dorsal foot proximal to toes 1-2: 15.8 ± 14.4, dorsal foot proximal to toes 4-5: 13.6 ± 11.6, medial peri-malleolus: 10.0 ± 8.9).

Secondly, the data obtained using the MoistureMeter D® indicate that the hydration profile of the plantar and non-plantar tissues differ. The MoistureMeter D® measures taken from the plantar skin show increasing hydration from the shallower to deeper tissue depth. For example, hallux plantar values (mean value (measurement depth)): 31.6 (0.5 mm), 33.9 (1.5 mm), 38.1 (2.5 mm)). This is contrary to the measurements collected on non-plantar and non-palmar sites that displayed no significant hydration gradient at different depths of the soft tissues, or an inverse gradient where tissue hydration decreases with increased depth. As found on the forearm, forehead and three calf sites: forearm dorsum values (mean value (measurement depth)): 31.4 (0.5 mm), 29.2 (1.5 mm), 26.6 (2.5 mm)).

Thirdly, the MoistureMeter SC™ data collected on the plantar and palmar tissues correlate strongly with MoistureMeter D® data than on any other body site. The MoistureMeter D® measures collected from the plantar foot location displayed high correlation coefficients (1.5 mm probe - great toe plantar (r=0.786)) with the 'effective hydration' measures collected from the overlying SC, particularly evident within the shallower MoistureMeter D® measurement depths. This phenomenon is not observed to such an extent within non-plantar and non-palmar locations.

When these three findings are considered collectively, alongside the postulations on callus and skin hydration included in Section 2.3.2. it becomes evident that the mechanism of hydration measurement, and the unique structure of the plantar skin, is highly relevant to the interpretation of hydration data collected using commercially available hydration measurement devices.

The next section discussed the evidence-base of SC hydration gradients and related this to the data from the three review papers at different SC depths.

2.3.6 The SC hydration gradient

The SC hydration gradient refers to the changing concentration of water present at different SC depths. This was first detected by electron probe analysis on skin biopsies taken from the lower leg (Warner et al., 1988). This investigation revealed a gradual increase in the water content of the SC from approximately 15% at the surface of the SC, to 70% at the junction between the SC and the stratum granulosum (Warner et al., 1988).

The SC hydration gradient was further explored by Egawa et al (2007), who used Confocal Raman spectroscopy (CRS). This is a non-invasive method of examining the components of a biological substance using scattered light (Vandenabeele, 2013). Egawa et al suggested that the defined hydration gradient within the SC could be used as a proxy measure for SC thickness (Egawa et al., 2007). The hydration gradient in the cheek, upper arm, volar forearm, hand dorsum and palm skin were measured, and SC thickness was calculated. Palmar skin was 5-10 times thicker (173.0 μm) compared to the cheek (16.8 μm), the volar forearm (22.6 μm) and hand dorsum (29.3 μm) (see figure 10) (Egawa et al., 2007).

All hydration profiles followed the same pattern regardless of skin location, and displayed consistency between participants, starting at approximately 30% water concentration at the most superficial layer of tissue (twice the 15% value observed by Warner, Myers and Taylor in 1988) and becoming saturated at approximately 70%, the presumed interface between the SC and the stratum granulosum (Egawa et al., 2007) (See Figure 12).

Palmar skin, however, displayed a relatively shallow hydration gradient within the more superficial SC tissue layers until approximately 70% of SC thickness; at which point the hydration level increased rapidly to a constant level of between 55% and 70% indicating the start of the stratum granulosum layer (See Figure 12) (Egawa et al., 2007). This unique hydration profile is important as it demonstrates that the thickened SC is not just a drawn-out replica of a non-palmar SC, but a different profile entirely, characterised by an extended superficial portion of consistently low hydration. These data also displays less homogeneity between participants than non-palmar skin sites (Egawa et al., 2007).

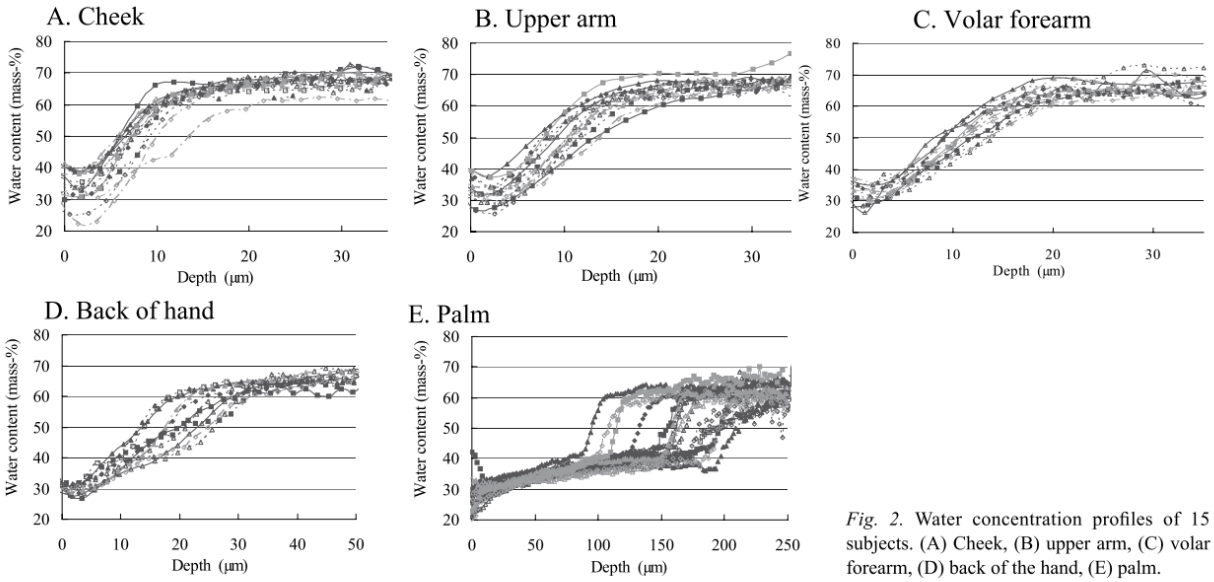


Fig. 2. Water concentration profiles of 15 subjects. (A) Cheek, (B) upper arm, (C) volar forearm, (D) back of the hand, (E) palm.

Acta Derm Venereol 87

Figure 12. Water concentration profiles of different skin locations of 15 subjects (Egawa et al., 2007). Used with permission.

2.3.6.1. Implications of the use of different commercially available electrical hydration measurement devices

The commercially available hydration measurement devices described in published research to data all collect data from different measurement depths (see Chapter 4). These become of major importance when the hydration gradient of the SC is considered.

The Corneometer® CM825 is described as measuring water content of tissues at approximately 15 µm or below (Courage and Khazaka electronics GmbH, 2010; Fluhr et al., 1999a) (See Figure 13. A. Cheek). With the added insight into SC hydration gradient afforded by CRS, it is clear that the hydration of the palmar SC rises much more gradually across its depth - rising from 30-40% across almost the full depth of the SC, followed by a rapid rise at the junction between the SC and SG (depth of 90-190 µm (See Figure 13. E. Palm)). If the Corneometer® CM825 is applied to the cheek and the palm the value returned for the cheek will represent the water content around the junction between the SC and the stratum granulosum (See the blue line inserted at 15 µm depth on Figure 13). In contrast, the same measurement taken from the palm skin will be representative of a superficial

layer of the SC and is therefore not comparable (See the blue line inserted at 15 µm depth in Figure 13 diagram “E. Palm”).

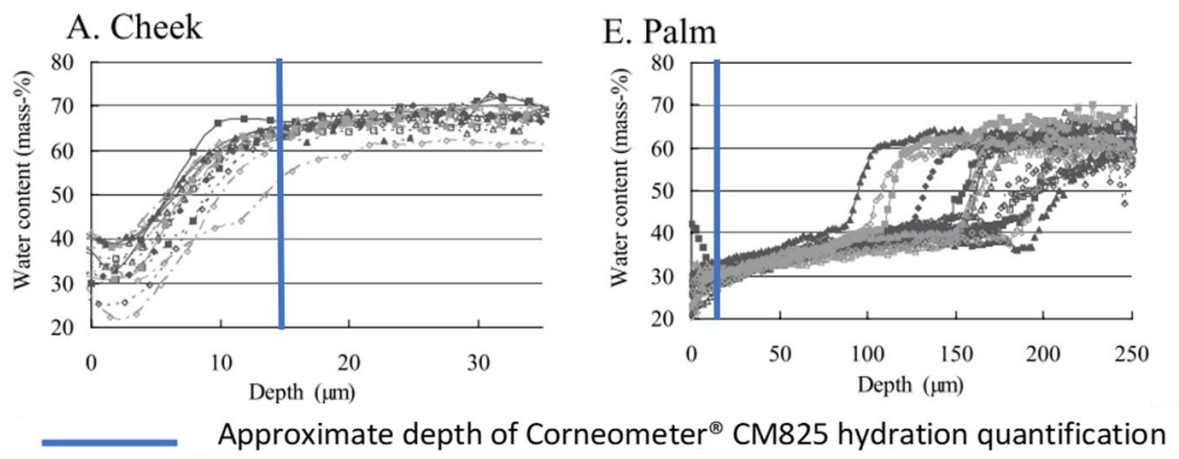


Figure 13. A diagram demonstrating the hydration measurement depth using the Corneometer® CM825 on check and palmar skin. Amended from Egawa et al 2007. Used with permission.

A similar premise can be applied to the data collected by the MoistureMeter D®. Depending on the thickness of the epidermal tissues, hydration measures will likely be collected from the epidermis (as opposed to the dermis) due to the devices depth measurement parameter (0.5 mm or 1.5 mm). This could offer some explanation for the inverse trend in TDC values between thin-skinned areas (the forehead and forearm) and thick-skinned areas (the palm and the plantar surfaces) (Mayrovitz, Bernal, et al., 2013).

As both devices mentioned above collect data at a fixed tissue depth, to quantify the water content of tissue at the same level, it would be pertinent only to compare data collected by these devices on skin surfaces that exhibit a similar structure, i.e., similar SC thickness. Alternatively, measures collected using these devices could be considered alongside existing knowledge of the thickness and hydration gradients of locational skin to contextualise these data.

These limitations could be addressed using the MoistureMeter SC™, which purports not to measure hydration at a set depth. Instead, it returns a value that represents the hydration of the tissue, derived from the dryness of tissues and the thickness of the SC (a full

explanation of the measurement principle may be found within Alanen et al., 2004). This device could therefore be used to compare skin sites, irrespective of their structure.

2.3.7. Relating outcomes from the literature to plantar SC

The hydration gradient of the plantar SC has not previously been examined. It is only possible to hypothesise its characteristics by considering the gradient displayed by its' most equivalent tissue – the palm.

Mayrovitz, Bernal, et al. (2013), showed the palmar and plantar surfaces displayed similar levels of hydration (measured by the MoistureMeter SC™) and hydration was correlated with shallow MoistureMeter D® measurements (1.5 mm depth probe - great toe plantar (r-value = 0.786)) (Mayrovitz, Bernal, et al., 2013). If the hydration gradient within the SC of the plantar skin is similar to the palm, but is extended over a thicker layer of tissue (palm: 600.9 µm ± 96.8 vs sole: 637.1 µm ± 186.0) (Lee & Hwang, 2002) the depth at which the Corneometer® CM825 measures would yield data is not comparable to data collected using the same device from any other body area.

Although the physiology of palmar and plantar tissue is comparable, the hands and feet perform different functions. They are exposed to different contact materials and forces that may result in these skin areas having dissimilar hydration gradients. For example, the hands are more often exposed to detergents, which may reduce the volume NMFs within the skin (Caspers et al., 2003) and the feet are often enclosed in hot and humid environments, such as hosiery and footwear, which can influence skin hydration (Katagiri et al., 2003; Nam et al., 2015) (according to measurements collected using a Corneometer®). For reasons of environment and thickness, amongst others, the hydration gradient within the SC of the plantar skin cannot be assumed to be analogous to that of the palm.

Numerous techniques have been used to quantify the thickness of the plantar epidermis i.e. ultrasonography (Yuet-Lan Chao, 2012) and histology (Boyle et al., 2019). These studies found different epidermal thickness at different foot locations (See Table 9). Therefore, hydration data collected using a device with a fixed measurement depth, may not

necessarily be collecting data from an equivalent anatomical level within the tissue when applied to other locations on the foot.

Table 9. Plantar epidermal thickness.

Location	Thickness (± SD where provided) (μm)	Measurement mechanism	Reference
Plantar Hallux	510 ± 180	High frequency ultrasound	(Chao et al., 2011)
	1070	High frequency ultrasound	(Strzalkowski et al., 2015)
1st Metatarsal head	550 ± 190	High frequency ultrasound	(Chao et al., 2011)
3rd Metatarsal head	620 ± 150	High frequency ultrasound	(Chao et al., 2011)
5th Metatarsal head	610 ± 130	High frequency ultrasound	(Chao et al., 2011)
	1210	High frequency ultrasound	(Strzalkowski et al., 2015)
Heel	660 ± 130	High frequency ultrasound	(Chao et al., 2011)
	1320	High frequency ultrasound	(Strzalkowski et al., 2015)
Medial arch	760	High frequency ultrasound	(Strzalkowski et al., 2015)
Lateral arch	920	High frequency ultrasound	(Strzalkowski et al., 2015)
Plantar aspect of the foot	741.7	Histological examination (results of meta-analysis)	(Lintzeri et al., 2022)
	511.9	High frequency ultrasound (results of meta-analysis)	(Lintzeri et al., 2022)
	724.4 ± 232 (non-specified metatarsal head)	Histological examination	(Vela-Romera et al., 2019)

Hydration measures of human skin are valuable as they provide information on its structural integrity. Well hydrated skin is elastic and provides an effective physical and chemical barrier. Currently, the Corneometer® CM825 is used widely to measure skin hydration, and the data captured by this device correlates with other skin characteristics (such as mechanical behaviour) (Hashmi, Nester, et al., 2015). No indication of how skin hydration across its depth influences biophysical characteristics exists. The biophysical characteristics of the plantar skin may be influenced to a larger degree by tissue hydration at a deeper level within the skin than the superficial layer.

Skin hydration measurement devices assess the effectiveness of products designed to relieve xerosis, for example, emollients and exfoliating agents (Jennings et al., 2003). Knowing how these represent the hydration gradient and their relationship with foot skin integrity (measured by proxy via biophysical characteristics), could be used to assess products more effectively. For example, the Corneometer® CM825 has been used to assess

emollient effectiveness on foot skin (Parker et al., 2017). Emollients that improve hydration within the uppermost 15 µm of tissue will be perceived favourably by a research team using this device. An emollient that delivers hydration at a deeper level could potentially have a larger impact upon skin integrity and mechanical behaviour, but this cannot be identified by the Corneometer® CM825 due to its fixed measurement depth.

2.3.8. Conclusion

There is a dearth of data available on the hydration of the foot skin, particularly on plantar locations. Whilst this remains, accurately assessing the efficacy of an emollient on plantar skin is challenging. It would be beneficial to collect a hydration dataset representative of healthy human skin across the plantar surface of the foot.

Such an investigation would be complimented by simultaneous collection of physical characteristics of skin, such as hardness, roughness, and elasticity, to aid the assessment of device suitability for use on the plantar skin. Finally, the application of CRS to the plantar foot would resolve the data-gap described surrounding the hydration gradient of the plantar skin and could provide valuable context to the use of the commercially available hydration measurement devices.

The review of the literature has informed the aims and hypothesis for the thesis (described in Chapter 3). The following section provides background data to support the interpretation of materials in section 2.5., where the previous application of CRS is described further.

2.4. The impact of age, diabetes status and emollient application on skin composition: a scoping review

2.4.1 Introduction

The remainder of this chapter focuses on CRS methods. CRS can measure the volume of many materials within the skin across its depth (Caspers et al., 2001). This presents an opportunity to broaden the scope of this project to include review of other skin composites (urea and NMFs) that are relevant to skin hydration and therefore integrity. These have not previously been examined on the plantar skin.

The novelty of these data and relevance to foot care (i.e. the potential for delivery of NMFs and urea via emollient provision), makes these data highly valuable. It would be remiss to undertake such data-collection without ensuring the output is directly applicable to contemporary issues facing foot health care in the UK, for example the ageing population and increasing rates of diabetes. For this reason, the participants recruited for the study described in Chapter 7, include young people, older people, and people with diabetes.

This section aims to provide the reader with the evidence of why urea and NMF are of such importance to the health of foot skin and review instances in which their volume within the skin has been measured and compared to age, diabetes status and recent emollient therapy. The relationship between skin water and age, diabetes status, and emollient application will also be explored.

The scope of this search is not limited to plantar skin due to the limited skin-research historically conducted on the foot. Instead, findings from the non-plantar skin are reviewed and considered in relation to the unique structure and function of the plantar skin where possible.

The research question for this literature review is: Do age, diabetes status, or emollient application have an impact on the skin biochemical composition?

Due to the relatively broad research question, and limited data available on this research area, a scoping methodology has been applied in this review. Similarly to the previous literature search, a PCC framework has been used to inform the searching strategy: Age and diabetes are used to define population characteristics, measurement of NMF, urea and water is the concept, in the context of the skin with or without emollient application.

2.4.2. Search strategy

The following search terms were utilised: stratum corneum OR epidermis OR skin AND urea OR water OR natural moisturising factor AND age OR diabetes OR emollient. The results of this literature search, and the screening process undertaken are displayed in Figure 14.

2.4.3. Search approach and literatures screening

In September 2022, a literature search was undertaken within the CINAHL database. The findings of which are presented below, following a brief explanation of the role of each composite in maintaining skin integrity. These data provide context to the review of instances in which CRS has been used to undertake similar investigations (Section 2.5) and inform the development of hypothesis described in Chapter 3.

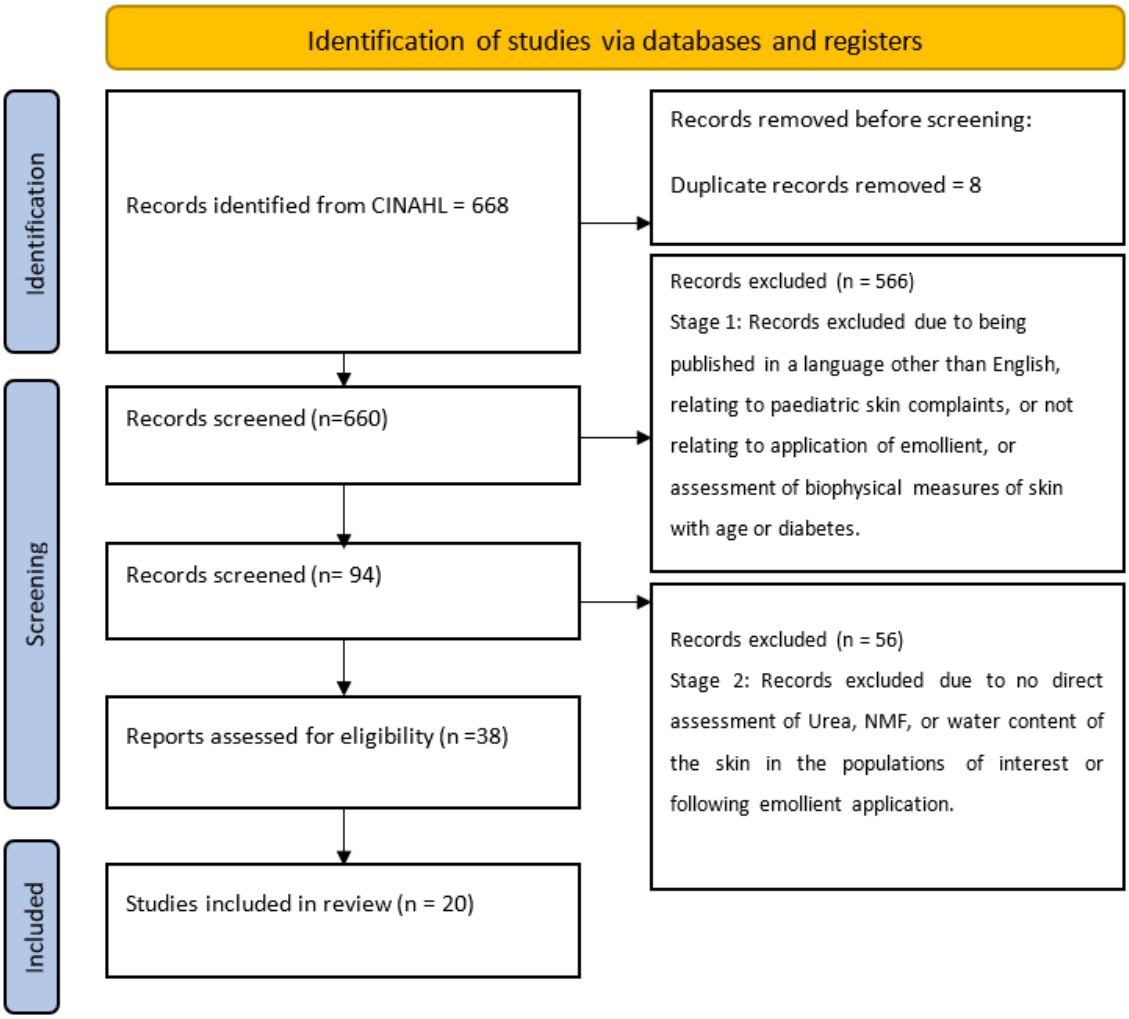


Figure 14. A record of the literature search and screening undertaken. PRISMA diagram format (McKenzie et al., 2021).

2.4.4. Discussion of compounds and findings from literature search

2.4.4.1. Natural Moisturising Factors (NMF)

NMF is a collective term for the amino acids and other small molecules generated following the breakdown of filaggrin in the epidermis (Del Rosso & Levin, 2011). These are found within the corneocytes and account for between 20-30% of the dry weight of the SC. Due to their hydroscopic nature, they tightly bind water within the corneocytes, acting as a natural humectant (Verdier-Sévrain & Bonté, 2007).

Several factors may influence the volume of NMFs within the skin: NMFs can be washed out of the skin through the use of surfactants (Caspers et al., 2001), and due to the role of water in facilitating the breakdown of filaggrin, low environmental humidity can reduce NMF production (Katagiri et al., 2003).

A reduction in NMF in the skin can lead to SC dehydration as it is no longer binding water in the SC, further reducing the availability of water to facilitate filaggrin breakdown and feeding into the 'dry skin cycle' (Rawlings & Matts, 2005; Thomas et al., 1985). This cycle can lead to the incomplete differentiation of keratinocytes, and reduced digestion of corneodesmosomes, creating a thickened SC with a rough surface due to corneocytes not sloughing off, instead forming clumps (i.e. visible skin flakes) (Rawlings & Matts, 2005).

NMF volume in the SC has historically been thought to reduce with age due to the increased incidence of xerosis. A. V. Rawlings et al. (1994) and several studies support this – reporting reduced NMF content in the aged (dos Santos et al., 2019a; Horii et al., 1989). However, several studies have also described an increase in NMF in the SC with age (Boireau-Adamezyk et al., 2021; Choe et al., 2018; Egawa & Tagami, 2008a; Takahashi & Tezuka, 2004), and some authors report that, although some components of NMF vary with age, the overall volume of NMFs is unaffected (Jacobson et al., 1990).

The instances in which NMF has been found to decrease with age, however, either have extremely small particulant number (n=4) (Horii et al., 1989), or discuss the alternative interpretation of their data that modify their findings. Dos Santos et al (2019) found that the NMF gradient within the forearm SC of young (n=11) (mean ± SD: 24.1 ± 3.3 years old) and elderly (n=11) (mean ± SD: 68 ± 5.8 years old) people were very similar, although the water content of the the SC in the young group was consistently higher than the elderly group (no values provided), this difference was only statistically significant ($p \leq 0.05$) at depths of 22 and 24 µm into the SC (dos Santos et al., 2019a). The author suggests that this may not indicate a true difference in the NMF content of the SC with age, but an indication of changing SC thickness, this idea is discussed in further detail within the Section 2.5. Boireau-Adamezyk et al (2021) and Choe et al (2018) normalise the NMF content of the SC they examined by it's depth, and conclude that NMF content increases with age, supporting the theory, and indicating that SC NMF does actually increase with age when SC thickness is considered.

Exploration of the NMF content of the skin of people with diabetes is currently limited to studies that have examined the biomarkers extracted from the skin surface. Berg et al (2023) found that people with type 1 diabetes have lower levels of NMF (nmol/µg protein) within skin-surface biomarkers than people without diabetes at the buttocks (mean (95%

CI): diabetic: 0.67 (0.52-0.82); non-diabetic: 1.07 (0.87-1.27) (p value=0.012 (paired t-test))), but not the forearm (mean (95% CI): diabetic (n=9): 0.75 (0.61-0.88); non-diabetic (n=9): 0.85 (0.70-1.00) (p value=0.916 (paired t-test)). However, the authors suggest this may be a chance finding as this result is not consistent with the other skin barrier measures obtained within their study (for example, no change in TEWL associated with this) (Berg et al., 2023).

In 2019, Lechner et al conversely found that people with diabetes have higher levels of NMFs (μg/cm^2) within the surface biomarkers extracted at the dorsal foot and the plantar heel, although these differences did not reach statistical significance: Dorsal foot (mean ± SD): diabetic: 101.7 ± 70.4; non-diabetic: 65.0 ± 37.1, p value = 0.05: plantar heel diabetic (mean ± SD): 199.0 ± 113.2; non-diabetic: 148.4 ± 86.06, p value = 0.11. In the future, it would be beneficial to test in-vivo volumes of NMF within the SC of people with Diabetes, to examine how these relate to biomarkers and establish whether the volume of NMFs can be manipulated via emollient therapy, for example. Emollients with NMFs added are available and have been found to improve skin hydration and reduce xerosis symptoms (when used in conjunction with urea), but the penetration of NMFs into the SC has not been examined (Weber et al., 2012). It would be useful to investigate the penetration of NMFs applied topically into the skin and relate this to skin integrity, without the addition of urea.

From the instances in which the NMF content of the SC has been investigated, no information is available on the plantar SC. The importance of NMF to maintain SC hydration, and therefore skin integrity is highly relevant to foot health in people of advanced age and people living with Diabetes who are vulnerable to plantar skin pathology. It would be pertinent to investigate the NMF content of plantar skin in these populations, as well as the impact of emollient therapy.

2.4.4.2. Urea

Urea is often described as one of the NMFs (Egawa & Sato, 2015; Piquero-Casals et al., 2021; Wellner & Wohlrab, 1993) due to its hygroscopic properties (Egawa & Sato, 2015).

However, in this review, it will be considered separately for a number of reasons. As well as acting as a humectant, urea has many other functions within the epidermis. Urea regulates epidermal proliferation, facilitates the production of antimicrobial agents within the SC and acts as a keratolytic agent (Piquero-Casals et al., 2021). Urea is used as an additive to foot care emollients to reduce callus and prevent callus growth (Piquero-Casals et al., 2021).

Urea is a common additive to emollients (Piquero-Casals et al., 2021; Soesman et al., 2022). Due to its low molecular weight, it penetrates well into the skin and can be used as a penetration-enhancer for other emollient ingredients (Piquero-Casals et al., 2021).

Despite the complex and highly beneficial role of urea within the epidermis, very little data exists on how the endogenous volume of urea changes with risk-factors for skin pathology, such as age and diabetes status. There is uncertainty from the few instances in which that has been explored.

Hussain et al (2019), analysed the volume of urea within isolated corneocytes (using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry) of healthy young people (n=5) (age range: 18-40 years) and healthy older people (n=5) (age range: 65-75 years). They found that older people had more urea within their corneocytes. However, Egawa and Tagami (2008) investigated the urea content of the SC using in-vivo CRS in a young (age range, mean: 59-76, 67 years) and old (age range, mean: 22-40, 32 years) (total n=31) population and found that the younger group has higher urea in the SC of the forearm and cheek, although not significantly ($p>0.05$). These results indicate that, although the urea content of isolated corneocytes increased with age, the urea content of the SC may be reduced overall due to a reduction of urea within the intercellular lipids. There are currently no instances in which both of these factors have been examined with age simultaneously, future research should aim to fill this knowledge gap as this could influence formulation of emollients.

The overwhelming majority of research concerning urea and the skin relates to using urea-containing emollients (Soesman et al., 2022). However, within these investigations, the change in urea volume within the skin is rarely quantified. Instead, the resolution of xerosis symptoms is judged via a visual scoring system for skin features associated with xerosis, or

through quantifying SC hydration using specific devices, or in some instances, both (Federici et al., 2015; Piquero-Casals et al., 2021).

Urea-containing emollients have been proven to increase the volume of water within the skin (when measures using commercially available hydration measurement devices) (Cobos-Moreno et al., 2021; Danby et al., 2016; Serup, 1992; Weber et al., 2012), and to reduce the visible features of xerosis (Federici et al., 2012; Jones & Lunn, 2020; Loden et al., 2001). Due to the commonality of xerosis on the foot skin, several of these studies have been undertaken on foot skin (Cobos-Moreno et al., 2021; Federici et al., 2012; Jones & Lunn, 2020).

In 2015, Egawa and Sato used CRS to measure the concentration of urea within the skin following application of an emollient. This study showed that urea applied topically via an emollient is able to penetrate the surface of the forearm skin, as well as demonstrating that different forms of urea within the SC can be quantified using CRS (urea-water solution and solid urea) (Egawa & Sato, 2015). This supports the use of CRS technology to measure the volume of urea in the plantar SC of individuals, to assess the penetration depth of emollients, and to observe for changes in the urea content of the SC in people of advanced age, or with diabetes.

2.4.4.3. Water

The importance of water within the SC has been discussed in detail in Section 1.3.

The volume of water within the skin with age, diabetes status and following emollient therapy, has been explored thoroughly, due to the accessibility and usability of hydration measurement devices. However, the outcomes of these investigations are not consistent across studies:

Lechner et al (2019) found that people with diabetes have higher numbers of superficial fissures in their skin than people without diabetes and their skin hydration (measured using the Corneometer® CM825) is lower on the foot dorsum (mean ± SD: Non-diabetic (n=20): 22.5 ± 10.1 AU; diabetic (n=40): 19.6 ± 6.2 AU (p value = 0.17) and plantar heel (non-diabetic (n=20): 2.6 ± 3.1 AU; diabetic 1.4 ± 2.2 AU (p value = 0.10)) but not the plantar 1st

metatarsal head (non-diabetic (n=20): 5.6 ± 4.2 AU; diabetic (n=40): 6.0 ± 4.9 AU (p value = 0.94). The glycaemic control of the participants was not noted. This is important as Sakai et al (2005) and Park et al (2011) have established a link between glycaemic control and skin hydration in people with diabetes with high fasting plasma glucose, have reduced skin hydration on the lower leg (Sakai et al., 2005) and long-standing hyperglycaemia reduces skin hydration (established using a rat model) (Park et al., 2011).

These data demonstrate that poor glycaemic control is associated with reduced skin hydration. The reason for this phenomenon, and the deleterious effects of this process on the foot of a person with diabetes aredemonstrated by Boulton (2014). To summarise, as the foot shape changes due to somatic motor neuropathy, areas of high-pressure develop and callus plaques form. These areas then become high-risk for development of DFU and resulting lower-limb amputation. This is supported by the work of Lane et al (Lane et al., 2020) who demonstrated a linked between glycaemic control and diabetic foot outcomes.

SC hydration has been found to decrease with increasing age, this has been demonstrated using a variety of hydration-measurement devices, such as the Corneometer® CM825 , Skicon (IBS, Hamamatsu, Japan), Dermalab hydration probe (Cortex Technology, Denmark) (Firooz et al., 2012; Man et al., 2009; Mehta et al., 2018; Park et al., 2011; Sakai et al., 2005). The age at which SC hydration changes, however is reported inconsistently. Sakai et al (2005), found a difference in skin hydration at the lower leg (mean high frequency conductance μs ± SD) (aged <45 years: 42.3 ± 19.7; aged >45 years: 29.5 ± 17.6 (p value= 0.020)), but not the forearm (aged <45 years: 54.7 ± 21.2; aged >45 years: 60.0 ± 32.8 (p value=0.511) in people aged above (n=26) and below (n=23) 45 years (Skicon 200). Whereas, Firooz et al (2012), only found hydration reduced after 50 years of age (mean+SD (AU) for all hydration measures form forehead, cheek, nasolabial fold, neck, forearm, palm and leg: 10-20 (years of age): 49.74 + 19.25; 20-30: 47.08 + 16.61; 30-40: 50.53 + 17.69; 40-50: 53.34 + 20.78; 50-60: 43.04 + 20.58 ($p<0.05$), and Man et al (2009), after 70 years of age on the on the forehead of women and the forearm in men ($p<0.05$ (no values provided)).

The analysis made within these studies of age-dependence of SC hydration is limited by their study design. The findings of Sakai et al (2005) provide a broad indication of hydration change with age, due to the broad age-range categories applied and the range of

participant ages (< 45 years group (mean+SD): 19.3+7.8; >34 years group: 68.2+7.9). Whereas the sampling used by Man et al (2009), breaks down ages into smaller ranges (36-50 years of age (n=142), 51-70 years of age (n=59), > 70 years of age (n=55)), and Firooz et al (2012) generates data only representative of a single decade of life, up to 60 years of age (10-20, 20-30, 30-40, 40-50, 50-60 (all n=10)). Although the statistical power of studies can be enhanced using a broad age-range due to increased participant numbers in each group, differences that may be evident between smaller age-ranges may be obscured using this method (i.e., women going through menopause may exhibit rapid changes in skin characteristics over a short time-period). In the future, it would be preferable to collect similar data using a larger number of participants and small age ranges or a regression analysis, as demonstrated by Boireau-Adamezyk et al in 2021.

Despite the large body of evidence supporting the reduction of SC hydration with age, there are some data to suggest otherwise. Hahnel et al (2007), investigated the biophysical characteristics of the skin of people in aged care facilities. This revealed a weak positive correlation (0.205 ($r \geq 0.02$)) between age and SC hydration (measured using the Corneometer® CM825) on the lower leg, but not the forearm. Marrakchi and Maibach (2007) and Wendling and Dell'Acqua (2003) also investigated SC hydration age dependence and found inconsistent and primarily insignificant results on the face and forearm using the Corneometer® CM825.

There are some limitations to these studies however, within the work conducted by Hahnel et al (2017), participants (n=223) were significantly older that individuals examined within previously discussed studies (mean ± SD: 83.6±8.0). No relationship was identified between age other facets of skin integrity, such as TEWL, or skin tear occurrence, increasing the likelihood that this was a chance finding (Hahnel et al., 2017). Marrakchi and Maibach (2007) had very few participants and inconsistent age-ranges ((mean±SD (range) young group (n=10): 29±3.9 (24-34); old group (n=10): 73.6±17.4 (66-83), and although Wendling and Dell'Acqua (2003) had a larger participant number (n=110), these were not equally distributed across age ranges: 40-50 (n=38), 50-60 (n=24), 60-70 (n=11), >70 (n=2). The limited data available from the older participant groups inhibits the authors ability to draw meaningful comparisons between the age ranges.

As has previously been discussed, skin hydration is commonly recorded following application of an emollient to assess its effectiveness. Parker et al (2017) published a systematic review of the instances in which foot skin hydration was assessed following emollient application using a variety of objective and subjective assessment methods, and Cobos-Morena et al (2021) published a clinical trial of a foot skin emollient assessed using the Corneometer® CM825. In neither of these papers is the suitability of the devices used expressly discussed in relation to the unique structure of the plantar skin or are alternative methods such as CRS described.

2.4.5. Conclusion

Within this section the role of urea and NMF within the skin has been detailed, and the limited instances in which the volume of these compounds (and water) within the skin has been investigated alongside age, diabetes and emollient use have been explored. These have been summarised below:

Albeit the limited and often conflicting information available, there is reason to believe that SC NMF increases with age and SC water decreases. Overall urea volume is reported to increase in cells with age but reduce in intercellular fluids.

Diabetes is associated with reduced SC water. Little information is available on the change of NMF and urea volume in the SC with diabetes status, and the limited biomarker data on NMF content is contradictory.

The impact of emollient on SC NMF and urea content is largely unknown as investigations assessing the impact of emollient primarily measure SC water content, which has been shown to increase following emollient application.

These data provide important context to the following discussion, in which the limited instances where CRS has been used to undertake similar comparisons are described.

2.5. In-vivo confocal Raman spectroscopy of the skin: A literature review

In this section, all instances in which in-vivo CRS has been used to quantify the volume of water, NMF, and urea within the skin of people of different ages and diabetes status will be reviewed.

A publication list shared by River Diagnostics (RiverD) (International B.V. Rotterdam, The Netherlands), the only company currently producing Confocal Raman Spectroscopes suitable for use in-vivo, has been searched for reference to patient age and diabetes status. A detailed review of the findings of relevant materials from this list is provided below.

This publication list is accessible at: https://www.riverd.com/skin-analysis-with-the-gen2-sca/learning-center/publications-index/.

2.5.1. Age

Five studies were published between 2007 and 2021 that compared skin composition between different age groups (See Table 10). All these studies used the 3510 Skin Composition Analyser (RiverD, Rotterdam, The Netherlands).

Table 10. Details of instances where CRS has been used to measure relationship between skin composition and age.

Title – authors (publication year) *Journal*	Participant group demographics	Skin sites measured	Comparison with age
Comparison of the depth profiles of water and water-binding substances in the stratum corneum determined in vivo by Raman spectroscopy between the cheek and volar forearm skin: effects of age, seasonal changes and artificial forced hydration - M. Egawa and H. Tagami (2007) *Acta Dermato-Venereologica*	31 female participants. No *n* given for individual groups. Young group (age range in years (mean age)): 22-40 (32) Older group (age range in years (mean age): (59-76 (67))	Cheek and volar forearm	Water Content NMF Content Urea content
Age related depth profiles of human Stratum Corneum barrier-related molecular parameters by confocal Raman microscopy in vivo - ChunSik Choe, Johannes Schleusenera, Jürgen Lademanna, Maxim E. Darvina (2018) *Journal of Biophotonics*	Young group: 7 people (3 male, 4 female) aged 23-34 years (Mean: 29) Older group: 4 females aged 45-62 years (Mean:50)	Volar forearm	NMF Content
Evaluation of penetration process into young and elderly skin using confocal Raman spectroscopy – L. dos Santos, V. Krishna Tippavajhala, T. Olivera Mendes, M. G Pereira da Silva, P. P. Favero, C. A. Tellez Soto, A. Martin (2019) *Vibrational spectroscopy*	All participants female. Young group (age range (mean + SD)): 11 people aged 18-28 years (24.1 ± 3.3) Older group: 11 people aged 62-80 years (68± 5.8))	Forearm	NMF Content
The stratum corneum water content and natural moisturization factor composition evolve with age and depend on body site - E. Boireau-Adamezyk, A. Baillet-Guffroy and G. N. Stamatas (2021) *International journal of dermatology*	All participants female. Four groups of 10 individuals aged 18-30, 30-40, 40-55, and 55-70 years.	Central cheek, dorsal forearm, upper inner arm.	Water Content NMF Content Urea Content

2.5.2. Impact of age on skin biocomposition.

2.5.2.1. Water

Egawa and Tagami (2008) and Boireau-Adamezyk et al. (2021) studied the relationship between skin water and age. Egawa and Tagami (2008) found 'old' (aged between 59 and 76 years) and 'young' (aged between 22 and 40 years) participants groups had a similar hydration gradient at the cheek and forearm. However, the old group demonstrated more variation between individuals than the young group. The 'young' participants were also found to have significantly lower skin hydration using a two-tailed Student's t test ($p<0.05$, no data provided) between 10 µm and 30 µm depth into the skin, than 'old' participants (See Figure 15).

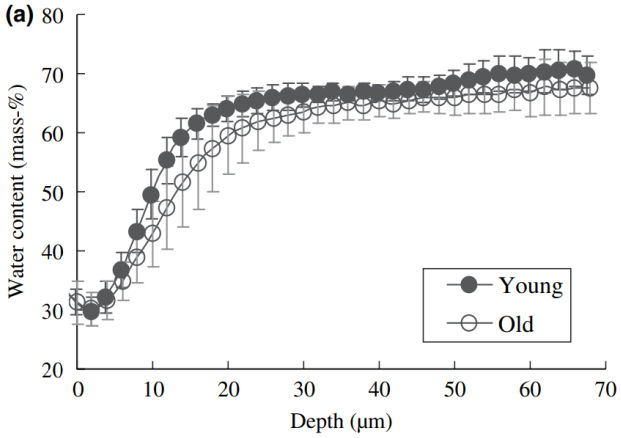


Figure 15. A graph demonstrating the water content of the SC of the forearm skin across its depth in two participant groups (Egawa & Tagami, 2008a). Used with permission.

The authors highlighted that the SC apparent thickness was greater in the young subjects at the forearm ($p<0.05$), indicating that the difference in water content at this depth could be the result of a shift in the gradient due to the thicker SC in young participants. No differences in tissue water content or SC apparent thickness between groups in the cheek skin were found in this this study.

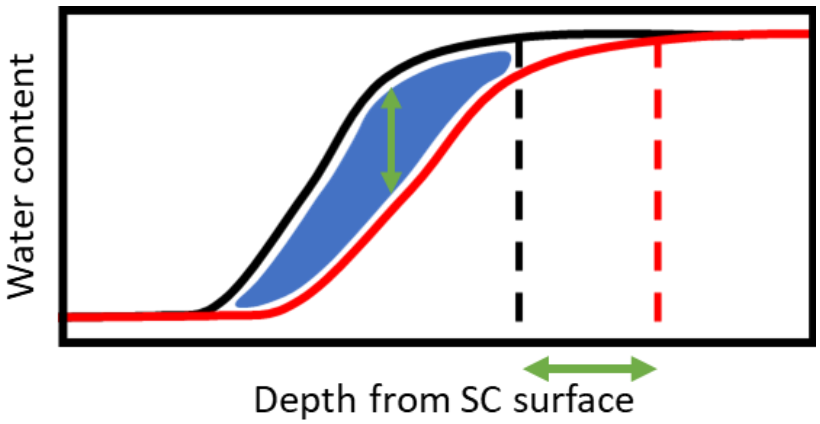


Figure 16. Diagram demonstrating SC content difference resulting from inequivalent SC thickness.

The SC thickness is key when analysing SC composition. Two hydration gradients are illustrated in Figure 16 (black and red lines) aligned to two different SC thicknesses (black dotted line and red dotted line, marked on the x-axis by a green arrow). When aligned to the same SC thickness, these hydration gradients are identical. However, when SC thickness is unequal, an area of inequivalence is created (the blue areas) where the black hydration gradient appears to have higher SC water (indicated by the green arrow). When the water content of the tissue is normalised over SC thickness, this difference is negated.

Boireau-Adamezyk et al (2021) employed an alternate method to examine the relationship between SC water and age. Average SC thickness was calculated (Böhling et al., 2014; Egawa et al., 2007) from 10 measurements, and this value was used to normalise the depth values for the concentration profiles that had been measured. The total volume of compounds was then used for statistical comparison with age (calculated as the integrated value between the normalised depths of the SC surface and junction with the viable epidermis).

Boireau-Adamezyk *et al (2021),* also used linear regression as a function of age for each compound they examined. This led them to find a significant correlation between SC water and age, where SC water decreases with increasing age represented by a regression equation with $R^2 = 0.14$ ($y = -0.1381*x+51.13$, $p < 0.001$), but only on the exposed arm site. No significant differences were found at the other measurement locations, the face, and the upper inner arm.

This investigation by Boireau-Adamezyk et al (2021) demonstrates that even with the confounding effect of SC thickness being removed, use of a regression analysis, and a sizable, well-proportioned participant group (n=40, 10 per age group: 23-30, 30-40, 40-55, 55-70 years), the effect of age on SC hydration is small and skin-site dependant. Although a statistically significant result is achieved within this study, the R^2-value is low. In future, it would be interesting to repeat this study with a larger number of participants to identify whether this would more effectively power the regression analysis and identify a similar pattern at the other skin sites.

When this analysis technique is considered in relation to measures of the plantar foot SC hydration, a limitation arises. The plantar SC thickness may extend beyond the measurement range of CRS (400 µm), making this exercise impossible for data obtained from the plantar foot.

Unfortunately, as Egawa and Tagami (2008) and Boireau-Adamezyk et al (2021) processed and reported their data in different manners it is not possible to directly compare these data. It would be an informative exercise to re-analyse each dataset according to the statistical model employed by the other authors to see whether this would modify the conclusions they drew from their data. Even simply plotting the SC hydration data obtained by Boireau-Adamezyk et al (2021), over SC depth (with or without SC thickness normalisation), would facilitate comparison between these two works and generate more nuanced understanding of the data obtained (i.e. an understanding of at what area/depth within the SC the hydration differed).

Despite this limitation, however, from these studies, it is consistently demonstrated that the water content of the SC can vary with age, although this is site-dependent, and the changing thickness of the SC must be considered. These results are broadly in agreement with previous investigations in which the SC hydration was examined for age dependence using commercially available hydration measurement devices, as described in section 2.4.

Consideration of water-binding

Choe et al (2018) also describe the difference in the hydrogen-binding status of the SC water and found significant differences between these in old and young skin. Between 10-30% of SC depth, older participants had higher strongly bound and lower weakly bound water molecules than young group. The authors suggest that this phenomenon may be due to drying of the SC within this region (Choe et al., 2018), citing Vyumvuhore et al (2015), who found that dehydrated SC increased partially bound water, which could result in the increase of hydrogen-bound water molecules, as shown in aged skin.

Unfortunately, little information is available to support this theory as this is the first instance in which water binding has been examined in-vivo, and the relevance of these data to the foot SC is unknown as the binding-status of plantar SC water has not been investigated.

2.5.2.2. Natural Moisturising Factors

There are four published instances in which NMF content has been examined using CRS between people of different ages. Each of these studies presents the NMF data from the SC slightly differently, which has implications on their interpretation.

Egawa and Tagami (2008) examined the volume of NMF within the SC on the cheek and the forearm in a young and old population. In this instance, the authors used the mean amounts of NMF in the SC for statistical comparisons, as opposed to the volume of material at each depth used within their equivalent hydration comparisons. The volume of NMFs within the SC of the ventral forearm of old participants was found to be higher than that of young participants ($p<0.01$) (Figure 17).

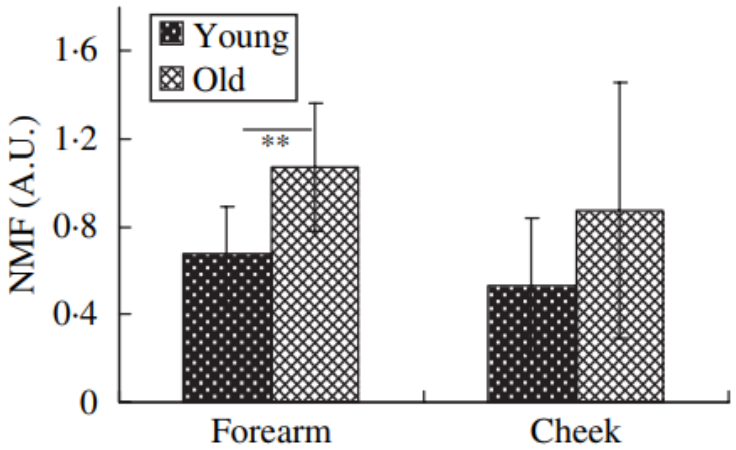


Figure 17. NMF content of the SC of the forearm and cheek in two different participant groups (Egawa & Tagami, 2008). Used with permission.

Choe et al (2018) also compared the volumes of NMF within the skin in two groups of different ages (See Figure 18). In this instance, the composition gradients were normalised to SC thickness and comparative analyses were conducted on values from equivalent percentage of SC thickness. i.e., for an SC thickness of 10 µm a 1 µm depth measure would be 10% of SC thickness. For a 15 um thick SC, 1.5 µm would be used for comparison.

Using this technique, an unpaired Student's t-test found that older people had significantly higher NMF content at 20, 30, and 40% of the SC thickness ($p<0.05$) no further detail provided). Although this cannot be directly compared to the data obtained by Egawa and Tagami (2007) due to their incommensurate analysis and reporting, these both indicate that the SC of older people contains more NMF.

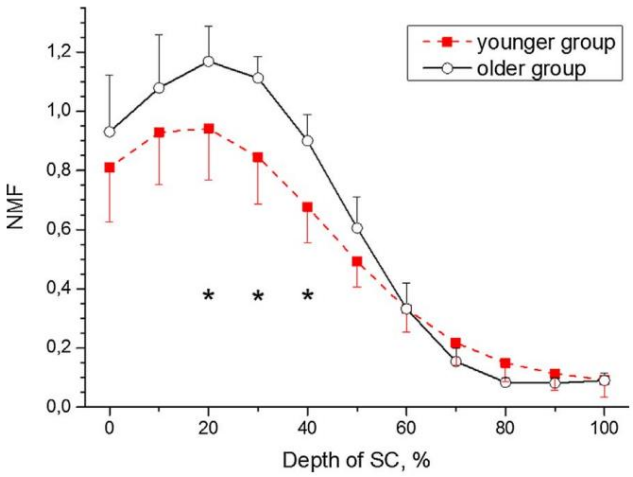


Figure 18. Diagram representing the volume of NMF within the forearm SC of two participant groups across SC depth (Choe et al., 2018). Used with permission.

However, in contrast to this, Dos Santos et al (2019) reported that the SC of younger people has higher NMF levels than older people. Dos Santos et al (2019) directly plotted the volume of NMF within the SC across its depth, up to 24 µm. Similar concentration gradients were observed for both participant groups (See Figure 18), but the authors noted that variation in NMF profiles decreased with depth, especially after 16um depth. At depths of 22 µm and 24 µm, the young age group had higher NMF content than the older age group ($p<0.05$).

The authors suggest that this difference could result from differences in the SC thickness between the two groups, however, they indicated that this could be due to the SC of older people being thinner (dos Santos et al., 2019a).

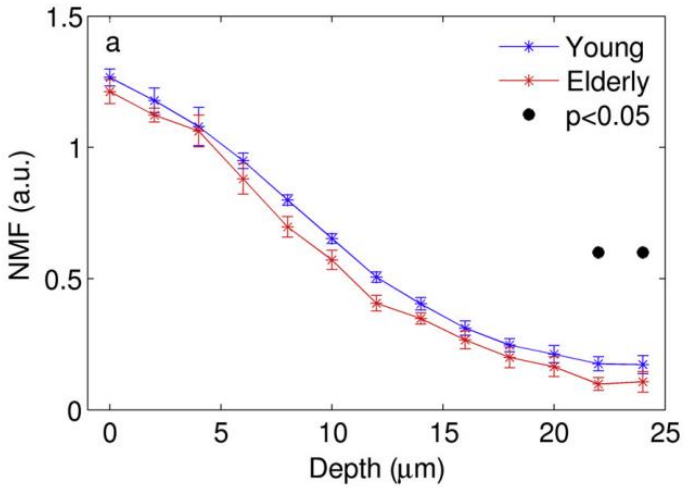


Figure 19. NMF content of the volar forearm SC in two participant groups (dos Santos et al., 2019a). Used with permission.

Boireau-Adamezyk et al (2021) also quantified the volume of NMF within the skin at the cheek, upper inner arm, and dorsal forearm (the same methods were applied to NMF data as water) (See Figure 19). On the cheek, the volume of NMF was found to increase with age (R^2=0.15, p<0.05), but this was not reflected at any other skin site.

There are a number of methodological differences between these studies, one of which is the materials considered to be a component of NMF within their analysis. Each of these studies has used the same device, the 3510 Skin Composition Analyser from RiverD. This is provided with software which contains a library of reference spectra that may be used to identify materials (and measure their volume) within Raman spectra, SkinTools 3 (RiverD, Rotterdam, The Netherlands). This software typically quantifies the amino acids alanine, glycine, histidine, ornithine, pyrrolidone carboxylic acid, proline and serine. Although not explicitly described, both Egawa and Tagami (2017) and Choe et al (2018) list components of NMF that align with the above, comprising amino acids and derivatives of amino acids (Choe et al., 2018; Egawa & Tagami, 2008a), and presumably used this software for analysis. These authors, however, also list lactic acid/lactate and urea, amongst other materials, as being included within the components that are considered within their NMF analysis (Boireau-Adamezyk et al., 2021; dos Santos et al., 2019b). This indicates that these studies are not using the same definition of NMF, and will be measuring different materials within the skin, limiting their comparability.

Despite this inconsistency, three out of the four instances in which SC NMF has been examined for age-dependency consistently report higher levels of NMF within the skin of older people (Boireau-Adamezyk et al., 2021; Choe et al., 2018; Egawa et al., 2007). The primary difference (participant demographics and methodology considered), between these investigations and that of Dos Santos et al (2019), is the incorporation of SC thickness into statistical analysis. Unfortunately, the application of these findings to data obtained from the plantar SC are limited due to the difficulties in measuring plantar SC thickness.

2.5.2.3. Urea

There have been very few instances in which the urea content of the SC has been examined between participant groups of different ages, although these results are consistent (Boireau-Adamezyk et al., 2014; Egawa & Tagami, 2008a).

Egawa and Tagami (2008) compared urea content of skin using the same technique they used for NMF (See Figure 20). They found that the urea content of the SC decreases with age, but not significantly ((p>0.05). This may be partly due to the small participant number for this study. Only 31 women participated, and the authors did not describe the distribution of these people between the groups, so it cannot be assumed that these were balanced. In future, a larger number of participants may increase the likelihood of obtaining a statistically significant difference between the two age groups observed.

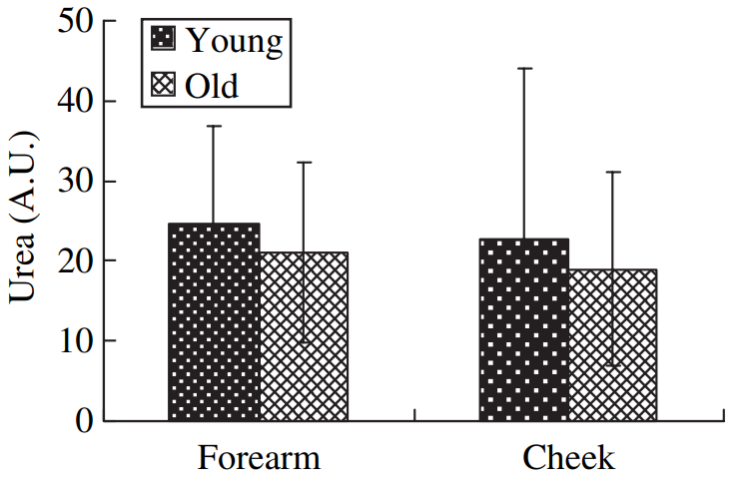


Figure 20. Volume of urea in the SC in two participant groups (Egawa & Tagami, 2008a). Used with permission.

Urea was also studied by Boireau-Adamezyk et al. (2021). Through a linear regression of SC materials (quantifying urea volume in the same manner as water) with age, urea content was found to decrease with increasing age on the upper inner arm skin ((p<0.05) (Boireau-Adamezyk et al., 2021). This same correlation was not found on the cheek or the dorsal forearm skin (Boireau-Adamezyk et al., 2021).

Despite the limited data available, the instances in which urea has been examined for age-dependence consistently report a decrease in urea volume with age. However, in each of these instances this data has been modulated using the SC thickness which is anticipated to be problematic when applied to the plantar SC.

2.5.3. Considerations of method design

2.5.3.1. Participant groups

Each of these studies used female participants only for comparisons between age groups, except for Choe et al (2018). Although, gender is not understood to influence skin hydration measurements, so this is not necessarily of importance (See Table 1) (Du Plessis et al., 2013).

2.5.3.2. Acclimatisation and environmental control

All but one study (Egawa & Tagami, 2008b) described a skin acclimatisation period of 15-20 minutes prior to data collection (Boireau-Adamezyk et al., 2021; Choe et al., 2018; dos Santos et al., 2019b) and both dos Santos et al (2019) and Boireau-Adamezyk et al (2021) undertook data collection in temperature and humidity-controlled spaces (maintained at 23 °C and 50 ± 5% RH (relative humidity) and 20–25°C and 40% RH respectively).

2.5.3.3. Skin preparation and product usage

Control of product use and skin preparation was inconsistent between studies: Egawa and Tagami (2008) did not discuss any controls of product use prior to data collection, Choe et al (2018) forbid product use 72 hours before data collection, and dos Santos (2019) restricted product use in the 24 hours before. Boireau-Adamezyk et al 2021) indicated participants were not allowed to use self-tanning products, or skin care products or deodorants for an undefined period (intentional sun-exposure was limited for one month). No author reports having recorded use of skin product prior to data-collection, or long-term use of skin products by participants.

Interestingly, Egawa and Tagami purposefully washed the skin 4hrs before data-collection with hard soap (Egawa & Tagami, 2008b), whereas Choe et al asked participants to refrain from bathing in the 4 hours prior (Choe et al., 2018). Although washing procedures prior to data-collection do not necessarily have to be controlled, the author should record and consider these if any anomalous results arise (Du Plessis et al., 2013).

2.5.3.4. Further exclusion criteria

Only dos Santos et al (2019) discussed exclusion criteria relating to disease or physiological factors, excluding participants with skin disease, diabetes, pregnancy and lactating individuals. Although the impact of pregnancy or lactation on skin composition are not well understood, skin disease and diabetes are known to influence skin characteristics (Del Rosso & Levin, 2011; Lima et al., 2017), and as such should be considered in inclusion and exclusion criteria for all investigations of this kind.

2.5.4. CRS measurement protocol

2.5.4.1. Parameters for CRS data-collection

When collecting data using CRS, the parameters of measurements can be altered to evaluate different areas of tissue (by varying measurement depth), to vary the resolution of the data collected (i.e. at a high resolution a measure would be taken every 2um), and the compound material measured (data from the high wavelength region can be used to measure the volume of water within a tissue, and data from the fingerprint region can be used to measure the volume of other materials) (see Section 4.3.2.4.).

Due to the similar aim of each of these studies, very little variation is shown between the parameters used for CRS within these four papers (See Table 11). Each study did, however, conduct a different number of measurements at each skin site which were combined for analysis. The highest number of repetitions is found in Choe et al (2018) who conducted 'at least' 10 measurements, followed by Boireau-Adamezyk et al (2021), who conducted 10. In 2007, Egawa et al did 2-3 measures and, unfortunately, no data was provided on the number of measures taken by dos Santos et al (2019). Although Raman Spectroscopy has been shown to generate consistent results (Bielfeldt et al., 2020), variation in human tissues necessitates the collection of multiple profiles at each skin measurement site, to allow for some to be discarded if results are abnormal, for example, if the data indicate that a skin structure, such as a hair, obscures the measurement area.

Table 11. Details of CRS data-collection parameters.

Authors and Date	High Wavenumber region (2500 – 4000 cm^{-1})	Fingerprint region (400-1800 cm^{-1})	Depth (µm)	Resolution (µm)	Additional detail
Egawa et al 2007	2600-4000	400-2200	80	2	
Choe et al 2018	2000-4000	400-2000	40	2	5s exposure in FP 1s exposure in HWN
Dos Santos et al 2019	1800-4000		24	2	10s exposure time 3 accumulations per frame
Boireau-Adamezyk et al 2021	2600-3800	400-1800	32 (HWN) 24 (FP)	4	

2.5.4.2. Data Processing

All but one paper within this review specify that they use the software provided by RiverD for analysis (SkinTools 3). Egawa and Tagami (2008) do not, however the formula they describe for quantifying material volume and SC apparent thickness are identical to those used within the software (Boireau-Adamezyk et al., 2021; Choe et al., 2018; dos Santos et al., 2019b).

2.5.4.3. Statistical Testing

A different statistical approach was employed within each of these studies to make comparisons between groups. Egawa and Tagami used 2-tailed student's tests, Choe et al used unpaired t-tests, and dos Santos used Mann-Whitney U Tests. These tests were applied to data from each measurement depth separately (with the exclusion of NMF and urea comparisons conducted by Egawa and Tagami et al (2008)), generating a list of results indicating significant differences between groups at each depth. When analysed in this manner, data are treated as 'discrete' from one another (McErlain-Naylor, 2020). In reality, they are part of a continuum and inherently linked – i.e., the hydration of the SC at 4 µm depth is related to the hydration of the SC at 2 µm depth and 6 µm depth.

In addition to this problem, this technique results in the conduction of multiple comparisons, which increase the likelihood of a Type I error occuring. The Bonferroni correction can be used to compensate for this risk (Binder et al., 2017) but is not utilised within any of these studies.

The technique utilised by Boireau-Adamezyk et al (2021) however, is more statistically robust. They used linear regression as a function of age. This is facilitated by their data-processing technique (described in Section 2.5.2.1.) which enabled them to use a single data-point to represents SC material volume within their analysis, rather than multiple values from different depths.

This technique does have some limitations. Firstly, it requires the SC thickness to be known, which is not always possible. For example, within the heel where the SC thickness extends beyond the measurement depth of RiverD devices. Secondly, this technique does not indicate the differences between groups at different measurement depths. In some instances, this is important, such as when considering using a commercial electrical measurement device with a limited measurement depth: For example, if it was found that the SC hydration was different between two groups within a specific region of the SC, a hydration measurement device that took measures hydration at that SC depth may be used explore this phenomenon (without the cost and additional time associated with use of CRS). i.e., if SC hydration differed between groups at the superficial 10-20µm of the SC, in theory, the Corneometer® CM825 could be used in future to observe for this difference.

2.5.5. Diabetes

A publication list provided by RiverD (available at https://www.riverd.com/skin-analysis-with-the-gen2-sca/learning-center/publications-index/) was searched for instances in which skin composition had been investigated alongside an individual's diabetes status. Unfortunately, only two examples of this were found, and these did not examine the volumes of water, NMF and urea in the tissue, but rather sought to identify Advanced Glycaemic End-products within the tissues of people with diabetes (Martin et al., 2017; Téllez Soto et al., 2016).

2.5.6. Conclusion

As described within section 2.4. with increasing age and diabetes, the skin becomes increasingly at risk of pathology, and this is, in-part, due to its changing composition. On the foot, this can be particularly detrimental to an individual's health and mortality (Menz & Lord, 2001; Mickle et al., 2010; National Cardiovascular Intelligence Network, 2022). Despite this,

CRS, a new method capable of quantifying skin composition in-vivo, has not yet been applied to the human foot to investigate these important changes.

The studies described in this section collect data from the volar forearm and cheek. These report conflicting information on how the volume of materials fluctuate in skin across age, and no data are given on how diabetes impacts the volume of specific compounds within the skin. However, these studies demonstrate that it is possible to observe differences in skin composition between participant groups using CRS and display a variety of ways these comparisons can be conducted. They also provide some useful insight into the direction of change that could be expected in equivalent investigations on the foot skin.

To understand the influence of age and diabetes on plantar skin composition, comparisons must be made between age – matched non-diabetic and diabetic people, and non-diabetic people of different ages. Additionally, it would be beneficial to observe the composition of the plantar SC following the application of an emollient, to provide insight into the penetration of emollient into the plantar skin and how this can modify SC composition.

These experimental design needs have been distilled into several aims and objectives listed in Chapter 3. These aims and objectives have been addressed through the conduction of a study described in Chapter 7. Several hypotheses have been generated for this study using the insight gained through the literature review presented in sections 2.4 and 2.5.

Chapter 3: Aims, objectives and hypotheses.

3.1. Aims and Objectives

The thesis aims are fulfilled by completing four studies, presented in chapters 5 to 8. Figure 21 demonstrated the aims and objectives of each study.

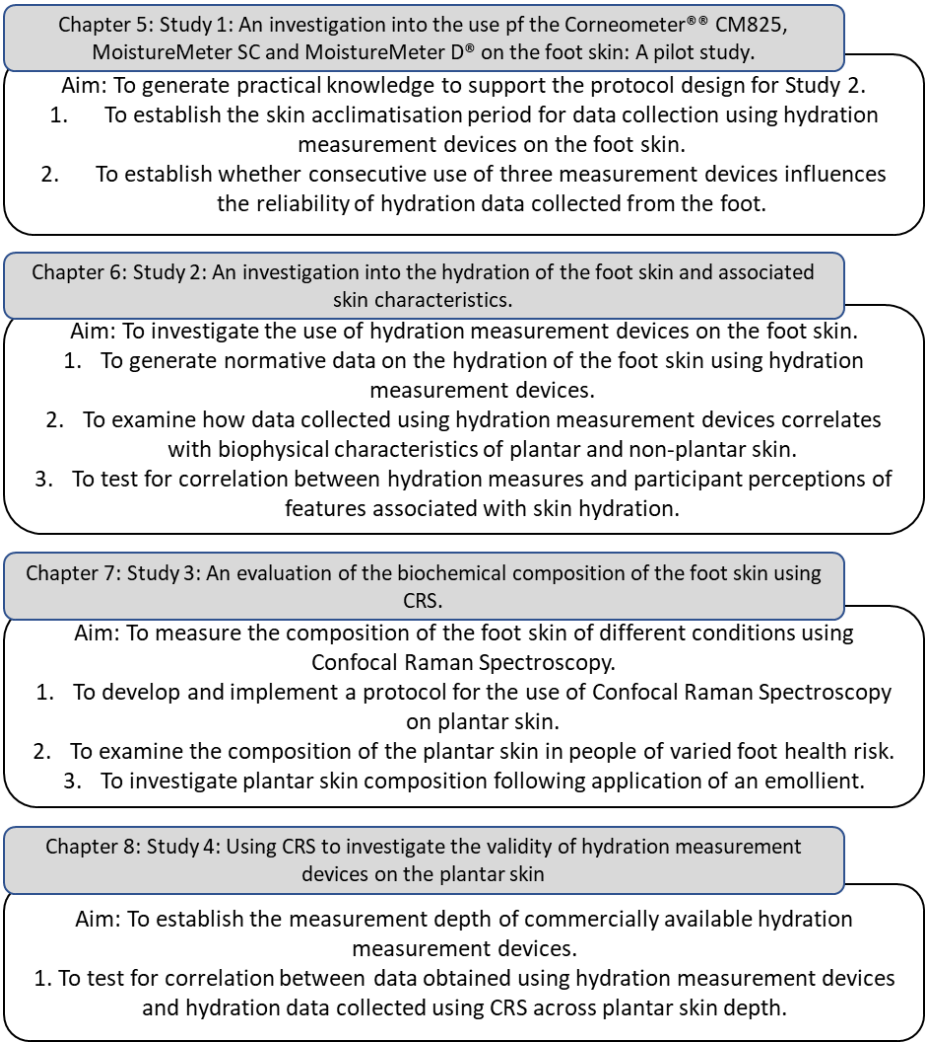


Figure 21. Project objectives.

3.2.Hypotheses

Additionally, hypotheses were generated for Chapter 7 objectives 2 and 3. These were generated following a review of the literature (see Section 2.4) and were used to inform the statistical design of Study 3 (see Section 7.3.10).

3.2.1. Objective 2 hypotheses:

1. The water content in the deeper layers superficial SC is significantly lower in the plantar skin compared to non-plantar skin.
2. The water content in the plantar SC of older people is significantly lower than that of younger people at the same measurement depths.
3. The NMF content in the plantar SC of older people is significantly greater than that of younger people at the same measurement depths.
4. The water content in the plantar SC of people with diabetes is significantly lower than that of age-matched non-diabetics.
5. The NMF content in the plantar SC of people with diabetes is significantly greater than that of age-matched non-diabetics.

3.2.2. Objective 3 hypotheses:

1. The water content of emollient-treated SC is significantly greater than untreated SC at the same measurement depths.
2. The NMF content of emollient-treated SC is significantly greater than untreated SC at the same measurement depths.
3. The urea content of emollient-treated SC is significantly greater than untreated SC at the same measurement depths.

Chapter 4. Instrumentation

4.1. Introduction

Across this project, a range of devices are used to measure the characteristics of the foot skin. This chapter describes and discusses the specification of these devices concerning their application to the foot.

In section 4.2**.,** the commercially available hydration measurement devices that are used within 'An investigation into the hydration of the foot skin and associated skin characteristics' and 'An evaluation of the biochemical composition of the foot skin using CRS' are discussed. Although these all use similar measurement mechanisms (electrical), slight variations in design are important to their use and interpretation of the data they collect.

In section 4.3., the principles and application of CRS to the foot skin are discussed.

In section 4.4. and 4.5., the instruments used to quantify the physical behaviour and topography of the skin are described, and the previous instances in which they have been applied to the foot are discussed.

In section 4.6., the visual assessment of foot skin via a self-administered questionnaire is discussed. This section includes a review of existing skin-scoring devices, followed by the development of a custom tool and the pilot testing process to validate its use.

4.2. Commercially available hydration measurement devices

Table 12 summarises the specifications of four hydration measurement devices used in this thesis.

Table 12. Quick reference table for commercially available hydration measurement devices.

Device	Corneometer® CM825	MoistureMeter SC™	MoistureMeter D®	DermaStat®
Image	 Image extracted from https://www.courage-khazaka.de/en/16-wissenschaftliche-produkte/alle-produkte/183-corneometer-e on 24/11/22	 Image extracted from https://delfintech.com/products/moisturemeters c/ on 24/02/23	 Image extracted from https://delfintech.com/products/moisturemeterd/ on 24/02/23	 Image extracted from https://www.dltpodiatry.co.uk/DermaStat on 24/02/23
Supplier	Courage and Khazaka	Delfin Technologies	Delfin Technologies	Arche Healthcare
Cost	£2770	£2750	£7650	£35
Calibration protocol	A calibration kit is provided with this device.	Calibration via an internal mechanism.	The device is calibrated by the supplier.	No information provided by supplier.
Cleaning instructions	The probe head may be wiped with a soft tissue between uses or cleaned with alcohol if required.	The probe head may be cleaned with a non-specified 'disinfecting cloth'.		The probe may be wiped with a soft cloth between uses. Alcohol may also be used to clean probe.
Measurement mechanism	Capacitance	Capacitance	Tissue Dielectric Constant	Capacitance
Measurement Depth	10-45 µm	No specific measurement depth given, instead the values represent the 'effective dryness' of tissues.	Four probes are available with different penetration depths.	No information provided by the supplier.
Accuracy	± 3%	± 3%	± 5%	No information provided by supplier.
Output	0-120 in Arbitrary Units	0-150 in Arbitrary units	1-80 Tissue Dielectric Constant Units	0-99.9% 'The moisture percentage of the measured skin area'
Optimal testing conditions	Ideal: 20°C room temperature and 40-60% relative humidity. Permissible: room temperature between 10-40°C and a relative humidity of 30-70% RH.	Ambient temperature and humidity should be maintained. The tested person should not be under any significant physical or psychological stress.		Room temperature between 5-40°C and a relative humidity of <70%.

Probe placement instructions	Probe head is applied to the skin for < 1 second. Data are displayed and stored in custom software. Three measurements should be taken for each skin site, and the mean of these used for analysis.	The probe head is applied to the skin for three seconds and a value is displayed on the screen of the device. Three measurements should be taken for each skin site, and the mean of these used for analysis.	The probe is applied to the skin surface for several seconds. A small 'beep' noise is emitted, and reading is displayed on a screen.
Skin acclimatisation period	10-20 minutes	20 minutes	None suggested
Additional considerations	A 5 second gap is required between repeat measurements on the same skin site to prevent influence from occlusion.	Skin should be free from significant levels of hair, scars, or folds. Skin surface should be dry and consistent pressure maintained on the probe head. The measurements obtained using these devices are not influenced by surface chemicals, salts, or by a period of occlusion according to the manufacturer.	Device is not to be applied to skin that is: Diseased, infected, injured, sweaty, dirty, wet, or calloused.
References	(Bare & Clarys, 1997; Courage & Khazaka electronics GmbH, 2010)	(Alanen et al., 2004; Delfin Technologies, 2016; EvaluLab, 2018; Miettinen et al., 2004)	(Arche Healthcare, 2018)

4.2.1. Corneometer® CM825 (Courage and Khazaka, Colne, Germany)

The Corneometer® CM825 is a widely used device that quantifies the water content of the superficial portion of the SC using the capacitance method (Serup et al., 2006).

The device probe consists of a grid of gold-covered electrodes, the 'active' portion of which is covered with a 20 µm thick layer of low dielectric material (glass). When applied to the skin surface this creates an electric field within the SC. The depth and form of the electric field are constant due to the stable components of the probe. The probe and the SC then function as a capacitor. The capacitance of the SC (its ability to store water) is variable as it is influenced by its water content, i.e., the more water in the SC the higher it's capacitance. The frequency of the electric field in the SC (indicating its capacitance) is measured by a resonating system which reports the frequency in arbitrary units (AU) which are used to represent skin hydration (Serup, Jemec, & Grove, 2006) (See Figure 22).

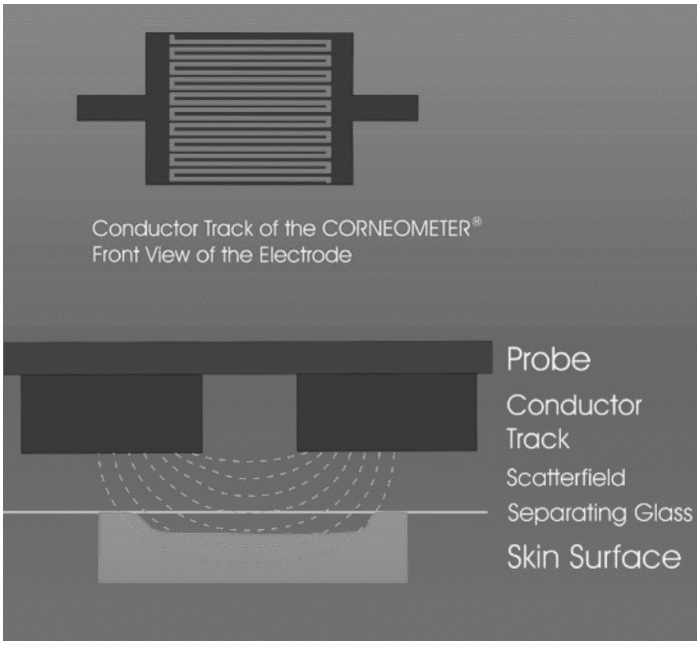


Figure 22. Diagram demonstrating the probe structure and signal penetration of the Corneometer® CM825, extracted from http://www.dproscientific.com/assets/brochure_cm825.pdf on 04/04/23.

An in-vivo model for the calibration of capacitance-based devices with materials of known hydration has not yet been developed. However calibration of these devices is carried out during its manufacture using three standard materials with known capacitance (Serup et al.,

2006), and can be checked by a user in a laboratory setting using a material with known capacitance provided by the manufacturer (Courage & Khazaka electronics GmbH, 2010).

The capacitance method has a high sensitivity which reduces at measures of 110 AU and above (Alanen, Nuutinen, Nicklén, Lahtinen, & Mönkkönen, 2004; Clarys, Barel, & Gabard, 1999; Fluhr et al., 1999b). This is of little consequence to the measurements collected on the human skin, as well-hydrated skin returns values below this threshold. According to Heinrich et al (2003) readings below 40 AU represent very dry skin, 40-55 AU dry skin and above 55 AU is normal skin (Heinrich et al., 2003). However, these guidelines were generated from data obtained solely on the forearm, with no consideration of how natural variation in skin hydration across the body would influence their suitability to application to other skin sites.

Within well-controlled conditions, capacitance based devices have been found to have good repeatability both within different locations on an individual, and within a large group of individuals of a similar age (Rogiers, Derde, Verleye, & Roseeuw, 1990). The intrarater and interrater reliability of the Corneometer® CM825 for the measurement of the foot skin hydration is reported to be between 0.88 and 1 for intrarater reliability, and >0.89 in interrater reliability, at all locations other than callused skin (intrarater reliability: 0.61 (95 % CI [−0.95 - 0.93]) and 0.40 (95 % CI [−3.17–0.90]) for two investigators) (Hashmi, Wright, et al., 2015).

The manufacturer reports the Corneometer® CM825 to measure the hydration of the SC at a depth of 10-20 µm (Courage and Khazaka electronics GmbH, 2010). Fluhr et al (1999) tested this by measuring the hydration of a filter saturated with a sodium chloride (NaCl) solution before and after being obscured with layers of 15 µm thick plastic foil. A single layer of plastic foil reduced the reading of the analog Corneometer® CM825 by approximately 95%. The authors concluded from this finding that the electrical field of measurement for this device is approximately 15 µm or less (Fluhr et al., 1999b).

Clarys et al (2012) undertook a similar experiment using layers of 15 µm thick polyurethane plastic foil to cover the surface of a filter pad saturated with distilled water before measurement using an analog and digital Corneometer® CM825. Conversely to the findings of Fluhr et al (1999), Clarys et al (2012) found that one layer of 15 µm thick plastic foil only reduced the capacitance values by 82.5% and 83.5% for the analog and digital probe,

respectively. In fact, the authors concluded that the penetration depth of both Corneometer® CM825 models was 45 µm (Clarys et al., 2012).

Although a similar method was used, some inconsistencies between these two experiments could contribute to these contradictory findings. Firstly, the permeability of the plastic film used as a barrier. Fluhr et al (1999) used Toppits® (Melitta, Germany) foil, which was specifically chosen due to its low water permeability (less than 0.1g/m^2 measured using the Evaporimeter EP1 (ServoMed, Stockholm, Sweden)). However Clarys et al (2012) used a non-specified polyurethane plastic foil. No information is provided on the permeability of this material. If the barrier used by Fluhr et al (1999) was less permeable than that used by Clarys et al (2012), this would decrease the readings obtained by the Corneometer® CM825. In future, the permeability of the barrier material used for this kind of study must be reported in order to support application of findings. Secondly, the fluid used to saturate the filter pad differed between studies. Clarys et al (2012) used ultrapure distilled water, whereas Fluhr et al (1999) used a NaCL (salt) solution.

Fluhr et al (1999) found that a variation in the concentration of a NaCl solution did not influence values obtained by the Corneometer® CM825 when used to saturate a filter pad of the same thickness (i.e. 140 µm thickness hydration measures (mean ± SD) for 0.15M NaCl solution: 121.2 ± 0.8, and 0.9%NaCl solution: 119.7 ± 1.4). Similarly, Clarys et al (2012) found that the NaCL concentration or purification level of an aqueous solution used to saturate a filter pad did not influence the readings: (mean ± SD) ultrapure distilled water: 117.7 ± 0.4, ordinary distilled water: 118.8 ± 0.4 AU, tap water: 118.2 ± 1.9, 0.15 AU NaCL: 119.1 ± 0.5 AU or Courage-Khazaka calibration solution: 119.8 ± 0.2 AU.

Alanen et al (2004), however, found that the in-vivo measurements collected using the precursor to the Corneometer® CM825 (the Corneometer® CM820) were influenced by the addition of NaCL solution to a base cream (Novalan®, Orion Corporation, Turku, Finland) when applied to the skin (Alanen et al., 2004). However, within this publication there is no discussion of the possible influence of NaCL on the efficacy of a moisturiser.

The inconsistencies in published works concerning the Corneometer® may be due to variations in the model used. Courage and Khazaka produce analog and digital versions of the the Corneometer® CM825. The analog and digital probes have a high correlation, analogous

penetration depth and similar sensitivity (Clarys et al., 2012; Heinrich et al., 2003). However when compared to the analog Corneometer® CM825 probe, the Corneometer® CM820 records higher values (± 60%) at the same skin sites (Serup et al., 2006).

In application to the plantar foot, the inconsistencies in the reported measurement depth of the Corneometer® CM825 are negligible as the reported penetration depths are less than the thickness of the plantar SC. The plantar SC has been reported to be around 500 µm thick – measured in ex-vivo tissue using a (Nikon Eclipse 90i) light microscope (Nikon Corp., Tokyo, Japan) and a scale (Vela-Romera et al., 2019). Although the hydration gradient within the SC has not been measured on the plantar foot, the palm (which is approximately 150um thick (Vela-Romera et al., 2019)) has a consistent, low hydration up to 100-150 µm depth (Egawa et al., 2007). If the plantar skin SC exhibits a similar hydration gradient to the palm, whether the penetration of the Corneometer® CM825 is 15m or 45um is inconsequential.

4.2.2. MoistureMeter SC™ (Delfin Technologies, Kuopio, Finland)

The MoistureMeter SC™ is a relatively new device (first described in 2004) with less supporting literature than the Corneometer® CM825 and the MoistureMeter D® (Miettinen et al., 2004). This device comprises an open-ended coaxial cable that, when applied to the skin, generates an electromagnetic field within the skin at a frequency of 1.25 MHz (application force is controlled by an inbuilt force sensor) (Mayrovitz, Bernal, et al., 2013) (See Figure 23).

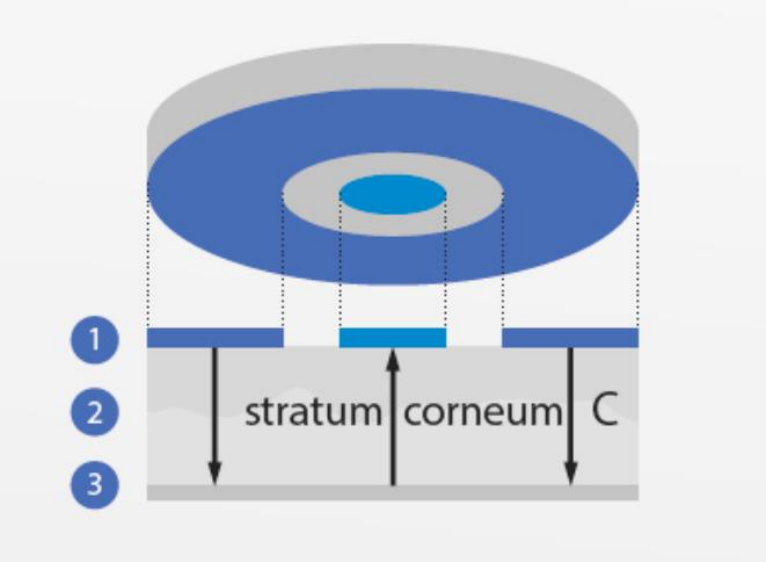


Figure 23. Demonstration of the probe head of the MoistureMeter SC™. Image extracted from https://delfintech.com/products/moisturemetersc/?gclid=EAIaIQobChMIo7aQm-qP_gIVh-vtCh102gUEEAAYASAAEgIDpfD_BwE on 24/02/23.

The measurement mechanism of the MoistureMeter SC™ is purported by the manufacturers to represent both the dryness of the dry layer of the skin (SC) and its thickness, through utilising the different water content of the layers of the skin (Delfin Technologies, 2016).

The SC has a low water content (reducing its ability to conduct electricity) and is therefore primarily capacitive, whereas the deeper layers of the tissue are more conductive. The manufacturers describe the total capacitance measured using this device as being a representation of the capacitance of the layers extending into the skin, which can be reduced both through the superficial dry layers being very dry or very thick (Alanen et al., 2004). The output of this device is therefore described as 'effective hydration' (described in section 2.4.4.1.)

The only commentary available on the function and performance of this device comes from the same publication from Alanen et al (2004), in which the MoistureMeter SC™ is compared to the Corneometer® CM20. The authors propose that the MoistureMeter SC™ is more sensitive to changes in skin moisture changes following application of a glycerin (a moisturising agent), and more independent to influence from NaCl being added to testing solutions (Alanen et al., 2004).

When considering the measurement mechanism described by Alanan et al (2004), very low measures of 'effective hydration' could be anticipated in a relatively this plantar SC (Vela-Romera et al., 2019).

4.2.3. MoistureMeter D® (Delfin Technologies, Kuopio, Finland)

The dielectric properties (ability to act as in electrical insulator) of human tissues are influenced by the amount of water within the tissue (Miettinen et al., 2004; Nuutinen et al., 2004). The tissue dielectric constant (TDC) method uses this principle to determine the hydration of tissues within the human body. The MoistureMeter D® uses an open-ended coaxial probe to generate and transmit a 300-MHz signal into the tissue, the dielectric constant of the tissue is determined through the portion of the electromagnetic wave that is returned to the device (Mayrovitz, Bernal, et al., 2013). This is then represented in arbitrary units ranging between 1 and 80. The dielectric constant of a dry protein is 3.3 AU (such as those found within this skin (Amin & Küpper, 2020)), pure water is 78.5 AU, meaning this measurement range represents the possible TDC range of the SC appropriately (Mayrovitz, Bernal, et al., 2013).

Each MoistureMeter D® probe has two concentric circular sensors, the distance between these sensors is directly proportional to the penetration depth of the electrical signal (i.e., the wider the probe, the deeper the measurement (See Figure 24). Four probes of different sizes are available to measure skin water content at different depths within the tissue (See Table 13). Each of these probes will be referred to by their 'effective' penetration depth throughout this document, rather than their names (i.e. the XS4 will be described as the 0.5 mm probe).

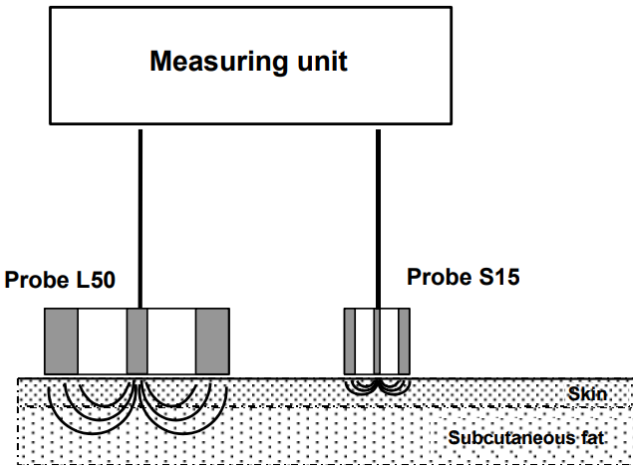


Figure 24. Schematic illustration of the dielectric measurement and induced electrical field in skin and SSF. Image provided by Delfin Technologies.

Table 13. Specification of the four probes associated with the MoistureMeter D®.

Probe name	Probe contact diameter (mm)	'Effective measurement depth' (mm):	Measures down to (mm):
XS4	10	0.5	2.5
S15	20	1.5	3
M25	23	2.5	5
L50	55	5	8

The data obtained using each of these probes is influenced by the water content of the tissue across a range of depths to varying degrees – i.e. The water content of superficial tissues has a higher impact upon the MoistureMeter D® readings than deeper tissues (Alanen et al., 2004). The 'effective' measurement depth indicates at what depth the water content of the tissue becomes markedly less impactful, calculated by a method devised by Meaney et al (2016).

The ability of the MoistureMeter D® to measure tissue-water has been validated through the work of Nuutinen et al (2004), in which the volume of fluid removed during haemodialysis treatment was found to correlate strongly with the tissue-water measured using the MoistureMeter D® (r= -0.99). In this same study, the repeatability of this device was found to be 3.0%, and was not dependant on the phase of haemodialysis (Nuutinen et al., 2004). A study by Miettinen et al (2006) in which the MoistureMeter D® was used to measure changes in water content of irritant exposed skin, found that the reproducibility of repeated measures was 2-5%, and it's standard error of measurement was 3-5%. Albeit a positive representation

of the use of this device, it is important to consider that these studies collect data from a layer of tissue which is potentially beyond that achievable on the plantar foot, as such their findings may not be entirely transferrable to this work. 1.5 mm (Nuutinen et al., 2004) and 2.5 mm (Miettinen et al., 2006) 'effective' measurement depth probes were used on the forearm within these studies. At this skin site, these are anticipated to collect data on the tissue water in the dermis (Miettinen et al., 2006).

4.2.4. DermaStat® (Arche Healthcare Ltd, Connecticut, United States)

Although there is no published data regarding the use of the DermaStat® it has been included within this body of PhD work for three reasons: it's expressly intended for use on the plantar foot, and it is low cost, and accessible to UK clinicians.

If this device is found to generate data comparable to that of a validated device such as the MoistureMeter SC2 or the Corneometer® CM825, this will make skin-hydration measurements affordable, enabling foot skin hydration health monitoring within low-resource settings.

Within the documentation provided with the DermaStat®, bioelectric impedance technology is referenced as its measurement mechanism (Arche Healthcare, 2018). However upon discussion with the supplier (Arche Healthcare) it was established that this is a capacitance-based device (personal communication with Arche Healthcare staff, Spring 2023). No information is provided on the measurement depth of this device or its sensitivity to salts on the skin surface.

To aid the interpretation of the data collected using this device, a "Skin Moisture Index" is provided in the device user leaflet (Arche Healthcare, 2018). This indicates a range of values purported to align with skin quality: 0-25% 'Very Dry', 25-50% 'Dry', 50-75% 'Good' and 75-100% 'Very good' (Arche Healthcare, 2018). Unfortunately, no evidence is supplied to support the interpretation of data in this manner.

Recently, a new device has been created by Arche Healthcare, also called DermaStat™ (although with the addition of a trademark indicator), which is intended for use by an individual on their own foot skin (Arche Healthcare, 2022). This device uses bioelectric

impedance to measure skin hydration (confirmed via discussion with Arche Healthcare) and is provided with a long handle to facilitate self-testing of the dorsal foot. A “Skin Moisture Index” has been generated for this device due to the change in measurement mechanism and skin testing site: 20-26% ‘Very dry’, 27-32% ‘Dry’, 33-38% ‘Good’, 39-45% ‘Very good’ (Arche Healthcare, 2022). Some example data are provided within the ‘DermaStat™ User Guide’ (2022) to support the accuracy of measures obtained using this device, however no context is provided on the circumstances of data-collection or system used for comparison.

Currently, no other commercially available hydration measurement devices are designed explicitly for home use. Access to foot skin hydration measurement at home could be advantageous in a number of ways. Empowering individual with diabetes to monitor and take control of their health has been shown to improve outcomes (Baldoni et al., 2017) and also aligns with the increasing focus on patient-centred care in the NHS (Alderwick & Dixon, 2019). Additionally, digital devices for remote monitoring of patient conditions are becoming increasingly popular and have been found to result in the reduced occurrence of DFUs, due to early identification of risk-factors for ulceration (Najafi & Mishra, 2021).

The suitability of this device and the skin site it is intended for measuring are yet to be determined. However, it is not known whether there is a strong correlation between the hydration of the skin on the dorsum of the foot other foot skin sites, such as the plantar skin, in any number of circumstances, i.e. in healthy skin, xerotic skin, or in its rate of change following emollient therapy. Also, although bioimpedance has previously been used to quantify skin hydration (Gidado et al., 2022), and devices using this technology demonstrate strong correlations with other devices such as the Corneometer® CM820 and Corneometer® CM825 (Fluhr et al., 1999a), bioimpedance is known to be influenced by the presence of salts on the skin surface (Gabard et al., 2006). It is yet to be seen how this device compares to those that are currently commercially available.

4.3. Confocal Raman Spectroscopy

In Study 3, CRS is used to measure the volume of compounds within the skin. Below, the underpinning science behind this method is described, followed by the specification of the device used within this study and a description of its application.

4.3.1. Principle of Confocal Raman Spectroscopy

A beam of light from a single or narrow wavelength is directed at a sample (in this instance an area of skin), the electric component of the electromagnetic light wave then interacts with the electron cloud within the molecules present (van der Pol & Caspers, 2014). Most of the laser light is reemitted at the same level of energy, but in a different direction, however, a small portion of the light is reflected with a different energy (van der Pol & Caspers, 2014).

Each compound in the skin is composed of a specific arrangement of atoms – the light reflected with a different energy represents the specific arrangement of atoms it encountered (van der Pol & Caspers, 2014). When the intensity of the reflected light is measured, a set of Raman signals are obtained that demonstrate shifts in the frequency of light from the original light source (van der Pol & Caspers, 2014).

These Raman signals can be compared to the known peaks that are representative of the vibrational frequencies of specific molecules, allowing the composition of the sample to be quantified. The size of these peaks is indicative of the volume of the compound. i.e., Vitamin A has a peak at 1593 cm−1, this peak is higher in the skin following application of a compound to the skin that contained Vitamin A (dos Santos et al., 2019a).

Through the use of confocal optics (spatial filtering), these data can be collected from a small area of tissue at defined depths (van der Pol & Caspers, 2014).

4.3.2. Second generation skin composition analyser (Gen 2 SCA)

The details included in this section are primarily extracted from the 'Gen2-SCA: 2nd generation skin composition analyser brochure' published by RiverD in 2013.

The 2nd generation skin composition analyser (Gen-2 SCA) is a Confocal Raman Spectroscope designed for use on in-vivo skin (RiverD International B.V. Rotterdam, The Netherlands). The specification of this device is described in Table 14.

Table 14. Gen2-SCA Specification. Data source: Gen2-SCA: 2nd generation skin composition analyser brochure (RiverD International BV, 2013).

Laser source	785 nm laser source for measurements in the 400-2500 cm^{-1} spectral (fingerprint) region 671 nm laser source for measurements in the 2500-4000 cm-1 spectral (high wavenumber) region
Charge Coupled Device (CCD)	2000 x 256 pixels nIR detector system, thermoelectrically cooled to approximately -45° C Quantum efficiency up to 90%
Microscope objective	1.2 numerical aperture NIR-Raman optimised microscope objective
Depth resolution	<3 µm
Depth range	0-400 µm
Associated software	Device control software: RiverICon 4 (RiverD, Rotterdam, The Netherlands) Data analysis: SkinTools 3 (RiverD, Rotterdam, The Netherlands)
Associated hardware	Power supply unit Windows 10 – compatible Medical PC (CE certified for use in Medical Devices)
Calibration	A calibration procedure is carried out at the beginning of each day of use. A calibration tool is placed over the optical window and a pre-programmed calibration file is run in RiverICon 4.

4.3.2.1. Internal mechanism

Laser light travels from a high-performance Raman module through a confocal pinhole to an inverted microscope stage. An immersion microscope objective then focuses the light through an optical window onto the skin resting on the measurement stage above. Raman scattered light returns to the high-performance Raman module, back through the microscope stage and confocal pinhole to the CCD detector system.

4.3.2.2. Operator training

Operators attend a 2-day training course on the use of the Gen-2 SCA with manufacturers at the RiverD headquarters in Rotterdam.

4.3.2.3. Data-collection templates

'Templates' within RiverICon 4 define the type of measurement taken:

Each template comprises of several depth ranges (called tracks) within which resolution, exposure time and frame number are varied. Custom tracks and templates are built by the operator to generate a testing protocol suitable for the skin site.

4.3.2.4. Establishing measurement parameters

Data can be collected from the fingerprint region (400-2500 cm^{-1}) or high wavenumber region (2500-4000 cm^{-1}) depending on the compound being measured. Water volume within tissues is quantified using data from the high wavenumber region, all other compounds quantified using the Gen2-SCA are calculated using data from the fingerprint region.

RiverICon 5 is the operating system for the Gen-2SCA. This software has a feature that allows the user to programme the Gen-2 SCA to automatically undertake multiple measurements in sequence in either the fingerprint or high-wavenumber region; i.e., to collect measures at a 2 µm resolution from the skin surface (0 µm) to 10 µm depth (generating data from 0, 2, 4, 6, 8 and 10 µm). The frame number and exposure time are also pre-programmed by the user.

A track is a series of measurements with these pre-set parameters. Several tracks can be programmed to be undertaken sequentially in a measurement template.

For example, a template consisting of two tracks could be:
Track 1: 2 µm resolution from 0 µm to 10 µm with a single frame and an exposure of 1 second.
Track 2: 5 µm resolution from 10 µm to 25 µm, with a single frame and an exposure of 3 seconds.
This would collect data from 0, 2, 4, 6, 8, 10, 15, 20 and 25 µm measurement depths.

The time required for each measure is cumulative. To run the above 'template' would take 14 seconds ((5*1 second) + (3*3 second)).

4.3.2.4.1. Measurement parameters within the context of Study 3

For the purposes of this project six templates were created, one of each measurement type (fingerprint and high wavenumber) per skin site measured. Maximum measurement depth (forearm: 50 µm, arch: 400 µm, heel 400 µm) was informed by published data on skin thickness for the forearm and heel (See Table 9). Remaining measurement parameters were established via an iterative process of conducting measures on each skin site and varying spectra resolution, exposure time and frame number to achieve high-quality (low noise) data within a reasonably short period.

Within the superficial portion of the skin, a high resolution and low exposure time were found to be suitable across all sites. Within deeper tissues, increased noise necessitated a longer exposure time which was compensated by taking fewer spectra (lowering resolution), especially within the heel. The final templates (See Table 15) maximised data-quality within the limited time available for data-collection within this study.

Table 15. Template parameters for skin site and measurement type fingerprint (FP) and high wavelength (HW).

	Templates 1+2 (HW + FP) Ventral Forearm			Templates 3+4(HW + FP) Medial Arch			Templates 5+6 (HW + FP) Heel		
Track number	Track depth (µm)	Resolution (µm)	Exposure (s)	Track depth (µm)	Resolution (µm)	Exposure (s)	Tracks (µm)	Resolution (µm)	Exposure (s)
1	0-20	2	1	0-20	2	1	0-50	5	2
2	20-50	5	5	20-400	40	2	50-400	50	10
Total time (s)	40			38			90		

The research team aimed to collect five good-quality measures from each skin location for each measurement type (fingerprint region and high-wavenumber region) as advised by the manufacturer (private communication, August 2022).

4.3.2.5. Use of the Gen-2 SCA

Skin is placed on the measurement platform, and a live video feed of the skin surface is displayed on the screen via the RiverICon software (See Figure 25Figure 25. RiverICon 5 Graphical Interface – extracted from "User Manual RiverICon 5.0 December 2021" RiverD). The probe can be slid along the skin by the operator using software controls until high-contact areas of skin are identified by the researcher. The level of contact between the probe and the skin is assessed subjectively by the operator. The high-contact areas appear dark grey. Suitable testing locations are selected using a 'point and click' function on the video screen. An appropriate template is selected, and the start button begins data-collection. Spectra are then displayed in real-time on-screen during measurement, and the output is automatically stored upon completion.

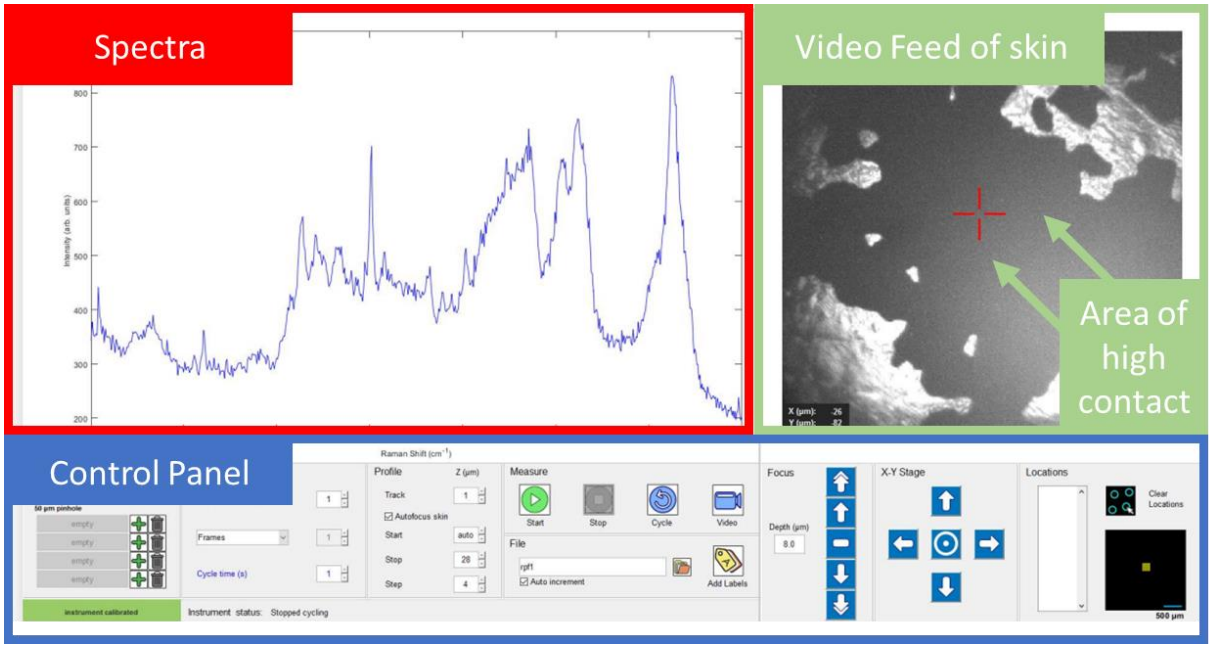


Figure 25. RiverICon 5 Graphical Interface – extracted from "User Manual RiverICon 5.0 December 2021" RiverD.

4.3.2.6. Data analysis

SkinTools 3 software facilitates the analysis of Raman spectra obtained using the Gen2-SCA by an operator with no specialised knowledge of CRS. Raman spectra files are exported from RiverICon software into SkinTools 3 by the operator, who then chooses a pre-programmed analysis protocol to be applied to selected files.

The analysis protocol selected is dictated by the spectral region of the data collected. Data from the high-wavenumber spectral range may be assessed for its water content which is calculated from the ratio between the signal intensities of two bands: the CH band (2910-2960 cm^{-1}) and the OH band (3350-3550cm^{-1}) that represent the water/protein ratio in the skin (RiverD International BV, 2020).

Within the fingerprint spectral range (400-1800cm^{-1}), spectral fitting is used to calculate the volume of compounds within the skin from a large range of reference spectra representing intrinsic and extrinsic skin composites (for example, ceramide, cholesterol, urea). The spectral fitting is detailed in research conducted by Caspers et al (2001).

The output of this analysis can either be exported to another software for further analysis or reviewed in SkinTools 3. SkinTools 3 presents the data as an index of compound volume over skin depth and allows the operator to overlay several trials over one another to compare

results from test conditions, i.e. following an intervention or at different skin sites. For the purposes of the study in this thesis results of SkinTools 3 analysis were exported into Microsoft® Excel® (Microsoft Corporation (Version 2208), Washington, United States) for analysis. A full demonstration of this process is described in section 7.3.9.2.

4.3.2.7. Application to the plantar foot

The Gen2-SCA has not previously been applied to the plantar foot due to problems anticipated with stabilising the limb and achieving adequate contact between the scope and the skin. These are reasonable concerns as the plantar skin is harder and rougher than other skin sites commonly tested using this device, such as the volar forearm (Hashmi, Nester, et al., 2015; Nam et al., 2015). This reduces the ease with which the skin surface would conform to the scope surface. Also, the Gen2-SCA must always be kept upright on a flat surface and the probe surface is on the uppermost surface of the tool, meaning the foot must be rested on top of the device and remain still for several minutes whilst measurements are taken. Remaining entirely still is challenge for some individuals and the current set-up of the Gen2-SCA offers no support or fixation for the foot.

For the conduction of 'An evaluation of the biochemical composition of the foot skin using CRS', a protocol was developed for the use of the Gen2-SCA on the plantar foot.

The issues described above were combatted through trial and error using different body positioning (flexed knee stabilised with a pillow, for example) and use of various stabilising tools (footbed orthoses with an aperture and footwear with an aperture, for example). The most effective method found is described below:

Participants were seated on a movable plinth during data collection with their leg hanging over the edge of the plinth. The heel was positioned over the Gen2-SCA scope, and the plinth was lowered until the heel rests on the scope with the knee bent at a 90° angle. This body positioning was found to reduce instability as the participants leg was fully supported by the plinth and the Gen2-SCA casing.

A rubber ring was placed under the heel (the inferior surface resting around the outside edge of the scope) to reduce the pressure on the scope and gently compress the plantar tissue

centrally. Rubber rings of different width and thickness are provided with the Gen2-SCA device to facilitate measurements on the scalp. This facet of the protocol allowed the full weight of the lower leg to be supported by the Gen2-SCA casing, whilst the scope surface was relatively unloaded. Excess pressure on the scope prevents sliding across the skin, so measurements are not able to be taken. The lateral pressure on the tissues caused the plantar heel to flatten on the scope surface, increasing skin contact and visibility.

The thickness of the ring, and height of the plinth were adjusted until sufficient contact was achieved between the plantar skin and the scope.

4.4. Measures of mechanical behaviour of skin

Two devices are used to quantify the physical behaviour of the skin, the DermaLab® Series SkinLab Combo Elasticity Probe (Cortex Technology, Hadsund, Denmark) and the SATRA STD 226 Digital Durometer (SATRA Technology, Kettering, UK). The former measures elasticity, and the latter measures hardness. The specification of these devices can be found in Table 16.

Table 16. Technical details of biophysical skin measurement devices.

Device	DermaLab® Series SkinLab Combo Elasticity Probe	SATRA STD 226 Digital Durometer
Image	 *Image extracted from https://www.deernz.org/sites/dinz/files/Instruction%20Manual%20SkinLab%20Z5010108%20UK.pdf on 04/05/21.*	 Image extracted from https://www.satra.com/test_equipment/machine.php?id=144 on 08/04/21
Supplier	Cortex Technology	SATRA Technology
Calibration protocol	Calibrated by manufacturer	Calibrated by manufacturer
Cleaning Instructions	The probe front may be wired with a dry cloth. If required, a drop of alcohol may be used to remove glue from the probe surface.	No information given
Probe placement instructions	The foot of the probe is adhered to the skin using double-sided adhesive rings.	The base of the probe is pressed firmly onto the skin surface. An indenter that protrudes from the probe surface is depressed into the probe. A shore hardness reading is displayed on the digital screen that represents how far into the probe the indenter has been depressed.
Accuracy	No data provided	No information given
Output	Young's Modulus (MPa), viscoelasticity (MPa) and retraction (msec).	Shore Hardness OO Scale (0-100)
Optimal testing conditions	Room temperature 10-35°C	No information given
Additional considerations	30-60 minutes must be allowed between repeat measurements.	Additional considerations relevant to the data collected using this device are explored below.
References	DermaLab Series SkinLab Combo Instruction Manual (Cortex Technology 2015)	SATRA website: https://www.satra.com/test_equipment/machine.php?id=144

4.4.1. SATRA STD 226 Digital Durometer

This device is not explicitly designed for use on human skin. Durometers are intended for use on materials of uniform density of minimum 6 mm thickness, as per ISO 7743:2011 (International Standards Organisation, 2011). As described in section 1.2.2 , no skin surface on the human body is consistently thicker than 6 mm (Lee & Hwang, 2002). The deformability of soft tissues underlying the skin therefore also influence the data collected using a durometer. A recent finite element model generated by Chatzistergos et al (2022) demonstrated that data from the SATRA STD 226 Digital Durometer primarily reflects bulk deformity of tissue, not the characteristics of the skin alone, on the plantar heel. However, this study also showed that a change in skin thickness or hardness can also influence readings from the SATRA STD 226 Digital Durometer, although to a smaller degree than underlying tissues (Chatzistergos et al., 2022).

In one instance, durometer data from the plantar foot have been found to decrease with skin hydration (Schmidt et al., 2018). However, this study did not involve direct comparison with skin hydration measures, but instead comparison of plantar skin hardness between two sets of feet, half of which had been immersed in water for 45 minutes to artificially raise skin hydration (Schmidt et al., 2018).

A durometer has been used to assess the soft-tissue characteristics of the foot in diabetes (Charanya et al., 2004; Piaggesi et al., 1999; Thomas et al., 2003) and the association between plantar skin thickness and sensitivity (Holowka et al., 2019; Strzalkowski et al., 2015), and changes in skin hardness and age (Periyasamy et al., 2012).

4.4.2. DermaLab® Series SkinLab Combo Elasticity Probe Consideration for use:

This device works by applying negative pressure to the skin inside the probe head (the circular probe head is adhered to the skin) for 2-3 seconds. The rate at which the skin is sucked into the capsule, its volume, and how quickly it retracts following the release of pressure are measured and used to calculate the tissues Young's Modulus and viscoelasticity. Retraction speed following displacement is also provided as an output.

As described in the instructional manual, the Young's Modulus and viscoelasticity output for this device have skin thickness factored into their calculations:

Young's modulus is calculated as below:

$Young's\ Modulus\ (MPa) = \Psi.\ p.\ \frac{r^4}{\Delta \times .s^3}$ where:

$\Delta \times$ = elevation pf the skin measured at the middle of surface (in m)

Ψ = constant

P = surface pressure (in Pa)

E = elasticity modulus (in MPa)

R = radius of the surface (0.005 m)

S = thickness of the surface

Viscoelasticity is calculated by dividing Young's modulus by retraction time:

VE = Young's Modulus/$R_{normalized}$ where $R_{normalized = R/260\ ms}$

Skin thickness is entered as 1 mm as default within the software but can be modified (Cortex Technology 2015). As the thickness of the skin is not quantified in these studies, it is not possible to modify the skin thickness to accurately reflect the skin locations measured. This limits application of these outputs to the plantar skin as this depth is not representative of the thickened skin at this location. Using an estimation of skin thickness for the plantar skin would generate uncertainty and reduce comparability of data between plantar and non-plantar sites.

The retraction output, however, does not require skin thickness data to be input into the software. Retraction is the time taken for skin to retract from peak elevation to 33% of peak elevation given in milliseconds. This output is considered the most appropriate for use within this study.

To provide consistency between skin sites, the same suction forces are applied to all skin sites measured within this study: 400mbar. As per manufacturer's instructions, several (in this instance 5) measurements are taken within the same cycle (within a period of around 20 seconds) for each site and the mean value of these used for analysis.

4.5. Assessment of skin topography – The Visioscan® VC98

The Visioscan® VC98 (Courage and Khazaka electronic GmbH, Cologne, Germany) is a system consisting of a handheld probe and accompanying software that allows the surface topography of the skin to be measured (for technical details see Table 17). The probe contains two light sources that emit UVA light either side of a video camera; the spectrum of the light, and the layout of the light sources allow for non-glossy, high-resolution images of the skin to be captured (CK electronic GmbH, 2005).

The probe is placed on the skin and live images of the skin surface are transmitted to the software and still images are captured by the researcher. These images can then be analysed within the software to a set of parameters that are representative of different topographical characteristics (scaliness, smoothness, wrinkles, for example). These characteristics are calculated according to the colour of pixels within the image.

For the purposes of this investigation, an analysis method termed 'contrast' was used, which calculated the difference in the colour (levels of grey) between adjacent pixels (CK electronic GmbH, 2005). This indicates the roughness of the skin surface, which is of interest within this work due to the relationship between skin roughness and hydration.

Table 17. Technical details for the Visioscan® VC98.

Device	The Visioscan® VC98 (Courage and Khazaka)
Image	 Image extracted from https://www.courage-khazaka.de/en/scientific-products/all-products/16-wissenschaftliche-produkte/alle-produkte/150-visioscan-e on 06/04/21
Supplier	Courage and Khazaka
Calibration protocol	Calibration can be undertaken by the operator using a calibration procedure built into the Visioscan® VC98 software and a calibration head that is placed on top of the camera.
Cleaning Instructions	The device may be cleaned using a dry, soft tissue. An alcohol-soaked tissue may be used if very dirty.
Measurement mechanism	The contrast of the image collected is analysed using custom software to determine the volume and density of peaks and troughs in the skin.
Accuracy	No accuracy data provided
Output	Arbitrary units indicating smoothness, roughness, scaliness and wrinkles, or 'contrast'
Optimal testing conditions	Temperature between 20°C and relative humidity of 50%

Probe placement	The base of this tool is placed upon the skin and an image is captured of a 6X8 mm area of the skin surface using a high-resolution video camera mounted with UVA-lights.
Acclimatisation period	None suggested
Reference	Visioscan® VC 98 and the Software SELS: Information and Operating Instruction (CK electronic GmbH, 2005)

Hashmi et al (2015) used this device to quantify the roughness of the healthy plantar skin at the 1st or 4th metatarsal head (depending on the location of an adjacent hyperkeratotic lesion) and the base of the 5th metatarsal, as well as plantar hyperkeratotic lesions (callus, fissures, and corns). Calluses and fissures were found to have the greatest skin roughness, generating approximately 50% higher contrast data than healthy plantar skin or the surface of corns (Hashmi, Nester, et al., 2015). No significant correlations were found between hydration and skin roughness in this investigation despite the large number or participants involved (n=93) (Hashmi, Nester, et al., 2015).

4.6. Foot Skin Health Questionnaire: A pilot test informed questionnaire

4.6.1. Industry motivation

Within the Gap Analysis provided by Scholl and in conversation with the research and development team, several issues were raised that relate to individuals' perceptions of their foot skin and how these could be used to inform emollient marketing and efficacy testing. The development of a self-administered scoring system for foot skin health was proposed, the perceived benefits of this for Scholl are described below:

- Insight into how specific features of skin inform an individual's perception of foot skin health or pathology could be used to inform the marketing materials used for products: i.e., How does a consumer's perception of foot skin flaking, cracking, or hardness contribute to their perception of overall foot skin health and their propensity to seek treatment? If consumers are sensitive to skin cracking, then this skin feature may be more heavily featured or described on packaging for an emollient.
- The use of a self-administered questionnaire generates opportunity for remote product testing. Distributing a product and having participants record their perceptions of emollient effectiveness using such a questionnaire is more cost effective than conducting an on-site clinical trial. Although this would not generate the same evidence for product claims, for example, 'skin hydration increases by 20% in 24 hours'. This could be used as a method to conduct early assessment of efficacy or support product claims based around consumer experience (i.e., 'users found foot skin cracking reduced by 50% in 1 week').

Prior to the commencement of the below project, the researcher reviewed the process required for the design and testing of a formal patient reported outcome measure, however this was deemed to be excessive when a simple, pilot-test informed questionnaire is sufficient to provide the benefit described above and fulfil the requirements for use within a study described in this thesis: As part of 'An investigation into the hydration of the foot skin and associated skin characteristics' the participants perception of their skin features associated with skin hydration (hardness, roughness, dryness etc.) was compared to objective measures of these characteristics.

To streamline the questionnaire development process, existing tools developed for a similar purpose were identified via a literature search and assessed for inclusion within the questionnaire using a set of pre-determined requirements. Suitable components were extracted, compiled, and supplemented with additional questions. The design of the final questionnaire was informed by questionnaire design theory (Oppenheim, 1992) and feedback from a diverse panel of reviewers.

4.6.2. Introduction

Despite the commonality of skin pathology on the human foot (Farndon et al., 2006), and the importance of consumer perception in identifying and treating these (Schofield et al., 2009), no tool exists to enable an individual to self-assess the features of their foot skin in a standardised manner.

This section aims to develop a short questionnaire that primarily collects data that aligns with the objective measurements collected in 'An investigation into the hydration of the foot skin and associated skin characteristics', but also has the potential to be applied more widely within foot skin health research.

4.6.3. Aim and objectives.

Aim: To create a tool that collects data on consumer perception of foot skin features related to foot skin health and presentation of xerosis.

Objectives:

- To review existing tools and questionnaires available to score skin features on the foot or severity of skin conditions associated with xerosis.
- To collate appropriate items from existing tools and questionnaires and supplement these with additional relevant questions to develop a new tool.
- To collect feedback on the design and content of this tool from a diverse group of people and amend accordingly.

4.6.4. Literature search strategy

A literature search was conducted using the University of Salford Library search engine (containing databases: CINAHL, Cochrane Library, Covidence: Systematic and Literature Review Management, Department of Health, EBSCO: All databases, Evidence search (NICE), Hathi trust Digital Library, Highwire Press, JISC Library Hub Discover, JSTOR, Ovid Online, Oxford Reference Online, ProQuest Central, ProQuest Health and Medicine Databases, ScienceDirect, UK National Statistics, Web of Science: Core collection, Wiley Online Library). The following key words and Boolean search terms were used:

Skin **AND** Score **OR** Assessment **OR** grade **AND** Foot **OR** Callus **OR** Xerosis **OR** Dry

Eight skin assessment tools were identified: Xerosis Assessment Scale; Overall Clinical Cutaneous Score; Overall Cutaneous Score (Pham et al., 2002); Merriman Grades of Callus (Springett & Merriman, 1995); Efficacy Measurement of Cosmetics and other Topical Products clinical tools: Consumer and Expert evaluation of a selected anatomical region or test site via a Visual Analogue Scale, Overall Dry Skin Score (Serup et al., 2006), Specified Symptom Sum Score System: grading of scaling, roughness, redness and cracks and the Dry skin/ichthyosis area and severity index (Serup, 1995).

4.6.5. Requirements for foot-skin assessment tool

The existing tools vary considerably in their scope and purpose. To guide the discussion of these tools, and ensure selection of suitable material, a list of requirements for this studies' data-collection protocol was defined.

A suitable tool will:

1. Collect data that contributes to the fulfilment of objective 3 for the study titled 'An investigation into the hydration of the foot skin and associated skin characteristics': To test for correlations between objective measures of hydration and the subjective opinions on skin hydration.
2. Be applicable to non-foot skin areas.

   Despite the primary focus of 'An investigation into the hydration of the foot skin and associated skin characteristics' being the features of the foot skin; the comparator sites in the studies are the forearm and anterior shin.

3. Not be specific to skin pathology (excluding foot skin xerosis).

   Participants displaying symptoms of a skin pathology will be excluded from data collection in study 'An investigation into the hydration of the foot skin and associated skin characteristics', this is except for mild-moderate xerosis or hyperkeratosis.

4. Be written in layperson's terms and useable by non-clinicians.

   Questionnaire recipients may not have any prior knowledge about skin features or assessment. The Flesch-Kincaid method will be used to assess readability. As the intended audience for initial use of this tool are undergraduate-level university students, grade 7 or below will be considered acceptable (US college equivalent) (Flesch, 1948).

5. Provide primarily quantitative data.

   As in the first instance, the outcome of this data collection will be compared directly to objective biophysical measures of skin characteristics, these must be primarily quantitative in nature. Any qualitative data collected must be limited in volume and contribute to the improvement of the questionnaire in future. i.e., to collect data where this is missing from the quantitative components of the study that are relevant to the study outcomes. For example, if a specific symptom or product is repeatedly described within the qualitative feedback of this survey, this can be later included as a quantitative component.

6. Present descriptions of skin characteristics that are reflective of foot skin xerosis.

   Although participants involved in this study will not have significant skin pathology on the foot as judged by the researcher, this does not mean participants will perceive this to be the case. The language used must be appropriate for describing the characteristics of foot skin characteristics associated with xerosis accurately.

4.6.6. Evaluation of existing skin assessment tools

This section contains a review of the skin assessment tools and questionnaires identified by the literature search.

4.6.6.1. Xerosis Assessment Scale (XAS)

The XAS (See Table 18) is a 9-point scale (0-8) that can be used to assess skin xerosis symptoms independently or as a component of the Overall Clinical Cutaneous Score (described below)

(Pham et al., 2002). This tool, although not foot-specific, has been used to assess the efficacy of emollient therapy for xerosis on the human foot (Carter et al., 2013; Federici et al., 2015; Garrigue et al., 2011), often alongside another scoring system that indicates skin stiffness or hyperkeratosis i.e. the Overall Cutaneous Score (Federici et al., 2015) and the Merriman Callus Grades (Hashmi, Wright, et al., 2015) (See Table 20 and Table 21).

Table 18. Xerosis Assessment Scale.

Score	Descriptor
0	Normal skin
1	Few minute flakes
2	Many places many undifferentiated flakes
3	Some polygonal scales
4	Moderate number of polygonal scales
5	Large number of polygonal scales
6	Fissuring between scales
7	Moderate fissuring between scales
8	Deep fissuring between scales

Due to the narrow scope of this assessment tool and the complex language used, the XAS is unsuitable for use in this study. The skin characteristics assessed by this tool do not represent the breadth of skin changes associated with xerosis on the foot i.e., thickening and hardening (as indicated by combined use of the XAS with another tool when applied to the foot skin previously). Also, the output of this tool is a single data point that represents the severity of flaking, scaling, and fissuring of the skin as a composite measure. The propensity of the skin to form flakes does not necessarily linearly correspond to severity of fissuring, and as such, these features should be assessed independently. Finally, as this tool has been designed for clinician use, the complexity of language makes this inaccessible to the layperson, indicated by a Flesch-Kincaid readability score of 9.5.

4.6.6.2. Overall Clinical Cutaneous Score (OCCS)

The OCCS is a 15-point score, calculated as the sum of the XAS score and two values indicative of skin stiffness/roughness and the presence of hyperkeratosis (See Table 19) (Pham et al., 2002). This score is designed for use by a clinician when assessing foot skin pathology. This is evident in the technical language used, resulting in a Flesch-Kincaid rating of 14. The latter two categories of the OCCS are intended to capture skin characteristics associated with

xerosis on the foot that is not captured within the XAS. This tool has been used to evaluate the effectiveness of an emollient applied to xerotic skin on the foot of patients with diabetes alongside the XAS, both were found to be sensitive to changes in skin features following emollient therapy (mean XAS and OCCS scores decreased by 38.1% and 38.4% respectively following 14 days of emollient application) (Garrigue et al., 2011).

Table 19. Overall Clinical Cutaneous Score.

Score	Sensation on palpation
0	Supple skin
1	Stiff skin
2	Rough skin
	Assessment of keratosis (thickness of corneal layer)
0	No hyperkeratosis
1	Hyperkeratosis not severe
2	Severe hyperkeratosis (requiring chiropody treatment)

Although the addition of observation of textural skin changes makes this score a more comprehensive representation of xerosis features on the foot than the XAS alone, the way these are assessed is not synonymous with the requirements for this investigation:

Within assessment of 'sensation on palpation', the observed skin may be recorded as 'supple', 'stiff' or 'rough' (indicating advancing skin pathology). Although the stiffness and roughness of skin are found to change with hydration (Hashmi, Wright, et al., 2015) progression of disease severity does not necessarily advance from 'stiff' to 'rough'. A pliable skin surface can have a rough surface due to skin flaking (i.e. psoriatic plaques) or an extremely stiff area of skin to be relatively smooth (i.e. an established callosity within a long-distanced runner).

This issue is also reflected within 'Assessment of keratosis' section, in which 'severe hyperkeratosis' is indicated when the keratosis is judged to necessitate chiropody treatment. Propensity to access clinical management of a disease process does not linearly advance with disease severity, this is highly dependent upon individual circumstances. For example, one individual may experience significant pain from a small heloma durum and seek regular debridement, however an individual with palmoplantar keratoderma (extreme hyperkeratosis) may manage their condition using over-the-counter remedies with ease.

4.6.6.3. Overall Cutaneous Score (OCS)

The OCS is a tool for the assessment of hyperkeratosis severity also described by Pham et (2002) (See Table 20). The OCS is a 4-point scale, with the descriptors ranging from 'normal skin' to 'severe hyperkeratosis' (Pham et al., 2002). This tool has previously been used by researchers alongside the XAS to evaluate skin features following a trial of emollients with different compositions and, although there was no direct comparison undertaken between these two outcomes measures, these two measures displayed the same trends as the study progressed (reduction in mean scores similarly following emollient application) (Federici et al., 2015).

Table 20. Overall Cutaneous Score.

Score	Descriptor
0	Normal skin
1	Mild hyperkeratosis
2	Relevant hyperkeratosis
3	Severe hyperkeratosis

Although this tool is simple to use, it is not suitable for use by a layperson due to the use of technical language and the requirement to interpret what hyperkeratosis is 'relevant' (See Table 16. Score '2'). Further, the features of hyperkeratotic plaques are not described. In fact, no other indicator of skin health is examined, such as thickness or roughness, limiting the scope of this tool to the assessment of hyperkeratosis alone.

4.6.6.4. Merriman Grades of Callus (MGC)

Springett and Merriman (1995) present a system for grading foot skin callus according to severity (See Table 21). This is achieved by identifying a callosity and assigning it to one of two grades according to the descriptors in Table 17 (Springett & Merriman, 1995). This grading system was utilised by Hashmi et al (2017) when characterising hyperkeratotic skin. Merriman Grades may only be used to assess the severity of callosities and they provide very little information beyond a broad understanding of the severity of the lesion. As such, they are unsuitable for use within this study.

Table 21. Merriman Grades of Callus.

Grade	Descriptor
Grade 1	No specific callus plaque, but diffuse or pinch callus tissue present or in narrow bands

Grade 2	Circumscribed, punctate oval or circular, well-defined thickening of keratinised tissue

4.6.6.5. EEMCO clinical tools

In 1995, the European Group on Efficacy Measurement of Cosmetics and other Topical Products (EEMCO), published a guidance document for the assessment of xerosis and ichthyosis using clinical scoring systems of their own design (Serup, 1995). The intent of this publication was to encourage the standardisation of skin assessment techniques across cosmetology and medical science (Serup, 1995). Four tools were proposed in total; three of which are designed to be used by a clinician and the final for self-assessment by an individual:

4.6.6.5.1. EEMCO clinical tool 1: Overall Dry Skin Score (ODSS)

The ODSS is a 5-point scoring scale that is used to assess the severity of xerosis and ichthyosis pathology according to visible symptoms (See Table 22) (Serup, 1995). This tool is not foot-specific, and as such, the symptoms detailed in the descriptor do not align with skin features associated with anhidrosis of the foot skin. Although scales, roughness and cracking are often observed on the foot, redness would usually be associated with a pathology, i.e. erythema of skin underlying scales would be considered an indicator of bacterial or fungal infection.

Table 22. Overall Dry Skin Score.

Score	Descriptor
0	Absent
1	Faint scaling, faint roughness and dull appearance
2	Small scales in combination with a few larger scales, slight roughness, whitish appearance
3	Small and larger scales uniformly distributed, definite roughness, possibly slight redness and possibly a few superficial cracks
4	Dominated by large scales, advance roughness, redness present, eczematous changes and cracks

As in the XAS and OCCS, there is an assumption that advancing severity of one pathological skin characteristic is concurrent with the advancement of all pathological skin characteristics, indicated by the uniform increase in severity of features with each increasing score. In reality, anhidrosis can present differently between individuals, particularly on the foot. For the purposes of this study, a scoring system is required that enables the scoring of each skin feature independently.

4.6.6.5.2. EEMCO clinical tool 1: Specified Symptom Sum Score System: grading of scaling, roughness, redness, and cracks (SRRC)

The SRRC was devised to capture the severity of the four symptoms the EEMCO identified as the primary symptoms of Xerosis and Ichthyosis (Serup, 1995). Each symptom is scored out of 4 for severity, and the sum of all four symptom scores is used for the total SRRC score (See Table 23).

Table 23. Specified Symptom Sum Score System: grading of scaling, roughness, redness and cracks.

	Scaling (visual evaluation)	
0	Absent	
1	Slight	Small scales only, surface lightly dull in colour
2	Moderate	Small scales in combination with larger scales (>0.05 mm), surface opaque or whitish
3	Severe	Larger and large scales (flakes >1 mm) are prominent, surface whitish
4	Extreme	Larger flakes covering almost the entire skin surface in the examination field
	Roughness (tactile evaluation)	
0	Absent	Perfectly smooth and pliable
1	Slight	Slightly irregular and scratchy on tangential tactile evaluation
2	Moderate	Definitely irregular and scratchy and possibly slightly stiffened on vertical tactile evaluation
3	Severe	Advanced irregularly and scratchy feeling associated with some stiffening
4	Extreme	Gross irregularity and major disturbance of skin markings and definite stiffening
	Redness (visual evaluation)	
0	Absent	
1	Slight	Small areas of minimal redness or diffuse faint redness
2	Moderate	Limited areas of definite redness or diffuse and obvious redness
3	Severe	Larger areas of definite redness or diffuse and more pronounced redness
4	Extreme	Advanced redness in entire examination field (redness of cracks not included)
	Cracks fissures (visual evaluation)	
0	Absent	
1	Slight	Single and superficial cracks in the examination field
2	Moderate	Single or grouped superficial and more deep cracks
3	Severe	As 2 but with deep cracks
4	Extreme	Dominated by deep cracks

This device is intended for use by a clinician, as demonstrated by the technical language used (Flesch-Kincaid score: 8.3). However, the simple structure of this tool makes it more accessible to consumers than others that been discussed previously. As each symptom is scored separately, the evaluator can concentrate on one aspect of the skin appearance at a time, rather than evaluating several symptoms at once. Within some other tools (XAS, OCCS, ODS) numerous symptoms must be exhibited at uniform severity simultaneously to reconcile with a single score descriptor.

Despite the technical expertise expected of the user, the authors provide some guidance on undertaking tactile evaluation which is required for the roughness category: Investigators are advised that tactile evaluation may be influenced by roughness/relief of the palpating finger and using the extensor side of the middle phalanx may be preferable to the pulp. This is useful information for an inexperienced assessor.

As with all tools proposed for use by the EEMCO, this is not immediately appropriate for application to the foot skin due to the nature of the pathologies it is intended to assess. To be modified for use by a lay person to self-assess foot skin, the items would need to be modified to represent the primary symptoms associated with foot skin xerosis. The complexity of language would also have to be reduced significantly.

4.6.6.5.3. EEMCO clinical tool 3: Dry skin/ichthyosis area and severity index (DASI)

The SRRC is used to evaluate the severity of four most common symptoms of xerosis and ichthyosis on each body area (head and neck, upper extremities, trunk, and lower extremities) and multiplied by the proportion of the body that area is deemed to represent to calculate the DASI score (Serup et al., 2006). The DASI indicates the severity of the disease across the whole-body surface (See Table 24).

This tool is unsuitable for this investigation as its primary purpose is to provide an indicator of whole-body skin pathology severity, as opposed to evaluating skin symptoms on specific skin areas.

Table 24. Dry skin/ichthyosis area and severity index calculating system.

Column A	Column B	Column C	Column D
Area Involvement	% of total area	SRRC score (/16)	(Column B) *(Column C)
Head and neck	10%		
Upper extremities	20%		
Trunk	30%		
Lower extremities	40%		
DASI Score (Sum of all Column D values) (/1600)			

4.6.6.5.4. EEMCO clinical tool 4: Consumer evaluation of a selected anatomical region or test site

Within EEMCO guidance, the single tool proposed for self-evaluation by a consumer takes the form of a visual analogue scale (VAS) with no marked divisions (Serup et al., 2006). Within the

example given, the assessor indicates the hydration of their skin along a tangent ranging from "No dry skin at all" to "Extremely dry skin, worst ever". However, the authors also suggest this tool may be used to compare products used simultaneously on different limbs. i.e. Product A and B are placed at opposing ends of the line and the consumer is encouraged to mark where along the line is representative of the effectiveness of the products on skin hydration or scaliness, for example (Serup, 1995).

As this skin assessment tool is the only system explicitly intended for use by a consumer, and this investigation pertains to the consumer perception of their own skin, this tool is of relevance to this work. Serup (1995) suggests that it is possible to alter the data obtained using this device through changing the phrases used at either end of the VAS. By using this tool to collect consumer assessment of skin health, dryness, hardness, and roughness, alongside more objective assessments of specific skin features, it would be possible to evaluate how these interrelate. i.e. how skin flakiness influences perception of skin dryness, how skin hydration is related to perception of skin roughness.

4.6.7. Identification of objectives from section 4.6.6. in the skin assessment questionnaires.

Each tool has been assessed for fulfilment of the requirements described in Section 4.6.6. (See Table 25).

Table 25. Evaluation of visual assessment tools.

	1 - Collect data that enables the fulfilment of the project aim and objectives	2 - Be applicable to non-foot skin areas	3 – Not be specific to skin pathology	4 - Be written in layperson's terms and useable by non-clinicians	5 - Provide primarily quantitative data	6 - 6. Present descriptions of skin characteristics that are reflective of foot skin xerosis.
XAS	In part	Yes	No	No	Yes	No
OCCS	In part	No	No	No	Yes	No
OCS	No	No	No	No	Yes	No
MGC	No	No	No	No	Yes	No
ODSS	In part	Yes	No	No	Yes	No
SRRC	Yes	Yes	Yes	No	Yes	In part
DASI	No	Yes	No	No	Yes	No

4.6.8. Proposed modifications of the assessment tools:

4.6.8.1. SRRC modification of scoring categories

A common symptom of xerosis and ichthyosis on the body is erythema. However, redness of the foot skin associated with anhidrosis would typically indicate an infection (e.g. Tinea Pedis) or a systemic skin disease (e.g. psoriasis or eczema).

Separation of roughness score into two separate scoring systems: 1. roughness and 2. thickening and hardening.

Within the modified tool roughness is considered an independent feature, with the skin progressing from a smooth surface to a rough surface. Thickened and hardened skin are combined as in the case of xerosis, and hyperkeratosis, thickening and hardening are typically seen together. These modifications are shown in Table 26.

Table 26. Modified SRRC scores for 'roughness' and 'thickening and hardening'.

	Roughness (tactile evaluation)	
0	Absent	Perfectly smooth
1	Slight	Slightly irregular and scratchy on tangential tactile evaluation
2	Moderate	Definitely irregular and scratchy
3	Severe	Advanced irregularity and scratchy feeling
4	Extreme	Gross irregularity and major disturbance of skin markings
	Thickening and hardening (tactile evaluation)	
0	Absent	Skin is pliable
1	Slight	Skin is pliable but feels slightly thickened
2	Moderate	Skin is noticeably thickened and is of increased hardness
3	Severe	Skin is significantly thickened and hard
4	Extreme	Skin feels extremely hard and thickened with visible callosities

4.6.8.2. SRRC Simplification of language

This tool has a Flesch-Kincaid reading grade of 8.3. The process undertaken to reduce this is the target grade of 7 is demonstrated in Table 27.

Table 27. Readability Modification Process for the SRRC.

Original Score		Original descriptor	FK Grade	Modified descriptor	FK Grade
	Scaling (visual evaluation)				
0	Absent				
1	Slight	Small scales only, surface lightly dull in colour	5.2		
2	Moderate	Small scales in combination with larger scales (>0.05 mm), surface opaque or whitish	6		
3	Severe	Larger and large scales (flakes >1 mm) are prominent, surface whitish	6.4		
4	Extreme	Larger flakes covering almost the entire skin surface in the examination field	10.3	Large flakes (flakes >1 mm) covering almost all of the skin surface	5.6
	Cracks fissures (visual evaluation)				
0	Absent				
1	Slight	Single and superficial cracks in the examination field	11.1	One crack, or a few superficial cracks present	5.2
2	Moderate	Single or grouped superficial and more deep cracks	5.2		
3	Severe	As 2 but with deep cracks	0		
4	Extreme	Dominated by deep cracks	7.2	Numerous deep cracks	5.6
	Roughness (tactile evaluation)				
0	Absent				
1	Slight	Slightly irregular and scratchy on tangential tactile evaluation	17	Slightly rough skin surface	3.7
2	Moderate	Definitely irregular and scratchy	19	Rough skin surface	0.9
3	Severe	Advanced irregularity and scratchy feeling	17	Very rough skin surface	3.7
4	Extreme	Gross irregularity and major disturbance of skin markings	12.3	Skin surface is extremely rough and scratchy. Skin markings are disturbed.	5.4
	Thickening and hardening (tactile evaluation)				
0	Absent	Skin is pliable	5.6		
1	Slight	Skin is pliable but feels slightly thickened	6		
2	Moderate	Skin is noticeably thickened and is of increased hardness	9.2	Skin is of increased thickness and hardness.	6
3	Severe	Skin is significantly thickened and hard	8	Skin feels very thick and hard.	0.9
4	Extreme	Skin feels extremely hard and thickened with visible callosities	10.3	Skin is extremely thick and hard. Appears callused.	4.9

In brief, each descriptor was assessed for its Flesch-Kincaid grade and where this was unacceptably high, modifications were made to the language to retain original meaning but

lower complexity. The resulting descriptors are displayed in Table 28. This modified version has a Flesch-Kincaid reading grade of 4.5.

Table 28. Modified SRRC.

Original Score		Descriptor
	Scaling (visual evaluation)	
0	Absent	
1	Slight	Small scales only, surface lightly dull in colour
2	Moderate	Small scales in combination with larger scales (>0.05 mm), surface opaque or whitish
3	Severe	Larger and large scales (flakes >1 mm) are prominent, surface whitish
4	Extreme	Large flakes (flakes >1 mm) covering almost all of the skin surface
	Cracks fissures (visual evaluation)	
0	Absent	
1	Slight	One crack, or a few superficial cracks present
2	Moderate	Single or grouped superficial and more deep cracks
3	Severe	As 2 but with deep cracks
4	Extreme	Numerous deep cracks
	Roughness (tactile evaluation)	
0	Absent	
1	Slight	Slightly rough skin surface
2	Moderate	Rough skin surface
3	Severe	Very rough skin surface
4	Extreme	Skin surface is extremely rough and scratchy. Skin markings are disturbed.
	Thickening and hardening (tactile evaluation)	
0	Absent	Skin is pliable
1	Slight	Skin is pliable but feels slightly thickened
2	Moderate	Skin is of increased thickness and hardness.
3	Severe	Skin feels very thick and hard.
4	Extreme	Skin is extremely thick and hard. Appears callused.

4.6.8.3. DASI change of subject.

Following a simple yes/no/don't know tick box question to indicate patient identification of skin features, VAS scales will collect consumer perception of skin health, dryness, hardness, and roughness.

4.6.9. Collation of Modifications

The modified VAS scales and SRRC scoring tool from EEMCO guidance were combined to form the new FSkHQ (See Appendix 2). Some additional questions were added following this process to capture additional information on product usage and foot skin pain for the benefit of Scholl footcare.

4.6.10. Foot Skin Health Questionnaire (FSkHQ) – Readability and usability piloting work

In July 2021 a small group of individuals of varied age and mixed professional background provided feedback on the readability and usability of the pre-pilot FSkHQ.

4.6.10.1. Participants

Participants were recruited from the friends and family of the research team who fit the below criteria:

Inclusion criteria **-** People of any age or gender from a range of professional backgrounds who hold no specialist knowledge of foot-skin health or questionnaire design.

Exclusion criteria **-** Individuals who have specialist knowledge of foot-skin health or questionnaire design.

Participants were given written instruction via email to provide feedback on the 'readability and usability' of the form in a digital format.

4.6.10.2. Results

Ten individuals provided feedback on the readability and usability of the pre-pilot FSkHQ (See Table 29).

Table 29. Participant Demographics.

Participant Number	Age group (years)	Sex	Profession
1	25-30	Male	Economist
2	40-45	Male	Health Care Professional
3	18-20	Female	Student
4	18-20	Female	Student
5	35-40	Female	Health Care Professional
6	30-35	Male	Engineer
7	30-35	Female	Engineer
8	65-70	Female	Civil Servant
9	70-75	Male	Retired
10	60-65	Female	Retired

Eight participants provided feedback via free text within an email, and two participants (7 and 9) provided feedback by returning an annotated FSkHQ. The researcher reviewed all

feedback, most of which was favourable, and extracted comments that specifically identified an aspect of the questionnaire that needed amending. Upon careful review these comments were separated into two broad sections – 'Formatting' and 'Language'. This feedback is displayed in Table 30.

Table 30. Feedback from participants.

Theme		Content
Formatting	Order of questionnaire components	"The order of the skin scoring matrix doesn't match the Modified Specified Symptom Sum Score list"
		"Move the table before the user fills in the questions"
	Delineation between questions/sections	"Move the question numbers into the left tab so they can see where the question begins and ends"
		"Put the questions in boxes to make it clearer"
		Use of formatting tools to indicate different questions – use of bold text and indenting of question numbers.
		Addition of box around question guidance on Page 1.
	Guiding user input	"consider markers on the lines for pre-set levels" (referring to VAS score)
		"say it's okay to mention brand name of any products used for treatment"
	Navigating completion	"with questions 4, 6 and 8 you might want to direct them to the next question by putting the instructions next to the answer"
		"I thought the cells being boxed off forced the point that they have to do something here" (in reference to the user completed cells within 'Table 1. Skin Scoring Matrix')
		Addition of "*Please refer to table 2 for scoring guidance*" prior to the Modified Specified Symptom Sum Score – Reference Table
		Addition of 'If this is unclear, please contact your podiatrist' on Page 1.
	General formatting	Numerous format changes on Page 1 – titles underlines/italicised, addition of spacing, text justified.
		Addition of missing question number 11.
Language	"you say that you should use the side of your finger, not the pulp. If that is the fingertip then it might be better to say that" AND "What is the pulp?"	
	"I feel 'worst ever' is possibly unnecessary…Leaving it as extremely would work better for us oldies" (referencing VAS scale labels)	
	"the introduction is presented in the first person and the following section introduces a third party" (referencing the VAS scales section on Page 1. Participant then amends following section to direct the reader directly. I.E. "if you were feeling very tired, you may make a mark here")	
	Addition of ", please complete both sections." following "There are two sections within this questionnaire" (Page 1)	
	Replacement of "you will need to" with "will you please" (Section B)	
	Replacement of "please tick those applicable" with "In this section please tick all boxes that apply" (Section B)	
	Inclusion of a section on Page 1 to demonstrate the use of a Yes/No tick box with addition of "Simply place a tick in **one** box"	
	Amendment of "To complete this, you will need to…" with "To complete this, simply…"	

4.6.10.3. Implementation

All feedback was implemented, except for three points deemed unnecessary by the research team (the author and their supervisory team) (highlighted in grey). These changes were not

implemented as they were perceived to conflict with other formatting changes made as a result of feedback or to add unnecessary instruction or reduce clarity.

Following this process, and consideration of the locations examined within the other components of this work, an additional skin site was added to FSkHQ (Peri-malleolar area). During the research team discussion, the tasks for the remainder of the PhD were discussed and prioritised. It was agreed that due to the timelines for data collection in the other studies, the further development of the questionnaire would be continued as part of post-doctoral work.

4.6.11. Conclusion

A relatively short, user-friendly questionnaire (See Appendix 3) was designed that was suitable for use in Study 2. The questionnaire determines individual perception of foot skin features across the entire foot and at specific foot sites. It also records opinions on overall foot health.

4.7. Chapter Conclusion

Within this chapter, a large number of devices have been described that collect data on a wide range of tissue characteristics that are reflective of the health of skin. The application of these simultaneously to the foot generates novel data with wide-reaching application.

The rapid development and implementation of such devices over the last 30 years reflects a transition away from subjective assessment of skin hydration via questionnaires and scoring systems for the assessment of emollient efficacy. However, consumer perception of foot skin health remains a major driver for their decision to seek emollient therapy, and their assessment of its effectiveness. For this reason, a new device has been developed expressly for use on the foot skin - the FSkHQ. This device will be used in conjunction with objective assessment of foot skin characteristics to generate new data on consumers ability to detect changes in skin characteristics and inform product development and testing in future.

Chapter 5. An investigation into the use of the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® on the foot skin: A pilot study.

5.1. Introduction

Although many aspects of study design for use of hydration measurement devices are informed by the manufacturer's specifications for use of their devices (see Chapter 4), or existing guidance for the measurement of epidermal hydration (Rogiers et al (1990) and Berardesca and Cameli (2018) there are several methodological factors unaddressed or inconsistent within device guidance and supporting literature.

5.1.1. Temporal spacing of measurements and order of device use

Occlusion of the skin by any surface (including the probe surface of a device) prevents TEWL and artificially raises the hydration of the superficial area of the skin (Berardesca et al., 1997). According to the manufacturers guidelines, the Corneometer® CM825 requires a gap of 5 seconds between measures (Courage & Khazaka electronics GmbH, 2010). No information is available on the effect of occlusion on either the MoistureMeter D® or the MoistureMeter SC™.

5.1.2. Skin acclimatisation period

An acclimatisation period is a period of time proceeding data-collection when the participant rests in the data-collection area allowing them and their skin to become acclimatised to the environmental conditions and for any physiological response to their journey to cease (Serup et al., 2006). These vary from 20-30 minutes in the literature and 10-20 minutes within device guidance (see Section 4.2.) (Berardesca et al., 1997; Rogiers et al., 1990).

In addition, little information is available on the within-day and between-day variability of data collection. Skin hydration fluctuates throughout the day (Le Fur et al., 2001). This phenomenon has never been investigated on the foot. Although this is not directly relevant to this study as this is a cross-sectional study, it is a valid concern for future longitudinal studies.

In this pilot study, the impact of acclimatisation and order of device use was examined on the variability of data collected using three hydration measurement devices: the Corneometer® CM825, the MoistureMeter SC™ and the MoistureMeter D®.

5.1.3. Novelty Statement

This study represents the first instance in which skin acclimatisation period and consecutive instrument use are assessed for their impact on the variability of hydration data collected from the foot skin.

5.1.4. Aim and Objectives

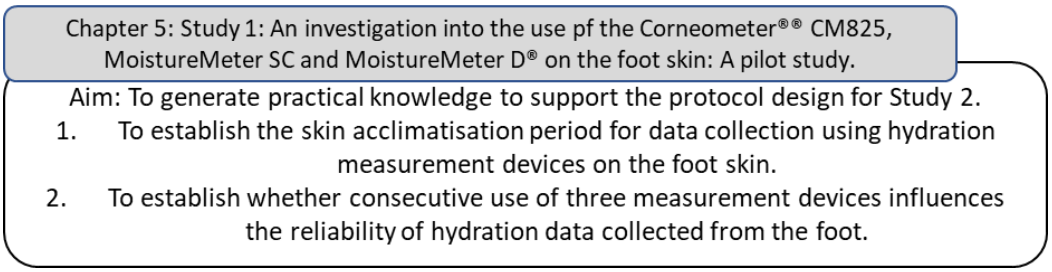


Figure 26. Objectives for 'An investigation into the use of the Corneometer® CM825, MoistureMeter SC and MoistureMeter D® on the foot skin: A pilot study.'

5.2. Method

Four stages of pilot data-collection were undertaken to resolve the methodological uncertainties associated with the use of the Corneometer® CM825, MoistureMeter D® and MoistureMeter SC™ for measuring plantar epidermal hydration.

The study protocol was reviewed and approved by The University of Salford Ethics Panel (application 3137).

5.2.1 Testing environment

Data collection took place in the Skin Laboratory (Podiatry Building, Frederick Road Campus, The University of Salford. This laboratory has an adjustable plinth, clinicians chair, equipment

trolley, a sink, and adjoins a podiatry clinic with a waiting room and toilet facilities. This area is heated via a central heating system and has been used for similar studies in which room temperature and humidity remained relatively consistent (Hashmi, Nester, et al., 2015). The temperature and humidity of the testing environment were recorded at the commencement and conclusion of each data collection period and monitored throughout. Data were collected during short periods to negate seasonal variabilities in temperature and humidity.

5.2.3. The Researcher

A PhD Student and a HCPC registered Podiatrist undertook data collection. This individual is familiar with the laboratory space and the equipment intended for use and can identify skin pathology that would exclude participants from the study.

5.2.4. Participants

Participants aged between 20 and 40 years of age were recruited from staff and students at the University of Salford. This narrow age range for participants was selected to minimise the influence of age on measurements (Cho et al., 2019; Egawa & Tagami, 2008a). This age range has also been shown to display peak skin hydration measures (when measured on the forearm and forehead) (Rogiers et al., 1990). Factors such as sex and race are not expected to influence skin hydration levels significantly, and as such, will not be considered in this study (Table 1) (Du Plessis et al., 2013; Rogiers et al., 1990).

5.2.4.1. Inclusion criteria:

People were included in the study if they:

Were healthy adults aged 20-40 years of any sex or race.

Had no current skin disease or systemic illness that is known to influence skin characteristics.

Were free of significant foot skin pathology, excluding mild dry or callused skin (as observed by the researcher according to skin appearance and texture).

5.2.4.2. Exclusion criteria:

People were excluded from taking part if they:

Were younger than 20 or older than 40.

Displayed symptoms of skin disease or had a history of skin disease.

Had a systemic condition that may influence the characteristics of their skin, i.e., diabetes or scleroderma.

Were displaying symptoms of COVID-19 (current or recent).

Were unable to refrain from washing skin on the lower limb for 5 hours prior to study.

Were unable abstain from using any topical applicants (other than cleansing products) on the skin testing locations in the 7 days before data collection.

5.2.5. Recruitment

Staff and students at the University of Salford were invited to this study via an internal online noticeboard system, email, and via a 'Tweet' published on social media site Twitter (X-Corp, California, United States) by the researcher. The recruitment message (See Appendix 4) contained instructions to contact the researcher via email for further information.

A Participant Information Sheet (PIS) (See Appendix 5) was dispatched to any individual who contacted the researcher for more information, followed (48 hours later) by an invitation to attend a data collection session. The exclusion and inclusion criteria for the study were confirmed with prospective participants prior to their appointment.

5.2.6. Measurement Locations

Data were collected at seven sites on the body, five of which are on the foot. These sites are described, and their significance is explained in Table 31 and these are demonstrated in Figure 27.

Table 31. Skin sites for examination.

Site Name (abbreviation)	Number	Significance	Location	Representation within literature (detail if an approximation)
Ventral Forearm (VF)	1	Control (or comparison) Site	Central forearm, 10 cm proximal to the wrist creases	(Mayrovitz, McClymont, et al., 2013) 'Forearm anterior'
Anterior Aspect of Tibia (AT)	2	Anticipated very low hydration levels	Central anterior surface, 10 cm proximal to the midpoint of the lateral and medial malleolus	None
Medial Peri-Malleolar Area (MPM)	3	Common non weightbearing location used in previous studies	Surface immediately behind the medial malleolus	(Kirkham et al., 2014; Mayrovitz, McClymont, et al., 2013)
Dorsal 3rd MPJ (D3)	4	Dorsal foot site	Dorsal Aspect of 3rd MTP joint	(Mayrovitz, McClymont, et al., 2013) 'Foot dorsum 1-2 and 4-5'
Heel (H)	5	Anticipated high hydration levels	2 cm inwards from posterior centre of the heel	None
Medial Arch (MA)	6	Anticipated low hydration levels	Plantar aspect of base of 1st metatarsal	None
Plantar 3rd MPJ (P3)	7	Anticipated intermediate hydration levels	Plantar aspect of 3rd MTP joint	(Hashmi, Wright, et al., 2015) 'Plantar metatarsal area'

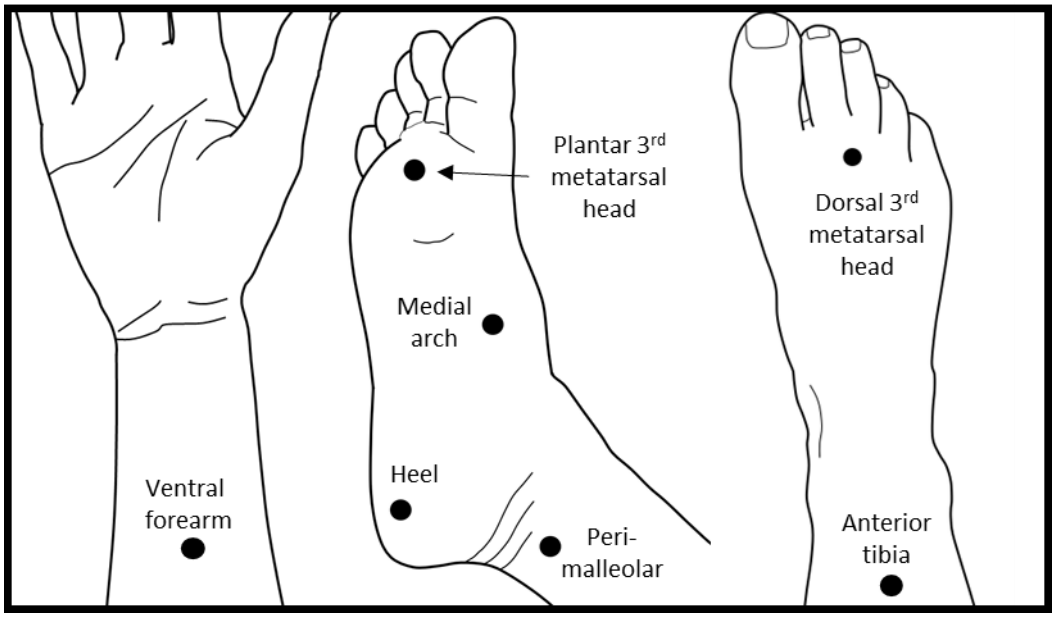


Figure 27. Demonstration of skin measurement sites.

5.2.7. Data collection protocol

Two data-collection protocols were used in this study. Protocol 1 was repeated three times, each time using a different device, and Protocol 2 was carried out once. Details consistent between the two protocols are described below, followed by a description of how they differ.

5.2.7.1. Protocol 1 + 2

5.2.7.1.1. Data-collection preparation

Upon entering the laboratory, participants were seated on the plinth and given a document pack containing the PIS (See Appendix 5) and completed the consent form (See Appendix 6). The acclimatisation period began once the participant removed their shoes, hosiery, leg and arm coverings and rested their legs on the plinth.

During the acclimatisation period, the researcher reviewed the exclusion criteria with the participant and located and marked the measurement sites using a surgical skin marker. Each mark was made approximately 1 cm lateral/posterior (depending upon the planar alignment of the skin surface) to the measurement site to prevent interference with measures. Measures were taken from an area adjacent to this mark during data-collection. During the

marking-up process, the researcher observed the characteristics of the skin and data-collection was ceased if any skin pathology was suspected.

5.2.7.1.2. Device use

Data were collected from all skin sites on both sides of the body for each study stage. Measurements were taken from the proximal skin sites first, ending at the most distal site (as numbered in Table 31) and alternated between the sides of the body. Participants were allocated an ID number (e.g. 1,2,3,4). Data collection for even-numbered participants began on the ventral right forearm (then ventral left forearm and the anterior aspect of the right tibia, and so on) and odd-numbered participants began on the left ventral forearm. This process was followed to negate any differences in right or left data resulting from unequal acclimatisation periods.

Three measurements were taken by each device and the mean used for analysis. To limit the influence of consecutive measurements on the same skin location, the full measurement sequence was repeated three times for each data set (rather than each skin site being measured three times consecutively). Where an error was made during data collection, the full measurement sequence was completed before any repeated measurements were taken to replace omitted or suspected inaccurate data-points (such as where the probe was not fully in contact with the skin surface). This was an infrequent occurrence due to the use of a written data-collection schedule by the researcher.

5.2.7.2. Protocol-specific details

5.2.7.2.1. Protocol 1 (Stage 1, 2 and 3): Assessment of impact of acclimatisation period length on values obtained using each device and within-day variability of plantar SC hydration.

For this study, each participant attended the lab four times over two days (AM and PM). Repeated measures were taken from the skin at numerous time-points at each of these appointments using a single hydration measurement device. This process was repeated for each device on a separate occasion: i.e. In a day 1 AM session, a single device would be used

to measure the skin hydration from each site (three times) at four time points. This would be repeated in a PM session in the same day, then both sessions would be repeated another day (day 2). If the participant was involved in a second stage of the study, they would attend again and undergo the same process, with a different device being used.

Corneometer® CM825 data were collected at 20,25, 30, and 35 minutes after acclimatisation started. MoistureMeter SC™ and MoistureMeter D® data were collected at 20, 30, and 40 minutes after acclimatisation (See Table 32). The difference in time-period is due to the speed of data-collection for each device. For all skin sites to be recorded three times the Corneometer® CM825 took approximately 3 minutes and the MoistureMeter D® and MoistureMeter SC™ took approximately 8 minutes.

The MoistureMeter D® 0.5 mm probe was used for this study, as this is perceived to be the Moisture Meter D® probe most-likely easily influenced by aspects of protocol design due to it measuring the hydration of the superficial layers of the epidermis.

Table 32. Stage 1 data collection schedule.

Time (HR:MIN)	Event Corneometer® CM825 only – MoistureMeter SC™ and MoistureMeter D®.
	Temperature and humidity of the testing environment is logged, shoes and hosiery are removed. Participants seated with feet raised.
00:00	Acclimatisation Commences
00:20	First dataset obtained
00:25/00:30	Second dataset obtained
00:30/00:40	Third dataset obtained
00:35	Fourth dataset obtained
	End of data collection, temperature and humidity logged, participant is excused, data stored.

Each two days of testing for each device were undertaken within a 7-day window to minimise the influence of weather changes. AM and PM data collection were undertaken between 10:00-11:30 (AM) and 14:00-15:30 (PM) for consistency and to allow participants to attend data-collection sessions within their usual working hours.

5.2.7.2.2. Protocol 2 (Stage 4): Assessment of use of devices consecutively

For this study, each participant attended the lab six times across three days (AM and PM each day). During these sessions, all three hydration measurement devices were used back-to-back

in quick succession (a 30-second hiatus between data-collection using different devices) (see Table 33). The results informed the acclimatisation period length of the first stage of this pilot testing. The order of device use was varied between days, as per the sequence shown in Table 34. The three shallowest-measuring probes from the MoistureMeter D® were used during this study from smallest to largest: 0.5 cm, 1.5 cm, 2.5 cm. The deepest-measuring probe (4 cm) was deemed excessively deep for assessment of the plantar SC and use of a fourth probe considerably extended data-collection time.

Table 33. Protocol 2 data collection schedule.

Time (HR:MIN)	Event
	Temperature and humidity of the testing environment is logged, shoes and hosiery are removed. Participants seated with feet raised.
00:00	Acclimatisation Commences
00:00 + Acclimatisation period	First dataset obtained (device as per Table 34)
30 second hiatus	
	Second dataset obtained (device as per Table 34)
30 second hiatus	
	Third dataset obtained (device as per Table 34)
	End of data collection, temperature and humidity of the environment are recorded, participant is excused, data stored.

Table 34. Sequence of device use in Protocol 2.

	Sequence of device use								
	Day 1			Day 2			Day 3		
AM	1	2	3	2	3	1	1	3	2
PM	2	1	3	3	1	2	3	2	1
Key									
1	Corneometer® CM825								
2	MoistureMeter D®								
3	MoistureMeter SC™								

5.3. Data analysis

Statistical Package for the Social Sciences (SPSS) 20 software (IBM, USA) was used for all data analysis. Data were assessed for normality of distribution through use of the Shapiro-Wilks test and review of Q-Q plots, measures of central tendency and histograms. The outcome of this normality testing informed the choice of statistical tests (See Table 35).

Data sets were assessed for differences between groups for:

- Data collected at different time points from the start of the acclimatisation period (Corneometer® CM825: T20, T25, T30 and T35, MoistureMeter SC™ and MoistureMeter D®: T20, T30, T40)
- Data collected using each device first and following the use of two other devices (i.e. Corneometer® first (CORN 1), Corneometer® CM825 after MoistureMeter SC™ (CORN after MoistureMeter SC™) and Corneometer® CM825 after MoistureMeter D® (CORN after MoistureMeter D®).
- Data collected in the morning and afternoon (AM and PM)
- Data collected on two different days (Day 1 and Day 2)

Differences between groups were identified using the statistical tests described in Table 35. Where participant numbers were too low to conduct meaningful analysis, or the data violated the assumptions of statistical tests (i.e., in the case of the Wilcoxon test, if differences between data sets were asymmetrical), data were reviewed visually through the use of box-plot graphs and Bland-Altman plots (Bland & Altman, 1999).

Table 35. Statistical analysis strategy.

Study component	Analysis Purpose	Parametric	Non-Parametric equivalent
Acclimatisation period and prior-device use investigation	Identifying differences between three or more groups	One-way ANOVA and Mann-Whitney test.	Friedmans test followed by post-hoc Wilcoxon signed rank tests.
Within and between-day variability investigation	Identifying differences between two groups	Dependent T-Test	Wilcoxon signed-rank test (assuming symmetrical differences between groups).

For each of these analysis multiple comparisons were made, i.e., data collected from 'T20' was compared to data from T25, T30 and T35. This increases the chance of type 1 errors

occurring. The Bonferroni correction has been applied to the chosen level of significance (0.05) in each instance where this occurs.

5.3.1. Outlier processing

Due to the limited number of participants involved in this study, and the small amount of data available from prior studies (most of which is from inequivalent locations) it is not possible to identify and exclude data using statistical methods. Instead, values that sit far outside of the IQR for each dataset will be reviewed in relation to the spread of the individual's data. Where data are consistent bilaterally or across similar skin surfaces (i.e., plantar or non-plantar sites) these will be retained.

This approach to outlier processing has been chosen to maximise the volume of data retained for analysis without reducing statistical confidence by including spurious results. Outliers have been highlighted in figures and discussed to ensure their inclusion is clearly presented.

5.4. Results

Ten individuals participated in the pilot studies between December 2020 and March 2021. Participant demographics and environmental conditions are provided in Table 36.

Table 36. Participant demographics and environmental conditions for each stage of 'An investigation into the use of the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® on the foot skin: A pilot study'.

Stage	Study Component	Protocol	n	% Female	Mean age (years) (SD) [3]	Mean start Temp (°C) (SD)	Mean end Temp (°C) (SD)	Mean start RH[4] (%) (SD)	Mean end RH (%) (SD)
1	Corneometer® CM825	1	4	0%	26 (2.7)	19.45 (1.08)	19.63 (1.13)	49.5 (6.08)	49.56 (6.16)
2	MoistureMeter D®	1	4	50%	27 (3.92)	20.28 (1.52)	20.88 (1.32)	45.69 (4.41)	43.88 (4.57)
3	MoistureMeter SC™	1	4	50%	28.5 (3.11)	19.53 (1.8)	19.7 (1.8)	47.44 (2.8)	47.38 (4.11)
4	All three devices	2	4	50%	29.75 (5.25)	19.73 (1.61)	19.69 (1.76)	39.58 (4.45)	39.88 (4.13)

[3] SD – Standard deviation

[4] RH – Relative humidity

5.4.1. Normality Testing

Non-parametric statistical methods were employed for analysis as all data were found to be non-normally distributed (p-value <0.05) apart from three sets of data obtained using the MoistureMeter D® (Output of Shapiro-Wilks test: MoistureMeter D® 1.5 probe following MoistureMeter SC™ use (p value = 0.164), MoistureMeter D® 2.5 probe following MoistureMeter SC™ use (p value = 0.140), and MoistureMeter D® 2.5 probe following Corneometer® CM825 use (p value = 0.258)). This result is not unexpected due to the small volume of data within each set, increasing the probability of non-parametric distribution (Altman & Bland, 1995).

5.4.2. Impact of acclimatisation period on data collected using the Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D®.

Fourteen Friedmans tests were conducted (one for each skin site) across data collected at different time-points for each device (See Table 37). The Bonferroni correction was applied to reduce the risk of a type 1 error arising as a result of this, generating a threshold p-value of ≤0.003 for statistical significance.

Table 37. Results of a Friedman's test analysing the influence of acclimatisation period on measurements obtained using the Corneometer® CM825. MoistureMeter D® and MoistureMeter SC™. Significant results highlighted in yellow (<0.003).

	Friedman Test outcome (p-value)		
Location (Abbreviation)	Corneometer® CM825	MoistureMeter D®	MoistureMeter SC™
Left ventral forearm (LVF)	0.599	0.76	0.939
Right ventral forearm (RVF)	0.643	1.95	0.015
Left anterior tibia (LAT)	0.078	0.432	0.449
Right anterior tibia (RAT)	0.428	0.269	0.294
Left peri-malleolus LPM)	0.1	0.054	0.459
Right peri-malleolus (RPM)	0.949	0.314	0.814
Left dorsal 3rd metatarsal head (LD3)	0.64	0.811	0.740
Right dorsal 3rd metatarsal head (RD3)	0.552	0.646	0.062
Left heel (LH)	0.009	<0.001	0.280
Right heel (RH)	0.332	0.010	0.223
Left medial arch (LMA)	0.46	0.174	0.066
Right medial arch (RMA)	0.264	0.005	0.009
Left plantar 3rd metatarsal head (LP3)	<0.001	0.014	0.113
Right plantar 3rd metatarsal head (RP3)	<0.001	0.068	0.022

Out of 42 Friedmans tests conducted, only three highlighted a significant (p-value ≤0.003) difference between the acclimatisation periods. These were found on plantar sites only: the left heel for the MoistureMeter D® and both plantar 3rd metatarsal heads for the Corneometer® CM825.

Below is a series of boxplot graphs showing the data collected at each time point for each device. Significant results of the post-hoc Wilcoxon signed-rank test are displayed on these. The p-value indicating the threshold of significance have been modified using the Bonferroni correction, according to the number of comparisons undertaken for each dataset.

Post-hoc Wilcoxon signed-rank tests identified three significant differences (p-value ≤0.003) between the hydration data collected using the Corneometer® CM825 at different acclimatisation periods on the 3rd metatarsal heads (left 3rd plantar metatarsal head: 20-30 minutes (p-value = 0.003), 20-35 minutes (p-value <0.001), right 3rd metatarsal head: 20-35 minutes (p-value = 0.001)) (See Figure 28). As acclimatisation period increased, the range of data collected on the 3rd metatarsal head decreased slightly. This same pattern is not reflected at other skin sites.

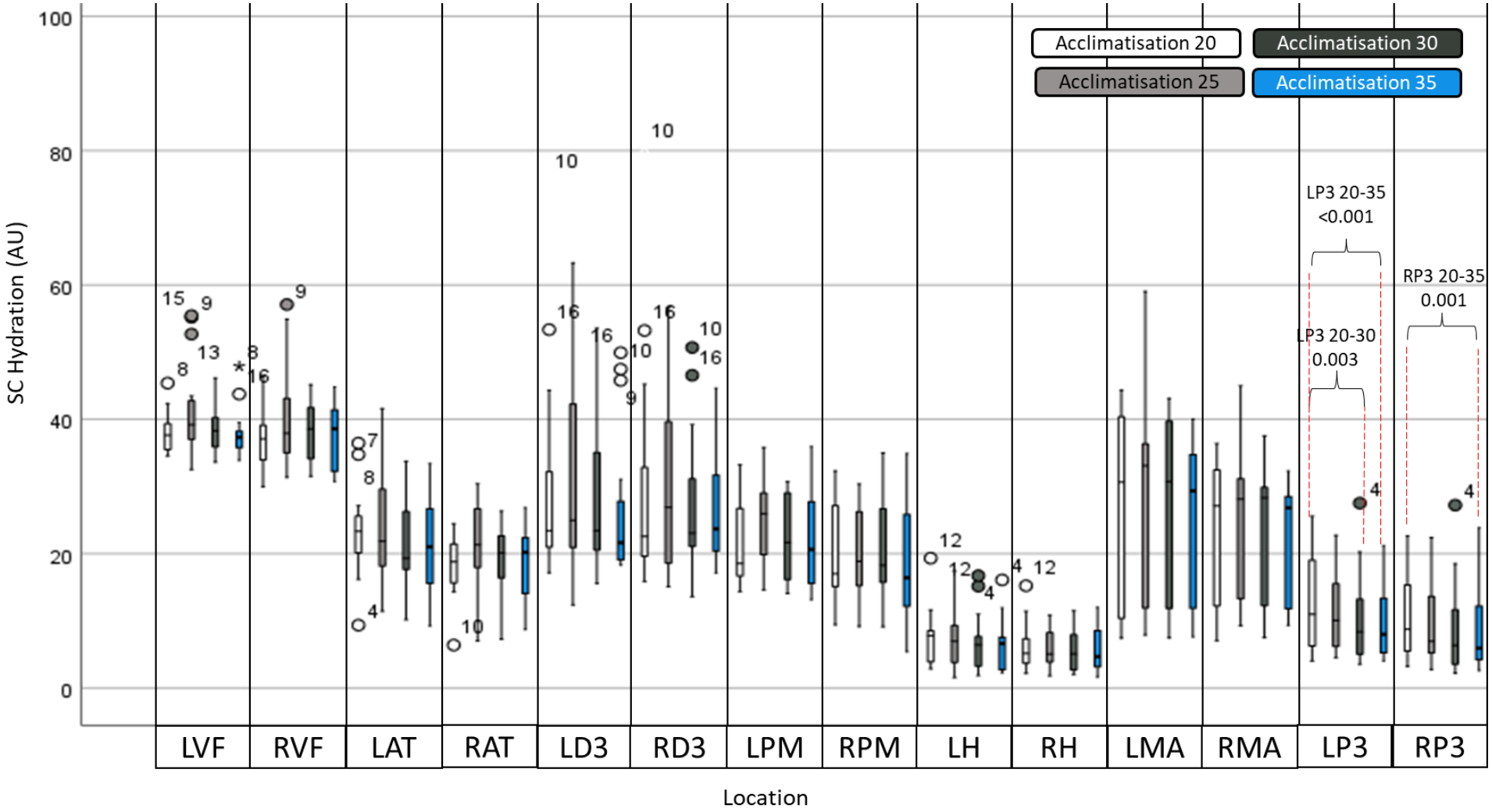


Figure 28. Corneometer® CM825 comparisons of data collected at each acclimatisation time for each location. Significant differences highlighted. Wilcoxon Signed-Rank Test p value = <0.0083

Two significant differences were identified using the post-hoc Wilcoxon signed-rank test on the data obtained from the left heel site using the MoistureMeter D® 0.5 mm probe at acclimatisation periods of 20, 30 and 40 minutes (See Figure 29).

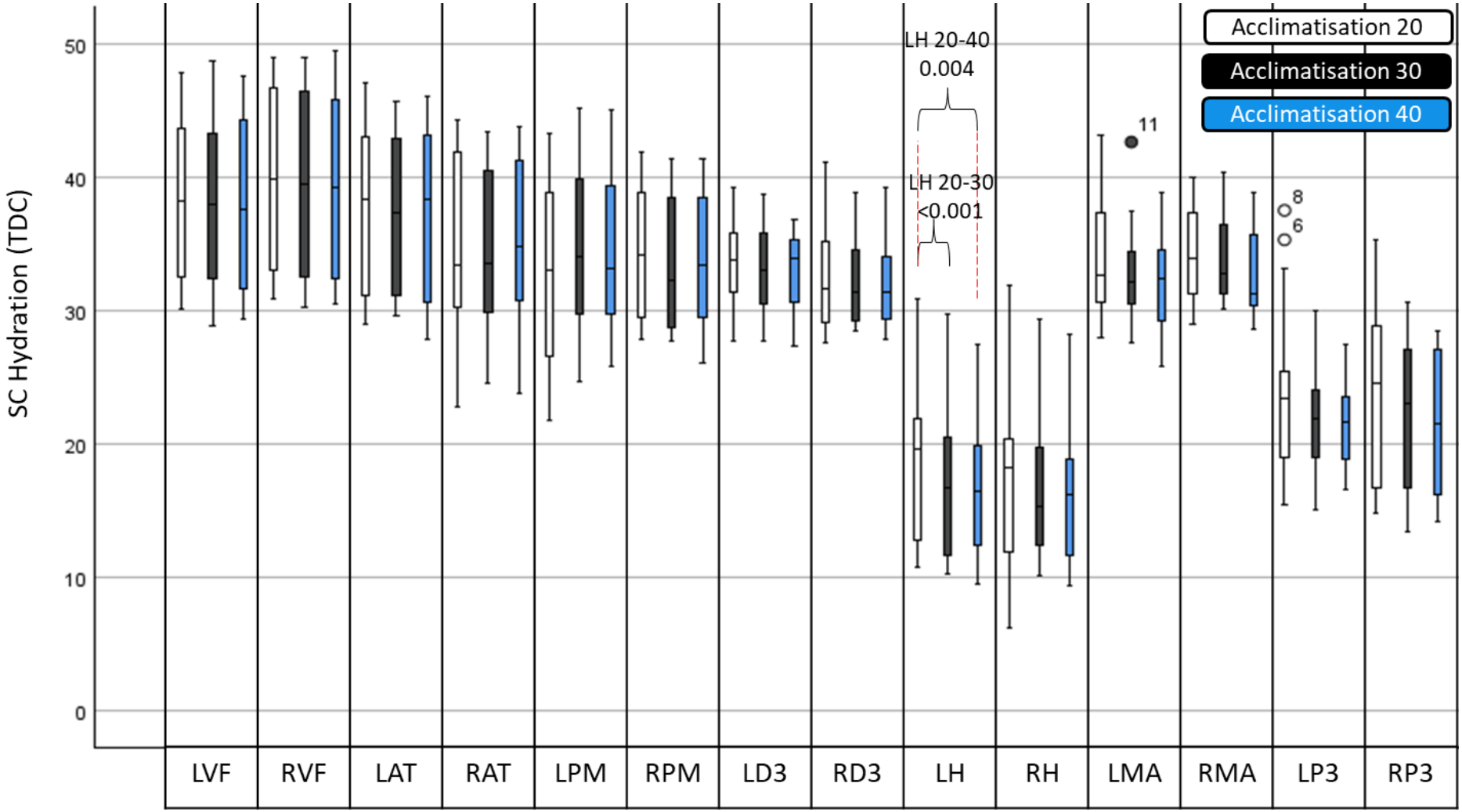


Figure 29. MoistureMeter D® (0.5 mm probe) comparisons of data collected at each acclimatisation time for each location. Significant differences highlighted. Post-hoc Wilcoxon Signed-Rank Test p value = <0.016.

There was a statistically significant reduction in hydration at 20- 30 minutes (p-value = <0.001) and 20-40 minutes (p-value = 0.004) for the left heel site as acclimatisation period increased (similarly to the Corneometer® CM825). This same pattern (albeit not identified statistically significant) is also reflected in the data collected from the 3rd metatarsal head.

5.4.3. Impact of prior device use on the data collected using the Corneometer® CM825, the MoistureMeter SC™ and the MoistureMeter D®

The Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D® were used in the same testing session in quick succession. The order of their use was rotated to generate three sets of data for each where each device was used first and following each of the other devices (i.e. for the Corneometer® CM82: Corneometer® CM825 first, Corneometer® CM825 after MoistureMeter D®, and Corneometer® CM825 after MoistureMeter SC™). The study design resulted in 8 data points being included within each data set (devices were used in each sequence twice (See Table 34) for four participants). The Friedman's test has been used to identify where differences exist between the data obtained by devices in these different circumstances (See Table 38). A p-value of ≤0.003 was considered statistically significant following application of the Bonferroni correction.

Table 38. Results of a Friedman's test analysing the influence of prior device use on measurements obtained using the Corneometer® CM825. MoistureMeter D® and MoistureMeter SC™.

Friedman Test outcome (p-value)					
Location (Abbreviation) Left ventral forearm (LVF)	Corneometer® CM825	MoistureMeter SC™	MoistureMeter D®		
			0.5 mm probe	1.5 mm probe	2.5 mm probe
Right ventral forearm (RVF)	0.140	0.008	0.068	0.687	0.607
Left anterior tibia (LAT)	0.072	0.325	0.417	0.417	0.417
Right anterior tibia (RAT)	0.135	0.093	0.417	0.325	0.303
Left peri-malleolus LPM)	0.325	0.417	0.417	0.882	0.417
Right peri-malleolus (RPM)	0.417	0.223	0.197	0.607	0.607
Left dorsal 3rd metatarsal head (LD3)	0.687	0.072	0.607	0.687	0.798
Right dorsal 3rd metatarsal head (RD3)	0.284	0.508	0.882	0.417	0.687
Left heel (LH)	0.206	0.687	0.882	0.417	0.687
Right heel (RH)	0.223	0.882	0.030	0.417	0.908
Left medial arch (LMA)	0.417	0.197	0.325	0.223	0.303
Right medial arch (RMA)	0.223	0.197	0.687	0.324	0.197
Left plantar 3rd metatarsal head (LP3)	0.197	0.223	0.250	1	0.053
Right plantar 3rd metatarsal head (RP3)	0.325	0.030	0.325	0.034	0.093
Location (Abbreviation)	0.223	0.607	0.417	0.159	0.687

Despite the lack of statistically significant differences between groups identified using the Friedman test, reviewing the data in more detail is worthwhile as the participant numbers are very low. This may reduce statistical power and cause differences between groups to be undetected using the Friedmans test. Due to the low number of data points for each dataset within this study (n=8), post-hoc Wilcoxon signed rank tests were not suitable (Mundry & Fischer, 1998). Instead, boxplots representing the data were reviewed below.

Some variability is shown between the data collected when the Corneometer® CM825 is used first, following the use of the MoistureMeter D®, and following the use of the MoistureMeter SC™. However, this does not demonstrate a consistent pattern between skin sites (See Figure 30).

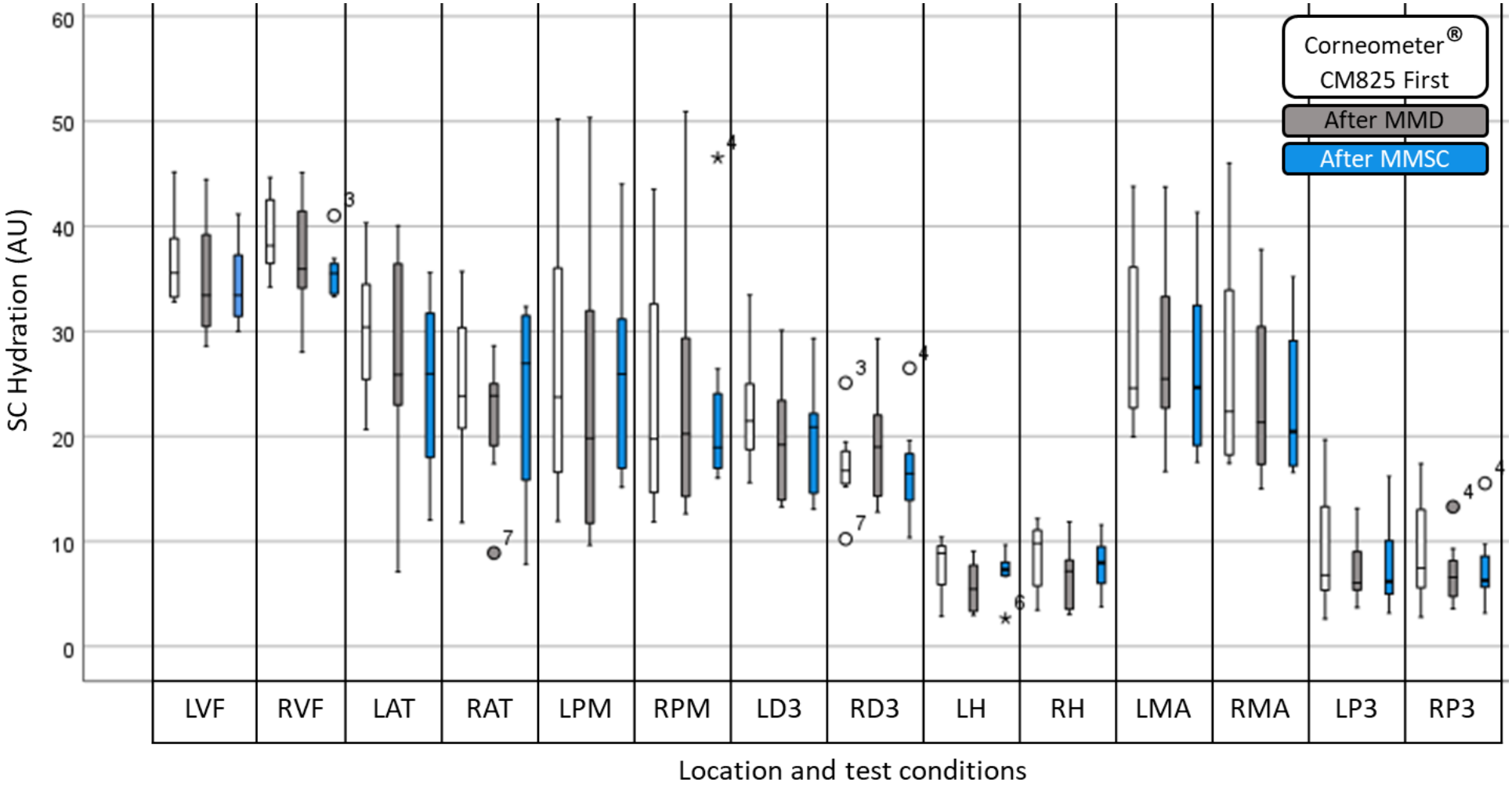


Figure 30. Data obtained using the Corneometer® CM825 with no prior device use, after MoistureMeter D® use (after MMD), and after MoistureMeter SC™ use (after MMSC).

At the 3rd metatarsal head site, the data collected by the Corneometer® CM825 without prior device use has a larger range than data collected after MoistureMeter SC™ or MoistureMeter D® use. The median values of the data, however, are very consistent: LP3 (Corneometer® CM825 first)(IQR): 6.78 AU (5.2 AU), LP3 after MoistureMeter D®: 6.05 AU (2.63 AU), LP3 after MoistureMeter SC™: 6.18 AU (3.85 AU), RP3 (Corneometer® CM825 first): 7.47 AU (5.33 AU), RP3 after MoistureMeter D®: 6.58 AU (2.35 AU), RP3 after MoistureMeter SC™: 6.27 AU (2.325 AU)).

This same pattern is somewhat reflected in the data collected using the MoistureMeter SC™ (See Figure 31).

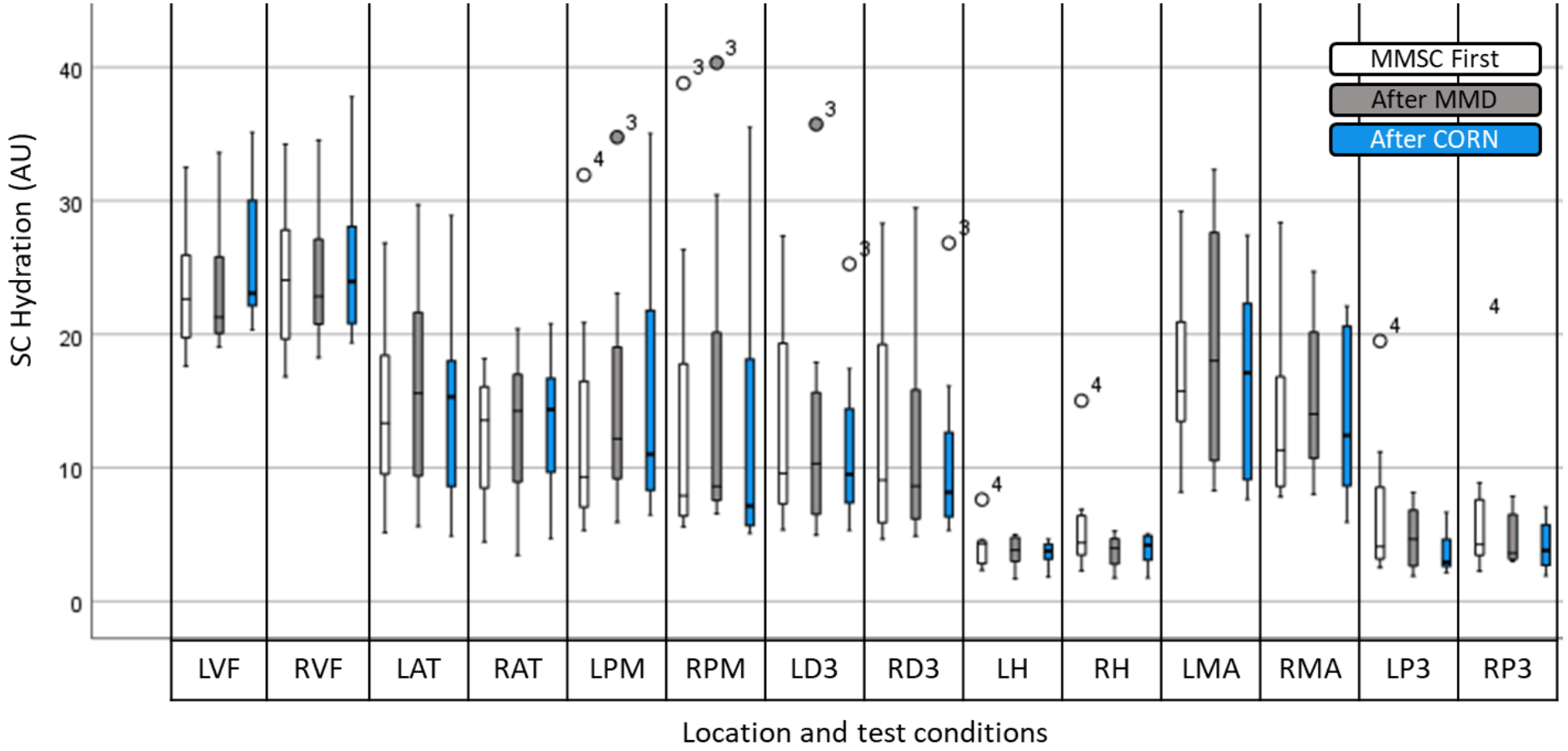


Figure 31. Data obtained using the MoistureMeter SC™ with no prior device use, after MoistureMeter D® use (after MMD), and after Corneometer® CM825 use (after CORN).

At the dorsal foot sites (LD3 and RD3) and metatarsal head (LP3 and RP3), the range of the data is larger when the MoistureMeter SC™ is used first than when it is used following the Corneometer® CM825 or MoistureMeter D®.

Data collected using the MoistureMeter D® (0.5, 1.5 and 2.5 mm probes) do not show any indication of being influenced by testing conditions in a consistent manner (i.e., being used first generating consistently higher or lower data than when used following the Corneometer® CM825 or the MoistureMeter SC™) (See Figure 32, Figure 33, and Figure 34).

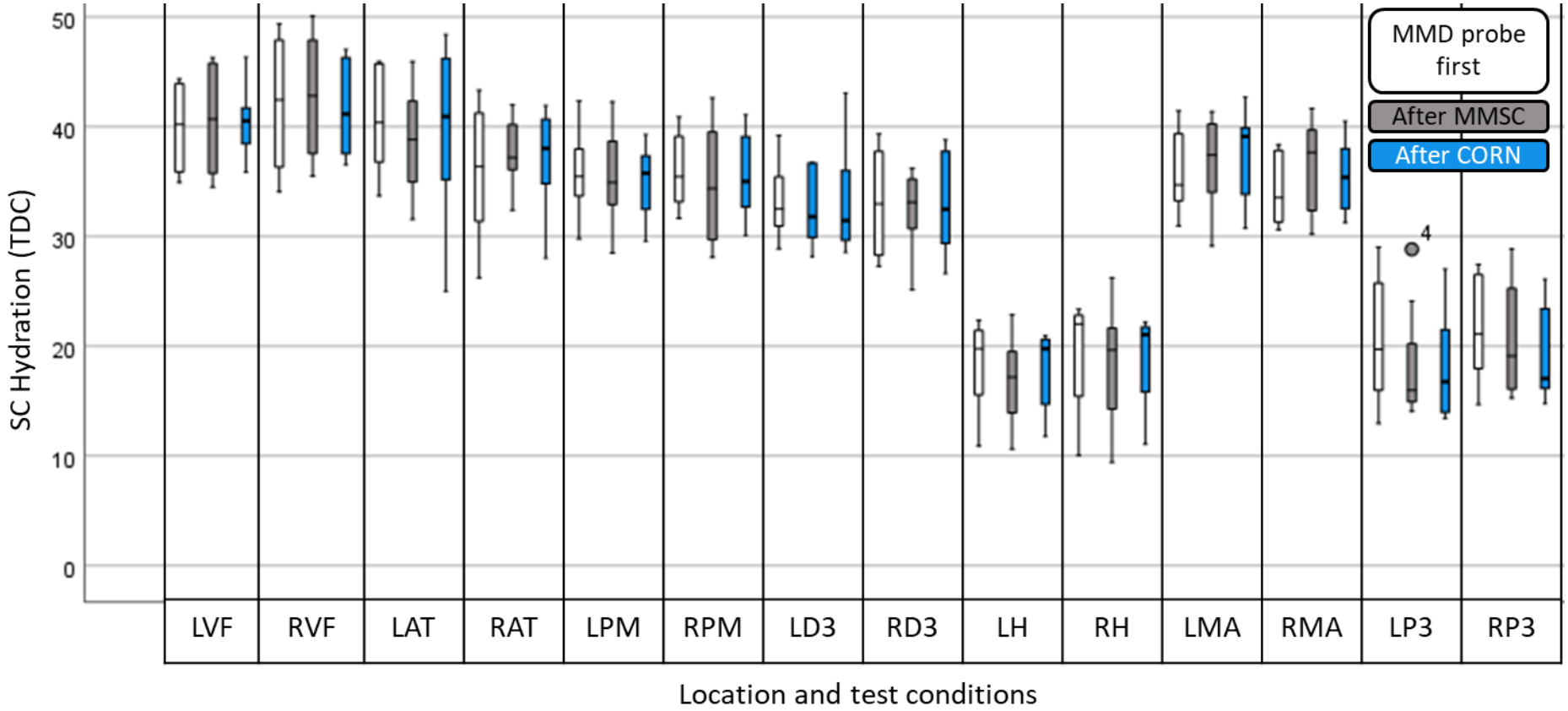


Figure 32. Data obtained using the MoistureMeter D® 0.5 mm probe with no prior device use, after MoistureMeter SC™ use (after MMSC), and after Corneometer® CM825 use (after CORN).

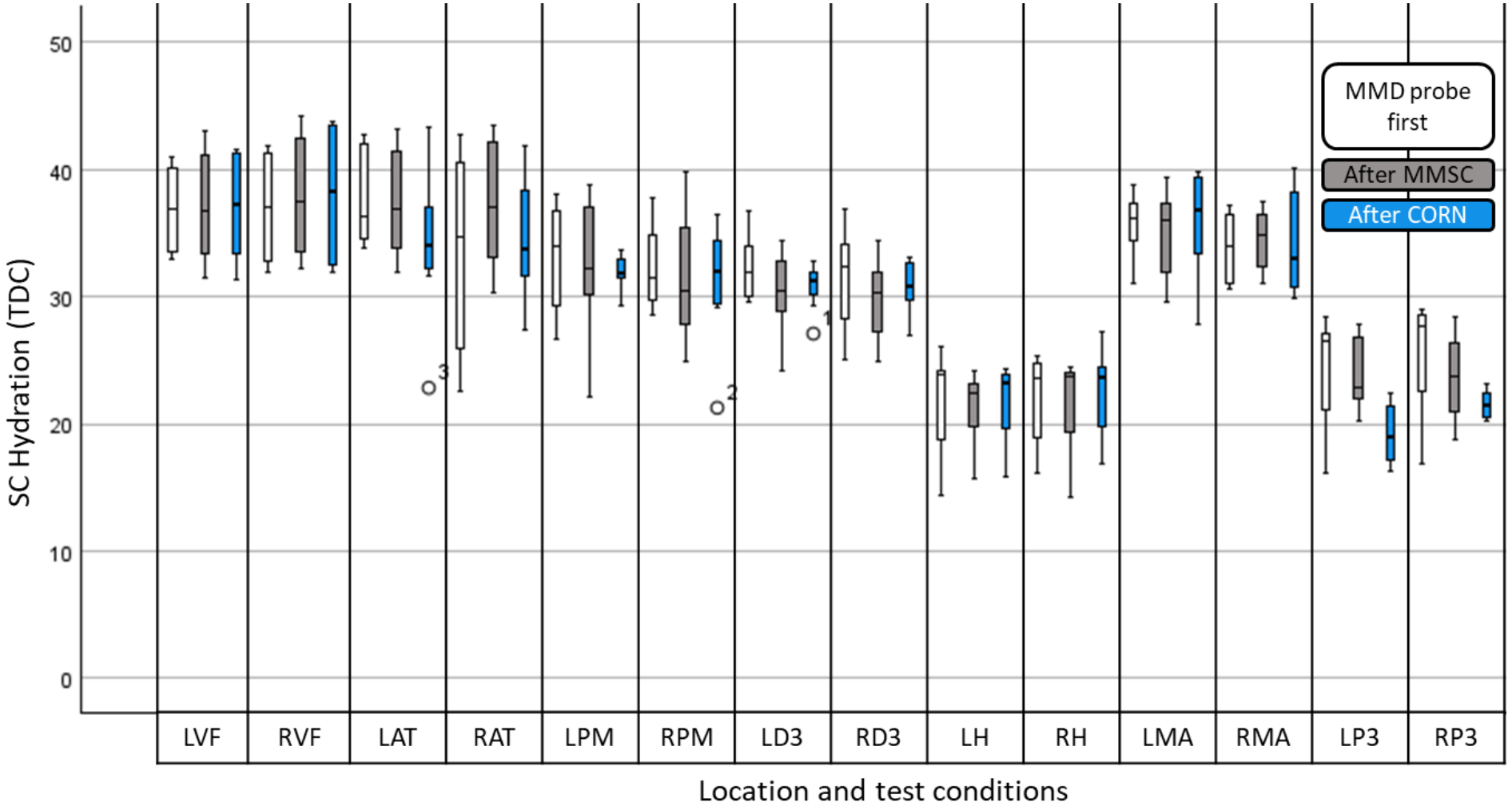


Figure 33. Data obtained using the MoistureMeter D® 1.5 mm probe with no prior device use, after MoistureMeter SC™ use (after MMSC), and after Corneometer® CM825 use (after CORN).

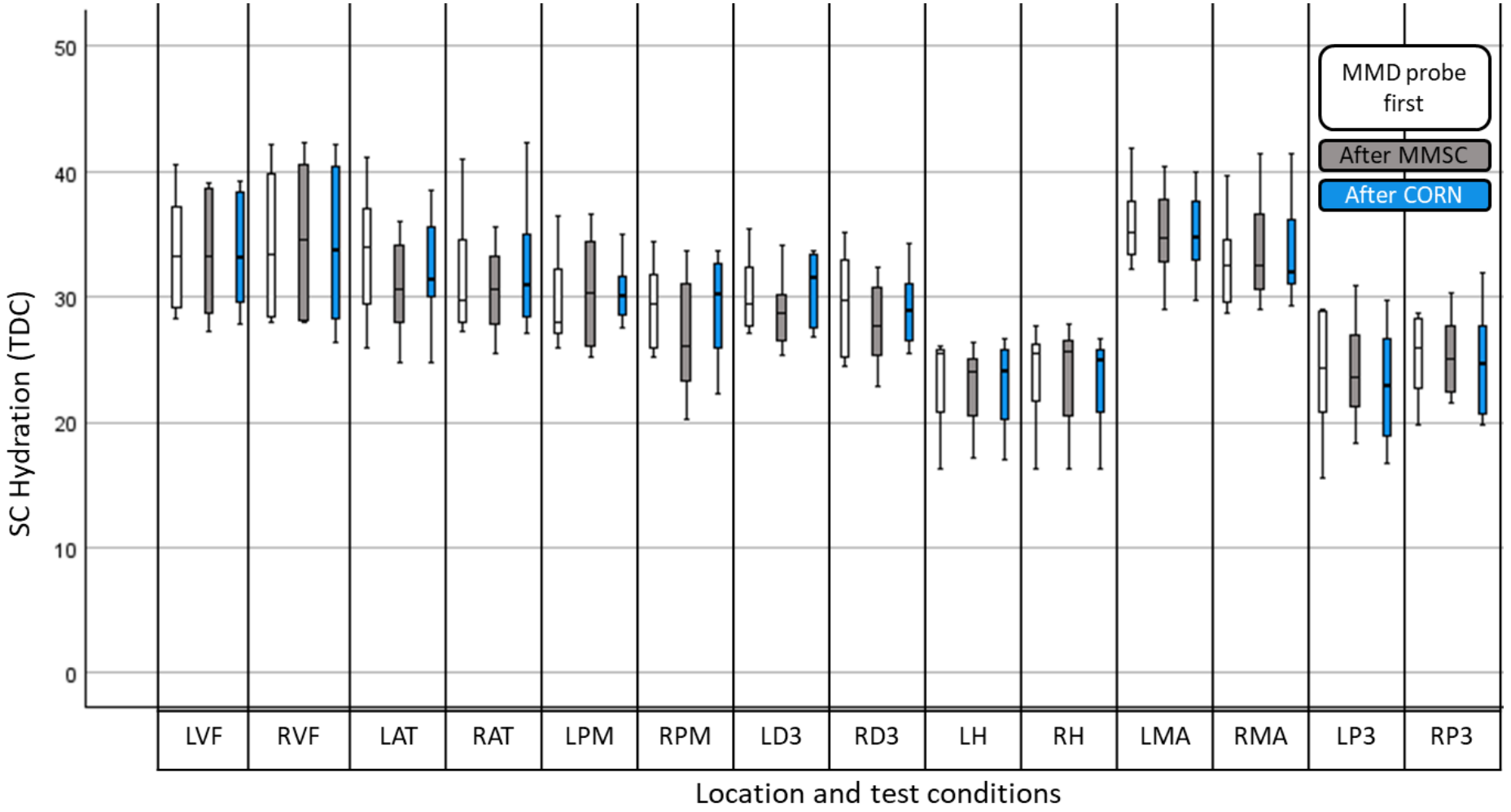


Figure 34. Data obtained using the MoistureMeter D® 2.5 mm probe with no prior device use, after MoistureMeter SC™ use (after MMSC), and after Corneometer® CM825 use (after CORN).

The above data indicate that the prior use of another device can influence the hydration data collected using the Corneometer® CM825 and MoistureMeter SC2™, primarily at the plantar skin sites. This phenomenon is not evident in the data collected using the MoistureMeter D®.

5.4.4. Between and within-day repeatability of data collected using Corneometer® CM825, MoistureMeter D®, and MoistureMeter SC™.

Due to the small number of participants involved in this study, the differences calculated between groups (for AM and PM data and Day 1 and Day 2 data) violated the assumptions of the Wilcoxon signed rank test (this requires the distribution of differences between the two groups to be symmetrical in shape). In lieu of this statistical test, box-plot graphs have been generated for each comparable dataset (Day 1 AM and PM data, Day 2 AM and PM data, AM Day 1 and Day 2 data, and PM Day 1 and Day 2 data) and have been visually assessed for differences between groups. For brevity, only half of these data are presented below (Day 1 AM and PM data and AM Day 1 and Day 2 date), the remainder of the equivalent comparisons can be found in Appendix 7.

This process is supported by the consideration of Bland-Altman plots generated for each comparison. Unfortunately, due to insufficient data, no threshold for 'clinically meaningful' change is included within these plots.

Within each data-collection session, multiple measures were taken from the same skin sites at different time points (representing different acclimatisation periods). Data collected from a single acclimatisation period have been used for these comparisons to prevent multiple measures confounding any differences observed between AM and PM, and Day 1 and Day 2 datasets (T20).

5.4.4.1. Within-day repeatability

Data collected using the Corneometer® CM825 display little variability between AM and PM data-collection sessions at any site (See Figure 35).

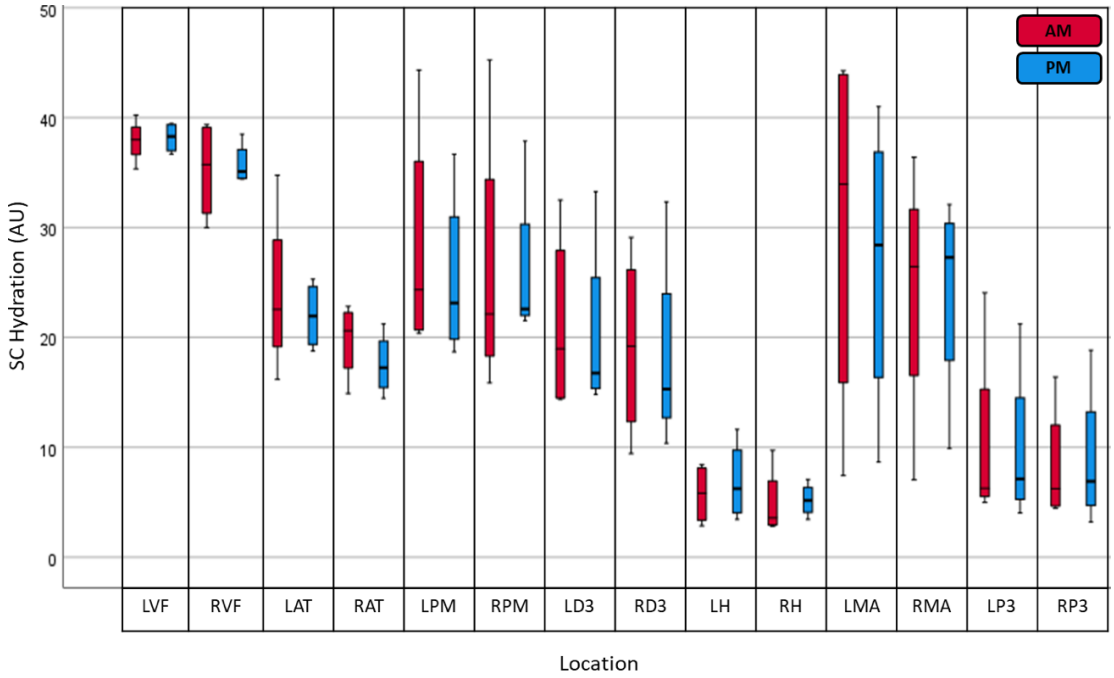


Figure 35. A boxplot graph demonstrating the skin hydration data measured using the Corneometer® CM825 in two data-collection sessions on the same day (AM and PM).

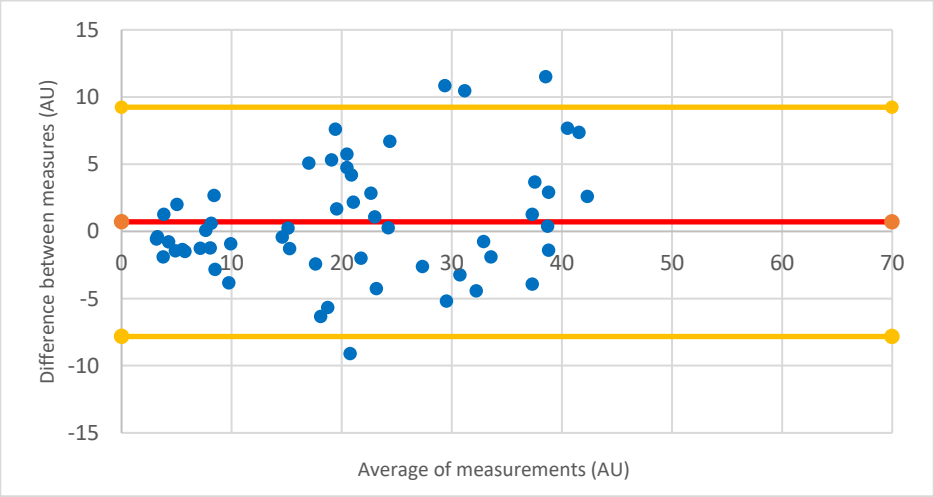


*Figure 36. A Bland-Altman plot demonstrating the agreement between data obtained using the Corneometer® CM825 from two data-collection sessions in the same day (AM and PM) from all locations.***

** The red line represents the mean difference between sessions, yellow lines represent the 95% confidence intervals (upper and lower).

For the Corneometer® CM825, the mean difference between sites across AM and PM sessions is 0.708, indicating data from one time-point are not consistently higher or lower than the other time point Figure 36. Four out of fifty-six data-points lie outside of the 95% confidence intervals.

Data collected using the MoistureMeter SC™ have a higher range in the AM than PM sessions on the plantar measurement sites, although the median values are similar (See Figure 37).

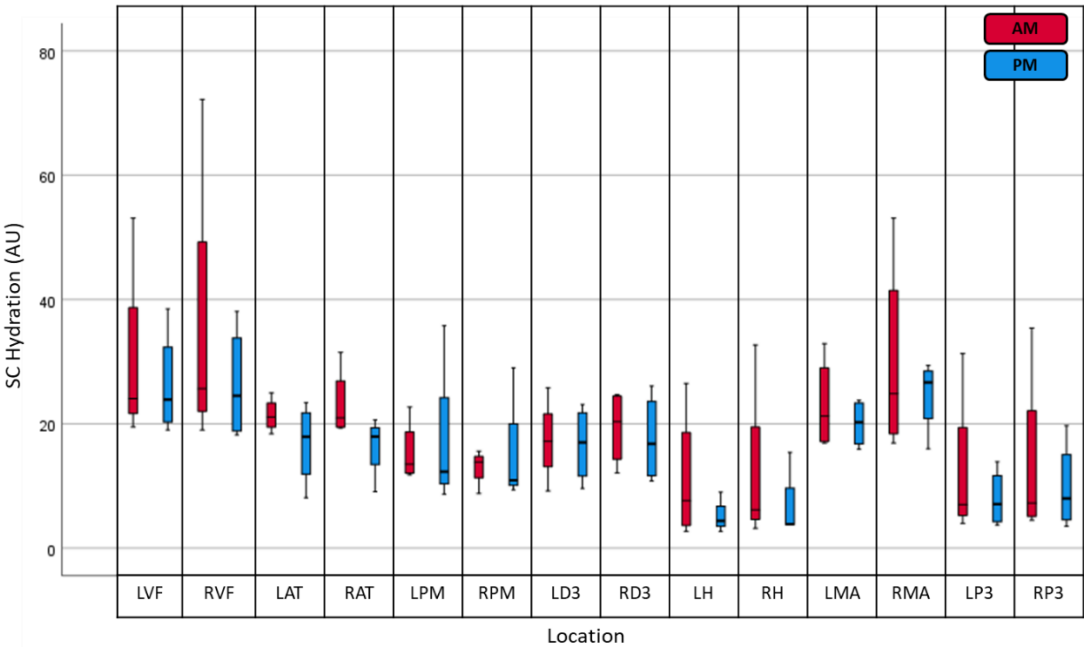


Figure 37. A boxplot graph demonstrating the skin hydration data measured using the MoistureMeter SC™ in two data-collection sessions on the same day (AM and PM)

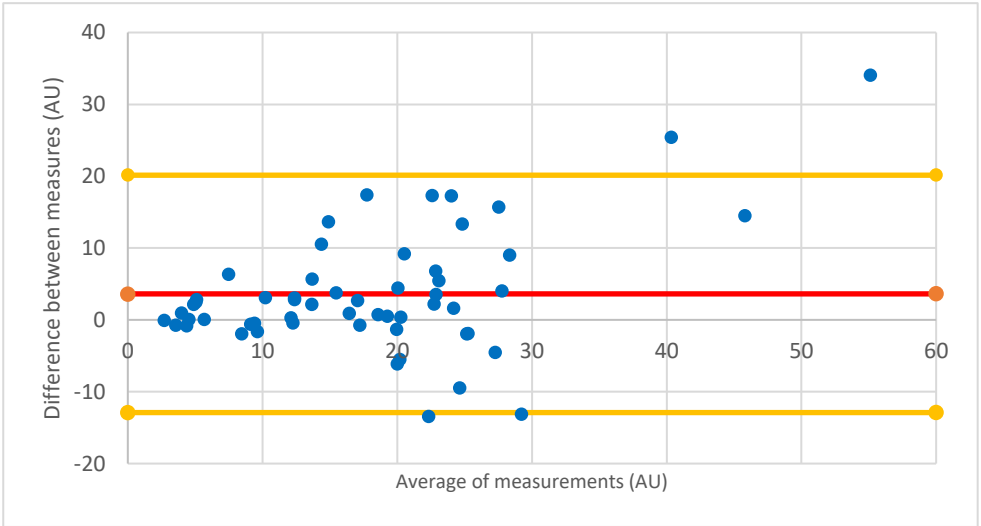


Figure 38. A Bland-Altman plot demonstrating the agreement between data obtained using the MoistureMeter SC™ from two data-collection sessions in the same day (AM and PM) from all locations.

Although most data-points are positioned closely to the mean difference or within the 95% confidence intervals, the mean difference (3.62 AU) is higher than the Corneometer® CM825 (0.708), reflecting the higher hydration data collected in the AM sessions (See Figure 38). This may also be contributed to by the large differences observed at the data points demonstrating the highest average of measures, which sit outside of the 95% confidence interval.

The MoistureMeter D® (0.5 mm probe) data has a higher range in the PM sessions than in the AM sessions, as demonstrated in Figure 39.

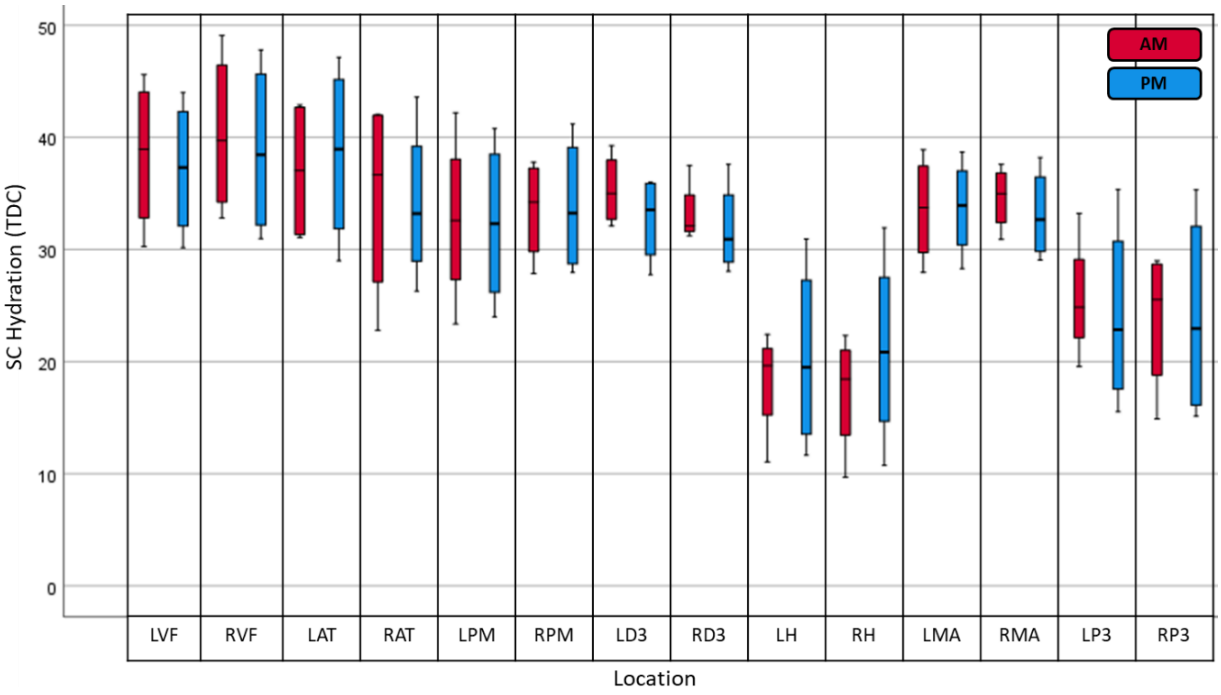


Figure 39. A boxplot graph demonstrating the skin hydration data measured using the MoistureMeter D® in two data-collection sessions on the same day (AM and PM)

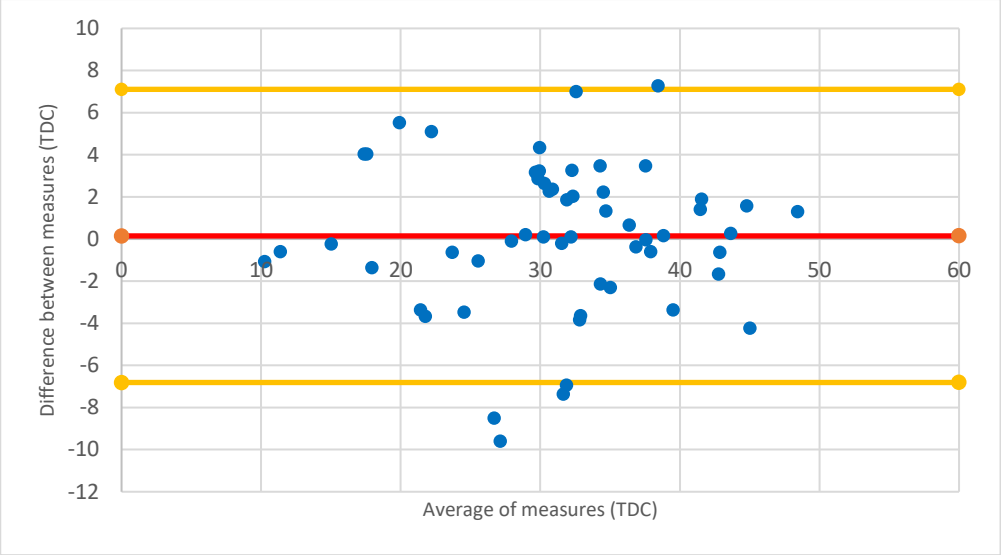


Figure 40. A Bland-Altman plot demonstrating the agreement between data obtained using the MoistureMeter D® from two data-collection sessions in the same day (AM and PM) from all locations.

Despite this irregularity, the Bland-Altman plot indicates that the mean difference between AM and PM values is very close to 0 (-0.022), indicating little overall deviation (See Figure 40). The even dispersion of the data points indicates that differences between measures (AM and PM) do not vary depending on water content of the skin (i.e., data are not more likely to exhibit large differences between AM or PM measures at areas of high or low skin hydration).

5.4.4.2. Between-day repeatability

Some variation is visible between data collected on different days using the Corneometer® CM825, however this is not consistent between sites (See Figure 41). Several high readings collected at the peri-malleolar site skew data collected at this site for Day 2, this is also evident in the Bland-Altman plot generated for these data (See Figure 42).

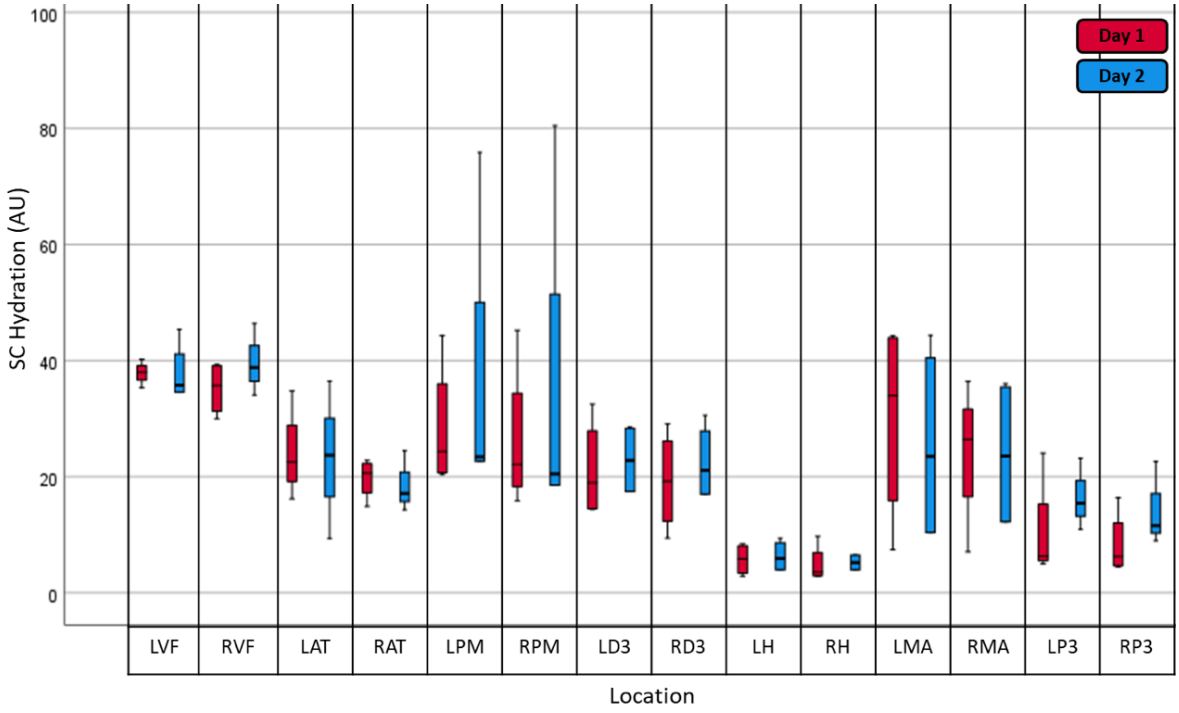


Figure 41. A boxplot graph demonstrating the skin hydration data measured using the Corneometer® CM825 in two data-collection sessions at the same time slot (AM) across two days.

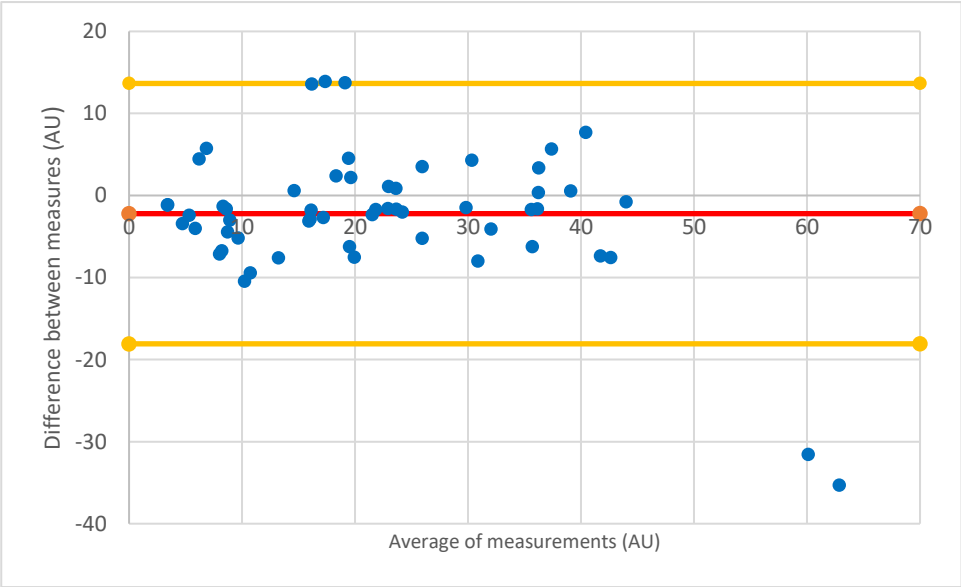


Figure 42. A Bland-Altman plot demonstrating the agreement between data obtained using the Corneometer® CM825 from two data-collection sessions at the same time slot (AM) across two days from all locations.

Between-day variability is small and very similar to within-day variability for the Corneometer® CM825 as demonstrated by the dispersion of the data points displayed in Figure 42, irrespective of the outliers which generate a small deviation in the mean difference between measures (-2.32).

MoistureMeter SC™ data shows some variability between days, with Day 1 values consistently higher than Day 2 values between sites (See Figure 43). The data dispersion appears skewed (the median is not central within the range). This could be due to one participant having higher skin hydration than the others.

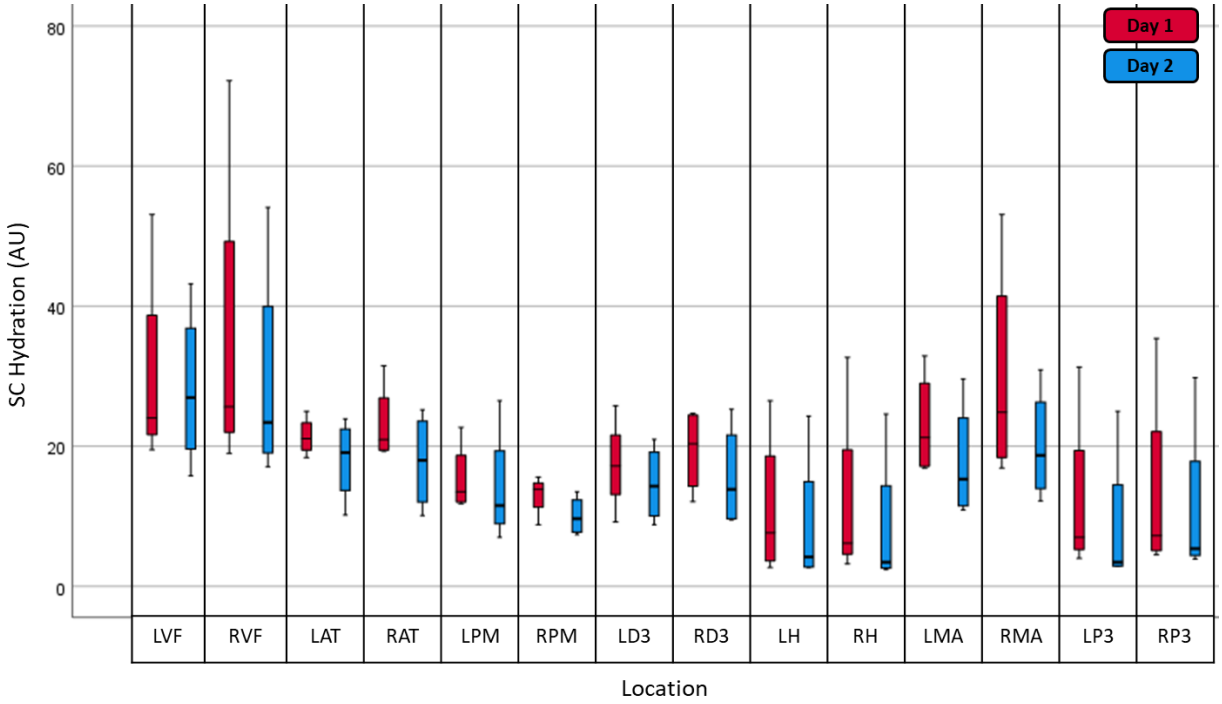


Figure 43. A boxplot graph demonstrating the skin hydration data measured using the MoistureMeter SC™ in two data-collection sessions at the same time slot (AM) across two days.

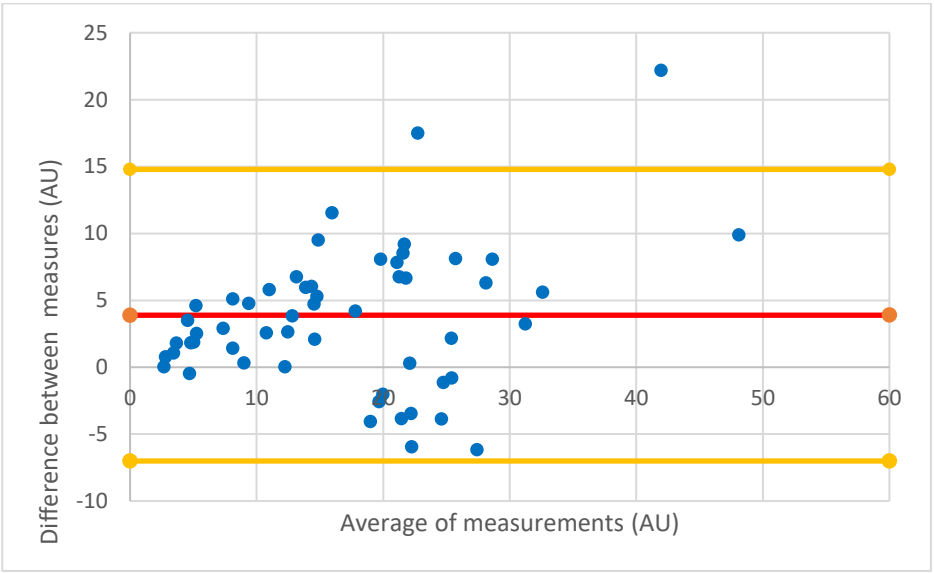


Figure 44. A Bland-Altman plot demonstrating the agreement between data obtained using the MoistureMeter SC™ from two data-collection sessions at the same time slot (AM) across two days from all locations.

This observed difference between days is reflected by the Bland-Altman plot (See Figure 44) which has a mean difference between measures of 3.89. Several datapoints with high

difference between measures could be contributing to this, but this finding does align with the variance observed between days in the boxplot (See Figure 43).

MoistureMeter D® data collected at different days display a small amount of variability, however, not in a consistent pattern (i.e., Day 1 data being consistently higher than Day 2 data) (See Figure 45).

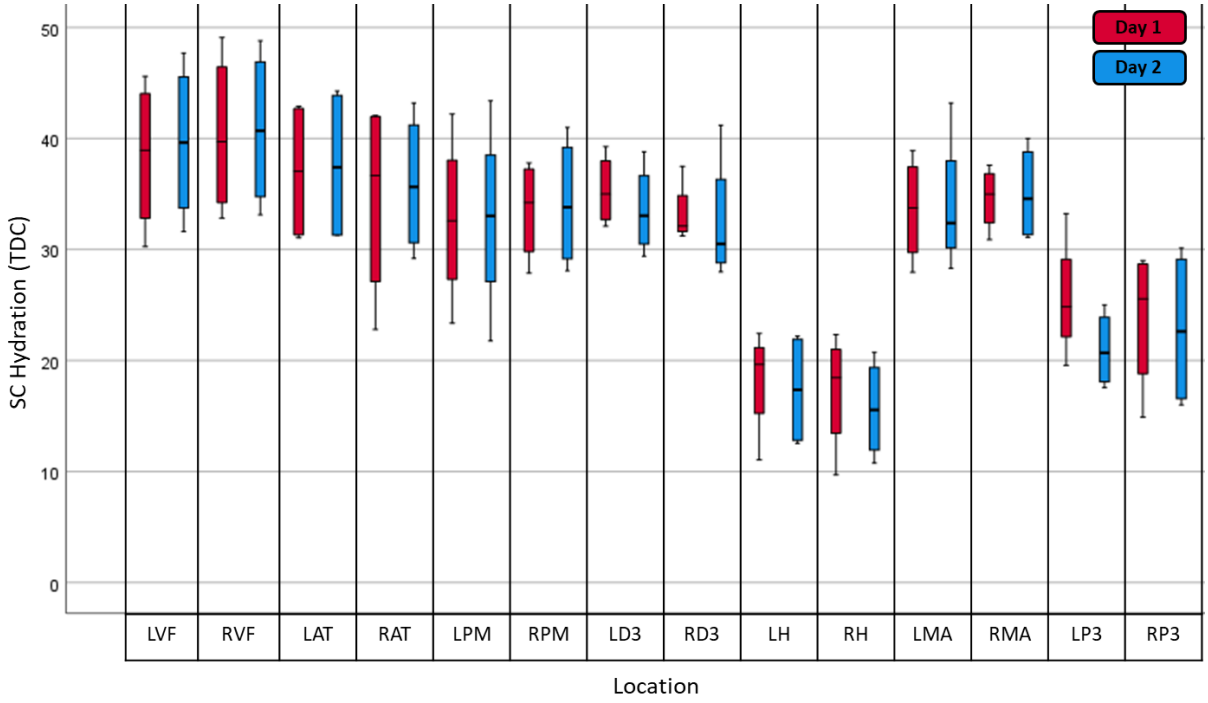


Figure 45. A boxplot graph demonstrating the skin hydration data measured using the MoistureMeter D® in two data-collection sessions at the same time slot across two days.

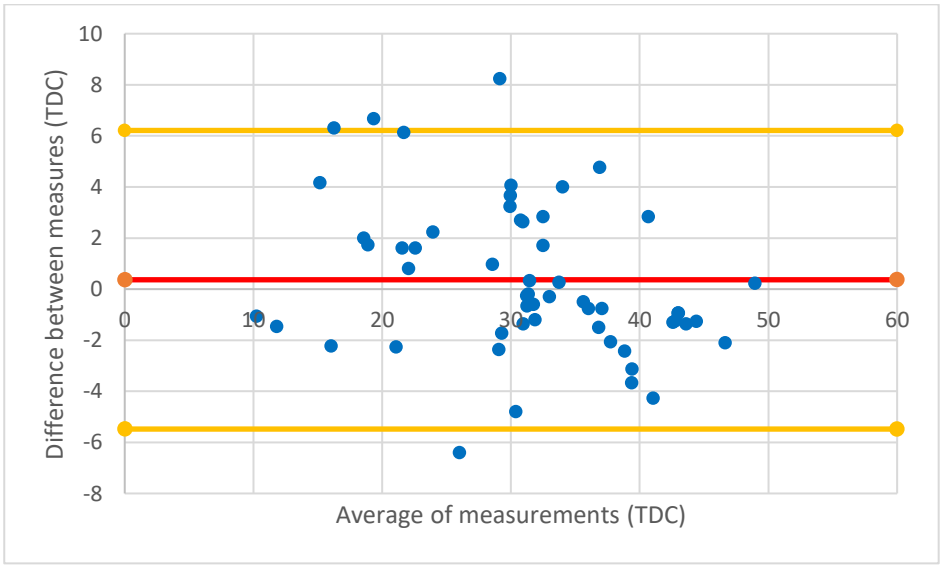


Figure 46. A Bland-Altman plot demonstrating the agreement between data obtained using the MoistureMeter D® from two data-collection sessions at the same time slot across two days from all locations.

This consistency between days is reflected in the Bland-Altman plot (See Figure 46), which has a mean difference between measures close to 0 (0.19) and has few data points outside of the 95% confidence interval barriers.

5.5. Discussion

5.5.1. Impact of acclimatisation period on data collected using the Corneometer® CCM825, MoistureMeter SC™, and MoistureMeter D®.

This data demonstrates that only the hydration of the plantar skin is influenced by acclimatisation period: Out of 42 Friedmans tests conducted, 3 highlighted a significant (p-value ≤0.003) difference in hydration data collected using the Corneometer® CM825 and MoistureMeter D® following different acclimatisation periods. Each of these was found on the plantar foot. No significant differences were found amongst the dorsal data or hydration data collected using the MoistureMeter SC™.

Post-hoc Wilcoxon signed rank tests demonstrated that statistically significant differences were identifiable between the 20-minute acclimatisation period, and subsequent time-points (Corneometer® CM825: LP3 20-30 (p-value =0.003), 20-35 (p-value <0.001), RP3 20-35 (p-value = 0.001); MoistureMeter D®: LH: 20-30 (p-value <0.001), 20-40 (p-value = 0.004)). From reviewing these data, it appears plantar skin hydration decreases and becomes less dispersed as acclimatisation period increases, when measured using the MoistureMeter D® (0.5 mm probe) and Corneometer® CM825.

The non-plantar skin observed in this study exhibited no such trend. This suggests that plantar skin requires a longer acclimatisation period than non-plantar skin for skin hydration to stabilise. This is not unexpected due to the thickness of the plantar SC (Vela-Romera et al., 2019) (presumably requiring more time to allow absorbed water to evaporate) and the excess of sweat glands on the plantar foot (Taylor & Machado-Moreira, 2013).

The acclimatisation period used for skin hydration measures varies across the literature from 15-30 minutes (Berardesca et al., 2018; Rogiers et al., 1990; Serup et al., 2006). As the

differences observed in the data collected in this study are observed between data collected at 20 minutes and later time-points (25, 30 and 35 minutes, and 30 and 40 minutes), it is conceivable that even larger variation would be evident in data collected following a 15-minute acclimatisation period. However, a 15-minutes acclimatisation period has been applied to the plantar foot within studies capturing foot-skin hydration previously and generated data with high intra and interrater reliability from all foot skin sites (other than callus) (intrarater reliability: 0.88-1, interrater reliability: >0.89) and that correlated strongly with physical skin characteristics known to relate to skin hydration (Hashmi, Nester, et al., 2015; Hashmi, Wright, et al., 2015). This indicates that consistency of acclimatisation period between data-collection sessions negates variability caused by a short acclimatisation period.

A skin acclimatisation period of 20 minutes has been used within each of the studies described later in this thesis. This acclimatisation period has been selected as a shorter acclimatisation time has been shown to generate reliable hydration data, and there is insufficient evidence from this pilot work to support extending this (therefore increasing time burden in future studies), particularly when consistency between data-collection sessions is proposed to be sufficient to reduce variability.

In future, it would be useful to repeat this studying using a wider range of acclimatisation periods to establish when or if foot skin hydration stabilises, i.e., by collecting data at 5-minute intervals for an hour.

5.5.2. Impact of prior device use on the data collected using the Corneometer® CM825, the MoistureMeter SC™ and the MoistureMeter D®

Within this study, no significant differences were identified between data collected when a device was used first or after using another device. Despite this, some differences were visible when the data were plotted as boxplot graphs.

In this study, hydration measures were found to decrease on the plantar skin when collected after using another device. This contradicts the work by Kottner et al (2014) which found no changes in SC hydration measures following other device use. It is proposed that the results of this study are in-fact associated with the shorter

acclimatisation period associated with the device being used first, rather than being the consequence of prior device use. For this reason, in the later studies described in this thesis these three hydration measurement devices are used in the same order starting from the device which takes the least time to collect data, to the most (to reduce variation in acclimatisation period between devices): Corneometer® CM825, MoistureMeter SC™, MoistureMeter D®.

In future, it would be useful to repeat this study in such a manner than differences in acclimatisation period were negated – i.e., by using an acclimatisation period beyond which plantar values are known to stabilise, or by staggering the use of devices to ensure a consistent acclimatisation period between trials.

Also of note within these data are the visible outliers in Figures 30 and 31. These have been included within the analysis as per the statistical analysis plan described in Section 5.3.1. On close review, these data all source from one participant, participant P06. This individual displays high skin hydration levels across all investigations, however these are most evident within this investigation of prior instrument use on the data collected using the Corneometer® CM825 and MoistureMeter SC™. The demographic details of this participant do not provide any indication as to why they may have higher foot skin hydration than the other participants: The participant is male (50% of participants in this investigation are male), 30 years of age (age range 25-37), and demonstrates typical washing, footwear, and hosiery behaviours within this participant cohort. From this, it may be assumed that the perceived 'high' skin hydration data observed as outliers within these datasets may be a reflection of natural variation within foot skin hydration, and not reflective of a participant demographic that should be excluded from future investigations.

5.5.3. Between and within-day repeatability of data collected using Corneometer® CM825, MoistureMeter D®, and MoistureMeter SC™.

Due to the requirement for participants to undertake multiple data-collection sessions, analysis of between and within-day repeatability was undertaken irrespective of that fact that the outcome of this work will not influence the other studies in this thesis as they have a cross-sectional design. Study of the variation in skin hydration within and between days

is more relevant to longitudinal studies, in which skin hydration is monitored to assess the efficacy of emollient for example. Circadian and ultradian rhythmic changes have been previously been found to be expressed in forearm skin hydration (Le Fur et al., 2001). However these have not been examined on foot skin. Although the design of this study does not facilitate a thorough assessment of the circadian or ultradian rhythm (the time-points used are too disparate), it could offer some insight into the between and within-day reliability of data for future data-collection.

Unfortunately, the limited number of participants who took part in this study, and the reduction of data to a single-timepoint for these comparisons (to prevent confounding effects from other aspects of study design), severely limit the meaning of the data. In some instances, data collected at different time-points appears to be consistently higher or lower than others, however the skew within these data indicate that this effect may be the rest of a single individuals data being raised in that session, as opposed to being a reflection in the data collected across the full cohort. Additionally, equivalent data do not demonstrate the same pattern (See Appendix 7).

5.6. Conclusion

The primary variability observed between datasets collected at different time points and with and without prior use of a device hailed from the use of a device at 20 minutes acclimatisation period, as opposed to 25/30/35 minutes (Corneometer® CM825), and 30/40 minutes (MoistureMeter D®).

The findings of this study have several implications:

- In relation to the wider literature base, this demonstrates increased variability of foot skin hydration on the plantar and dorsal skin, highlighting the need for close control of factors influencing foot skin hydration in future.
- For application by the industry partner, this demonstrates the need for further investigation into the use of commercially available hydration measurement devices within longitudinal studies conducted by the research and development team.
- For the purposes of this PhD project, this has demonstrated that the order of device use or the acclimatisation period used is not anticipated to impact hydration data

collected using these tools as long as these are kept consistent between participants. These results informed the design of the studies in Chapters 6 and 7 in which device use is ordered from the least to most time required to collect data: Corneometer® CM825, MoistureMeter SC™, MoistureMeter D® (0.5, 1.5, 2.5 mm probes). This method ensures minimal variability in acclimatisation period possible for each device. This could also be achieved by using each device at a set time: i.e., Corneometer® CM825 at 20 minutes, MoistureMeter SC™ at 20 minutes, MoistureMeter D® at 40 minutes, however this would unnecessarily extend data-collection.

Additional benefit:

In addition to the understanding of acclimatisation period and prior device use impact upon hydration data collected from the foot skin, this study has been beneficial to the researcher as it has provided valuable insight into the practical aspects of planning a study using these devices. For example: an understanding of the time required to use each device on every skin site and the optimal positioning of devices to reach all measurement locations.

Chapter 6. An investigation into the hydration of the foot skin and associated skin characteristics.

6.1. Introduction

The water content of the skin influences its physical characteristics: well-hydrated skin is smooth and elastic, with reduced risk of damage from mechanical trauma (Oe et al., 2012). The plantar skin is exposed to high levels of mechanical trauma during ambulation (Hosein & Lord, 2000; Jasiewicz et al., 2019; McKay et al., 2017; Vette et al., 2019), and is uniquely vulnerable to xerosis (Baird et al., 2003). Xerotic foot skin can lead to tissue damage, which can have catastrophic effects for vulnerable individuals (Collier & Brodbeck, 1993; Hashmi, Nester, et al., 2015; Kirkham et al., 2014; Murray et al., 1996).

To resolve the uncertainty surrounding the use of commercially available hydration measurements devices on the plantar skin, an understanding of how these relate to the biophysical characteristics of the skin influences by hydration is required. This study used three devices to measure skin hydration (Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D®), three measures of physical characteristics of the tissue (elasticity: DermaLab® Series SkinLab Combo Elasticity Probe, hardness: SATRA STD 226 Digital Durometer, surface features: Visioscan® VC98), and the questionnaire developed in Chapter 4, recording an individual's perception of their foot skin features and health (FSkHQ).

Data were collected from a cohort of young healthy people with no foot skin pathology, providing normative data on the foot skin hydration for each commercially available hydration measurement device, a demonstration of how these correlate with the biophysical characteristics of the skin and participants perceptions of their skin health.

Aspects of this study relating to the participants perception of their foot skin health are of particular value to the industry sponsor of this work, Scholl. A large range of Scholl's products are foot skin emollients. Insight into how features of foot skin appearance contributes to overall perception of foot skin health, and how this may drive product selection are of interest to Scholl. Additionally, an understanding of which commercially available hydration measurement device aligns most closely with consumers perception of

foot skin features are helpful for generating data in clinical trials that is reflective of the consumers experience.

6.1.1. Novelty statement

This is the first instance in which several commercially available hydration measurement devices are examined for correlations with objective and subjective indicators of foot skin health in a healthy population, therefore generating data on their suitability for use on the foot.

6.2. Aims and objectives.

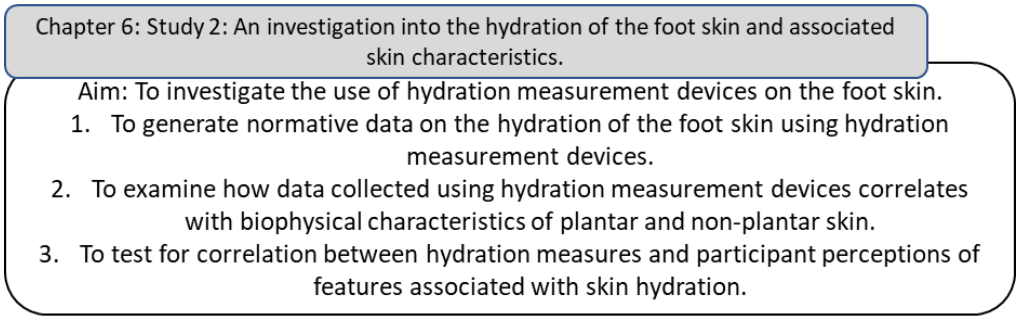


Figure 47. Objectives for 'An investigation into the hydration of the foot skin and associated skin characteristics.'

6.3 Method

A cross-sectional, mixed-methods study was conducted at The University of Salford, Manchester, UK. A single researcher collected all data. A group of participants aged 20-40 years were recruited to attend a single data collection session. Within these sessions, the hydration, biophysical characteristics, and participant perception of skin features of seven skin sites on the foot, leg and arm were quantified using non-invasive methods.

The study protocol was reviewed and approved by The University of Salford Ethics Panel reference number: 3137.

6.3.1 Participants

Participants aged 20-40 years were recruited from staff and students at the University of Salford. The narrow age range for participants (20-40 years of age) was selected to

minimise the influence of age on measurements of skin hydration (Cho et al., 2019; Egawa & Tagami, 2008a). This particular age range has also displayed maximal skin hydration measures (when measured on the forearm and forehead) (Rogiers et al., 1990). Factors such as sex and race were not expected to influence skin hydration levels significantly, and as such were not be a considered in this study (Rogiers et al., 1990).

6.3.1.1. Sample size

Table 39 summarises the relevant data sourced from the literature to inform the power calculation. GPower (Version: 3.1.9.7. Released: 17.03.20) statistical analysis software was used to conduct power calculations (Faul et al., 2007).

Table 39. Demonstration of power calculations undertaken using details from similar studies.

	'Biophysical measures of skin tissue water: Variations within and among anatomical sites and correlations between measures'(Mayrovitz, McClymont, et al., 2013)	Measurement of hydration in the stratum corneum with the MoistureMeter and comparison with the Corneometer® CM825 (Alanen et al., 2004)	Characterising the biophysical properties of normal and hyperkeratotic foot skin (Hashmi, Nester, et al., 2015)
Devices used	MoistureMeter SC2™ and MoistureMeter D® (1.5 mm probe)	Corneometer® CM825 and MoistureMeter SC™	Corneometer® CM825 and Cutometer® 575
Correlation coefficient (*p* value)	0.358 (p-value = 0.05)	0.75 (p-value <0.01)	0.25 (p-value = 0.01)
Details	Lowest correlation coefficient identified with p-value <5	Single value reported	Lowest correlation coefficient identified with p-value <5
Sample size required to recreate with 95% confidence interval	80	14	168

As is evident, the sample sizes required to observe these correlations vary considerably between data sets. It is not practical or ethically justifiable to undertake a large-scale study to pursue a significant result within a particular component of the study when a smaller group of participants may provide sufficient data to fulfil the aims and objectives of this study. As such, a pragmatic method involving an iterative design for data collection was adopted. Interim data analysis was conducted as data from groups of ten participants were processed to monitor the power of the study. However, as recruitment was relatively slow,

participant numbers did not become burdensome within the three-month timeframe necessitated by the rental of devices.

6.3.1.2. Inclusion and exclusion criteria, recruitment process, and testing environment.

The testing environment and exclusion and inclusion criteria used within this study are identical to those described in the pilot work in Chapter 4, as is the recruitment strategy (except for the wording of the recruitment material). The PIS and consent form used are in Appendices 8-11.

6.3.2. The Researcher

Data were collected by a PhD Student and a HCPC registered Podiatrist. This individual is familiar with the lab space and the equipment intended for use and can identify skin pathology that would exclude participants from the study.

6.3.3. Measurement Locations

Data was collected at seven locations on the body, five of which are on the foot. These locations are described, and their significance is explained in Table 40.

Table 40. Skin sites for examination.

Site Name (abbreviation)	Number	Significance	Location
Ventral Forearm (VF)	1	Control Site	Central forearm, 10 cm proximal to the wrist creases
Anterior Aspect of Tibia (AT)	2	Anticipated extremely dry	Central anterior surface, 10 cm proximal to the midpoint of the lateral and medial malleolus
Peri-Malleolar (PM)	3	Common location with previous studies	Surface immediately behind the medial malleolus
Dorsal 3rd MPJ (D3)	4	Dorsal foot site	Dorsal Aspect of 3rd MTP joint
Heel (H)	5	Anticipated high hydration levels	2 cm inwards from posterior centre of the heel
Medial Arch (MA)	6	Anticipated low hydration levels	Plantar aspect of base of 1st metatarsal
Plantar 3rd MPJ (P3)	7	Anticipated intermediate hydration levels	Plantar aspect of 3rd MTP joint

6.3.4. Measurement Devices

(Specifications of these devices and a review of relevant literature can be found in Chapter 4).

6.3.3.4.1. Commercially available hydration measurement devices

Corneometer® CM825 (Courage and Khazaka, Koln, Germany)

MoistureMeter SC™ (Delfin Technologies, Kuopio, Finland)

MoistureMeter D® (Delfin Technologies, Kuopio, Finland)

6.3.3.4.2. Measures of mechanical characteristics of skin

SATRA STD 226 Digital Durometer (SATRA Technology, Kettering, UK)

Dermalab Elasticity probe (Cortex Technology, Hadsund, Denmark)

Visioscan® VC98 (Courage and Khazaka, Koln, Germany) (Contrast parameter of output)

6.3.3.4.3. Measure of participant perception of skin

FSkHQ measures the perception of foot skin dryness and scoring of foot skin hardness, flakiness, cracking, and roughness.

FSkHQ further information

As part of the FSkHQ, participants completed a 'skin scoring matrix'. This matrix required participants to score the 'scaliness', 'cracking', 'roughness', and 'hardness' of four foot skin sites: dorsal 3rd metatarsal head, plantar 3rd metatarsal head, medial arch and heel. A reference table with descriptions for scores 0-4 was provided alongside this matrix. This process generated a score ranging from 0-16 for each skin site, which is defined as the 'FSkHQ skin score'.

6.3.5. Data collection protocol

Upon entering the laboratory, the participants were seated on the plinth and given a document pack containing the PIS (See Appendix 9. Study 2 participant information sheet) and consent form (See Appendix 10. Study 2 consent form). The participants then removed

any shoes, hosiery and rolled-up any leg or arm coverings and rested their legs on the extended plinth for fifteen minutes before data was collected.

During the acclimatisation period, the researcher reviewed the exclusion criteria for this study with the participant and ensured none of these apply to the individual. The researcher located and marked the skin sites using a surgical skin marker. As within the pilot work, each mark was made approximately 1 cm lateral/posterior (depending upon the planar alignment of the skin surface) to the skin to prevent interference with the data collected. During the marking-up process, the researcher checked the skin for any signs of skin disease that would exclude the participant from the study, and the participant completed the FSkHQ.

6.3.5.1. Order of data collection

The order of use for skin-measurement devices was determined by the perceived invasiveness of the instruments (least invasive to most invasive). The hydration measurement devices were used first, in the order described in section 5.5.2. The Visioscan® VC98 data were then obtained as this required no mechanical skin distortion and only a brief occlusion period. The DermaLab® Series SkinLab Combo elasticity probe was then used as these cause a small amount of tissue movement and use an adhesive disc. Finally, the SATRA STD 226 Digital Durometer was then be used as this applies the greatest amount of stress on the tissues (See Figure 48).

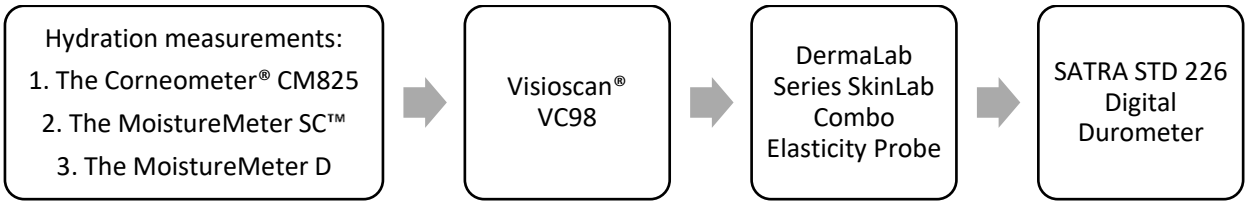


Figure 48. Order of device use.

6.3.5.2. Hydration measures

Each hydration measurement device was used to collect data from each skin site, working from those most proximal to the most distal (numbered in Table 40) and alternating between left and right body areas. Participant numbers were used to determine whether

these measurements were obtained from the right or left-hand side of the body first – i.e. even numbered participants had measurements taken right-left and vice-versa.

6.3.5.3. Mechanical testing

Whilst the participant was still seated on the plinth, measurements of skin surface texture and mechanical behaviour of skin were obtained as described in Chapter 5.

6.3.6. Data analysis

Descriptive statistics were used to display data collected from each skin site using three hydration measurement devices: Corneometer® CM825, MoistureMeter SC™, MoistureMeter D® (range, measures of central tendency, for example). Inferential statistics were used to test for correlations between hydration data and the following variables:

1. Hardness, retraction speed of skin following suction, and roughness data and skin hydration.
2. FSkHQ score and skin hydration measures.

The tests used are described in Table 41. The Shapiro-wilks test, Q-Q plots, measures of central tendency and histograms were used to determine the normality of data distribution.

Table 41. Statistical testing procedures.

Analysis Purpose	Parametric	Non-Parametric
Correlation Assessment	Pearsons Correlation Coefficient	Spearmans Rank Correlation Coefficient
Assessing for difference between groups	Student T test	Mann Whitney-U test

Due to increased risk of Type 1 errors with multiple comparisons, the Bonferroni correction was applied.

6.3.3.6.1. Outlier identification and processing

Outlier data was processed in same manner as in Chapter 5 (see Section 5.3.1.), in order to retain as much data as possible and maximise statistical confidence for analysis.

6.4 Results

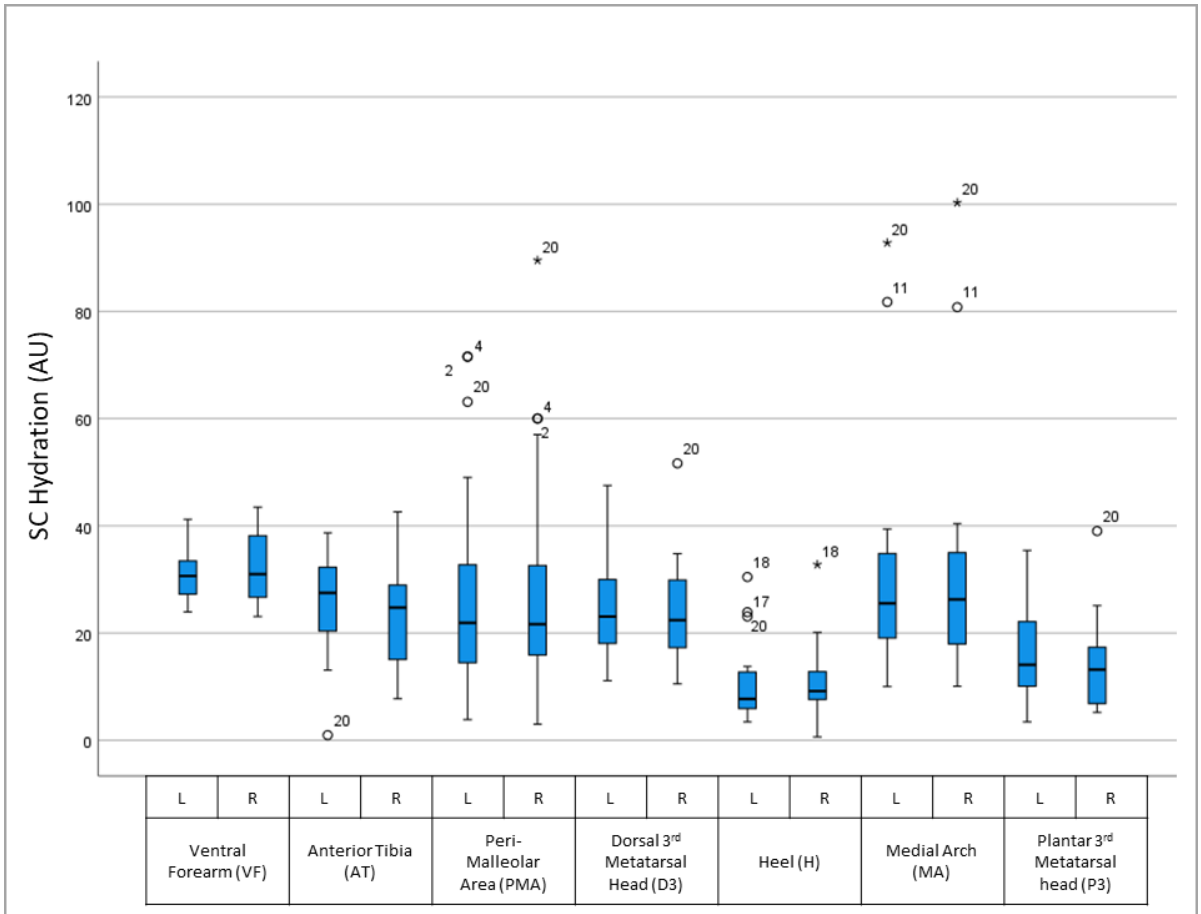
Data was collected between April 2021 and May 2022. Thirty-two participants were recruited (mean Age: 27.9, SD: 4.8 years, 53% female). Environmental conditions remained relatively stable throughout data-collection appointments (See Table 42).

Table 42. Participant demographics and environmental conditions.

N	Age (years) (SD)	% female (n)	Mean Start Temp (°C) (SD)	Mean End Temp (°C) (SD)	Mean Start RH (%) (SD)	Mean End RH (%) (SD)
32	27.9 (4.8)	53% (17)	20.4 (1.3)	20.4 (1.6)	41.5 (6.6)	40.8 (7.3)

6.4.1 Hydration of the plantar and non-plantar SC measured using the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D®.

Data were collected from 20 participants using the Corneometer® CM825. Data have been presented below for each measurement site (See Figure 49). Fewer participants were tested using the Corneometer® CM825 than the other devices included due to malfunction during the data-collection period.



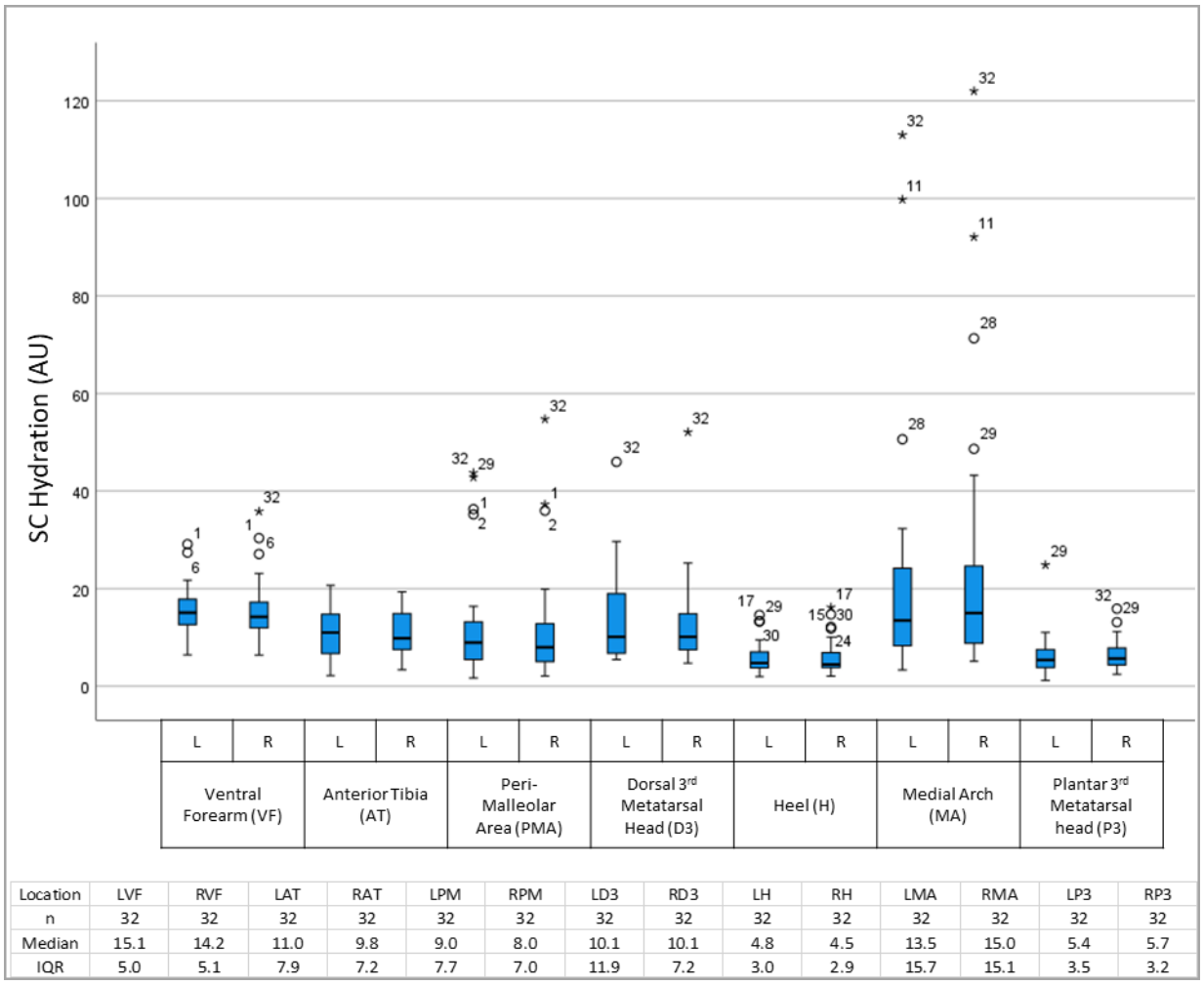
Location	LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Median	30.7	31.0	27.5	24.8	21.9	21.7	23.1	22.4	7.7	9.2	25.6	26.3	14.1	13.2
IQR	5.4	10.7	10.9	13.0	16.3	14.4	11.8	11.6	6.2	4.7	13.9	16.7	10.9	10.1

Figure 49. Corneometer® CM825 location data.

There is a difference in hydration levels as measured using the Corneometer® CM825 between non-plantar skin sites and the heel (LH and RH) and forefoot locations (LP3 and RP3). The values from these sites are lower and have a smaller IQR (LH (median (IQR): 7.72(6.2), RH: 9.2 (4.7), LP3: 14.1 (10.9), RP3: 13.2 (10.1)). Data from the medial arch (MA) which are more analogous to non-plantar data than plantar data (similar median values and IQR) (LMA: 25.6 (13.9), RMA: 26.3 (16.7)).

Several outliers are visible in these data. These data points were found to be the result of three consistently high hydration measurements taken using the Corneometer® CM825 (generating a high mean value) and were consistent between skin sites of different sides of the body from an individual participant (20 Figure 33). As such, these were considered indicative of the natural variation in plantar skin hydration and were included in analysis.

Data were collected from 32 participants using the MoistureMeter SC™. These data are presented in Figure 50.



Location	LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
n	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Median	15.1	14.2	11.0	9.8	9.0	8.0	10.1	10.1	4.8	4.5	13.5	15.0	5.4	5.7
IQR	5.0	5.1	7.9	7.2	7.7	7.0	11.9	7.2	3.0	2.9	15.7	15.1	3.5	3.2

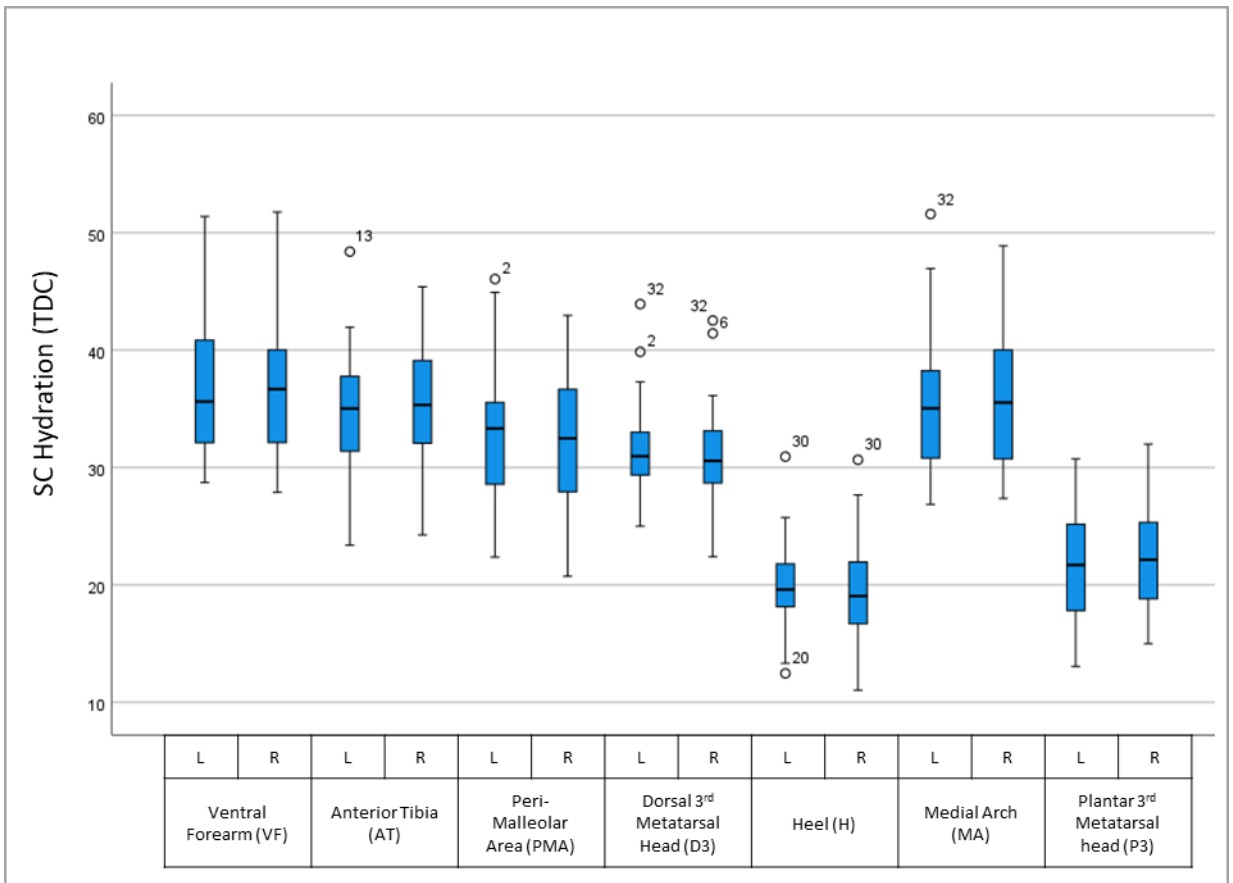
Figure 50. MoistureMeter SC™ location data.

Similarly to the Corneometer® CM825 dataset, the heel (RH and LH) and the forefoot (RP3 and LP3) sites display lower values with a smaller range (LH (median (IQR): 4.8 (3.0), RH: 4.5 (2.9), LP3: 5.4 (3.5), RP3: 5.7(3.2)). The median value of the medial arch sites, however, are higher than all but one non-plantar site (VF) and exhibit a much larger IQR than all non-plantar sites (LMA: 13.5(15.7), RMA: 15.0(15.1)), which all display a relatively consistent IQR and median value.

More outliers are found within the MoistureMeter SC™ data than in Corneometer® CM825 data, however there were also found to be the result of repeatedly high measures and consistent between body-sites. The participants who generate most of the outliers within the MoistureMeter SC™ data (P11 and P32) are the same individuals that appear outliers

within the Corneometer® CM825 dataset (P11 and P20) (variation in participant numbers has led to inequivalent participant identification number between studies for these individuals). Outliers within the MoistureMeter SC™ data are further outside of the nonoutlier data than those in the Corneometer® CM825 data. For example, the medial arch measures from Participant 32 are approximately 4 times the median hydration value in the Corneometer® CM825 data, whereas these are approximately 7 times the median value when the MoistureMeter SC™is used.

The MoistureMeter D® was used to collect data from 32 participants. Figure 51 illustrates the data obtained from each skin site.



Location	LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
n	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Median	35.6	36.7	35.0	35.3	33.3	32.5	31.0	30.6	19.6	19.1	35.1	35.5	21.7	22.2
IQR	8.6	7.8	6.2	6.8	6.7	8.3	3.6	4.1	3.6	4.8	7.3	8.6	7.3	6.4

Figure 51. MoistureMeter D® 0.5 mm probe location data.

Data collected using the 0.5 mm probe is used to measure SC hydration across the foot, similarly to the Corneometer® CM825 and the MoistureMeter SC™, the heel and forefoot (H and P3) sites display markedly lower median values (LH (median): 19.6, RH: 19.1, LP3:

21.7, RP3: 22.2), whilst the medial arch skin (MA) has a median value and IQR more akin to the non-plantar sites (LMA: 35.1 (7.3), RMA: 35.5 (8.6)). However, the forefoot (P3) has a similar IQR to non-plantar sites, and the dorsal foot skin (D3) displays a smaller IQR (LP3 IQR: 7.3, RP3: 6.4).

In comparison to the Corneometer® CM825 and the MoistureMeter SC™ data, the MoistureMeter D® data has fewer outliers and where these are evident, they are much closer to the main dataset. For example, the outlier from the left medial arch P32 data is 1.5* the median value of at the same location within the MoistureMeter D® dataset, the equivalent outlier for the Corneometer® CM825 and MoistureMeter, however, was 4* and 7* the respective median value).

6.4.2. Skin hydration and physical behaviour and surface texture

The data collected using hydration measurement devices have been compared to measures obtained simultaneously on the biophysical characteristics of the skin (hardness, elasticity, and roughness). Below, the relationships between these skin features are explored on plantar and non-plantar skin sites.

Spearman's rank order correlation has been applied as all data were found to be non-parametric in distribution through the Shapiro-Wilks test ($p<0.05$) and review of Q-Q plots, measures of central tendency and histograms.

The Bonferroni correction was applied to reduce the risk of a type 1 error arising as a result of the multiple comparisons undertaken within this analysis, generating a threshold p-value of ≤ 0.003 for statistical significance.

6.4.2.1. Skin hardness and hydration

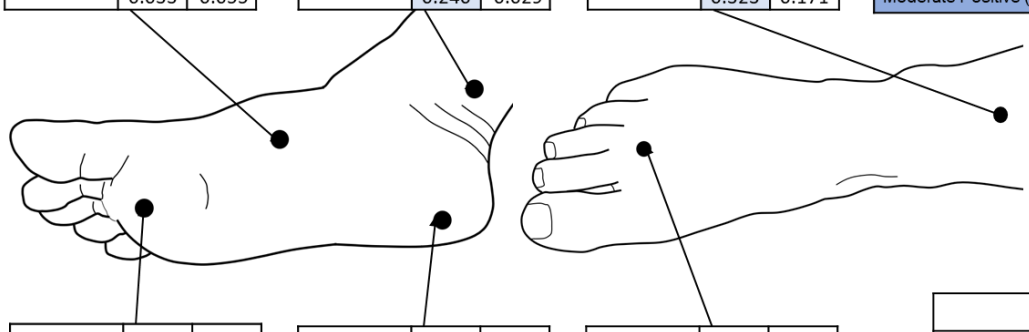
A statistically significant, moderate-strong negative correlation was identified between skin hardness and hydration data at the right plantar 3rd metatarsal head for the 0.5, 1.5, and 2.5 mm probes of the MoistureMeter D® (all p-values <0.000), the left heel for the Corneometer® CM825 (p-value: 0.003) MoistureMeter SC™ (p-value: 0.001) and MoistureMeter D® 0.5, 1.5 and 2.5 mm probes (all p-values <0.000) (See Figure 52).

MA	Left	Right
Corneometer	-0.468	-0.234
MMSC	-0.277	-0.259
MMD 0.5	-0.080	-0.021
MMD 1.5	0.009	0.005
MMD 2.5	0.033	-0.055

PM	Left	Right
Corneometer	0.280	0.146
MMSC	0.352	0.072
MMD 0.5	0.316	0.131
MMD 1.5	0.299	0.006
MMD 2.5	0.240	-0.029

AT	Left	Right
Corneometer	-0.117	-0.525
MMSC	0.069	0.009
MMD 0.5	0.364	0.338
MMD 1.5	0.282	0.427
MMD 2.5	0.323	0.171

'Significant' Correlation (p≤0.003)
Weak Negative (r-value = -0.21 to -0.4)
Moderate Negative (r-value = -0.41 to -0.6)
Strong Negative (r-value ≥-0.61)
Weak Positive (r-value = 0.21 to 0.4)
Moderate Positive (r-value = 0.41 to 0.60)



P3	Left	Right
Corneometer	-0.314	-0.528
MMSC	-0.273	-0.434
MMD 0.5	-0.235	**-0.611**
MMD 1.5	-0.301	**-0.658**
MMD 2.5	-0.266	**-0.593**

H	Left	Right
Corneometer	**-0.633**	-0.491
MMSC	**-0.552**	-0.378
MMD 0.5	**-0.692**	-0.494
MMD 1.5	**-0.696**	-0.241
MMD 2.5	**-0.619**	-0.288

D3	Left	Right
Corneometer	0.292	0.132
MMSC	0.109	-0.097
MMD 0.5	0.072	-0.033
MMD 1.5	0.216	0.006
MMD 2.5	0.283	0.162

Forearm		
	Left	Right
Corneometer	0.185	0.341
MMSC	0.387	-0.052
MMD 0.5	0.483	0.084
MMD 1.5	0.463	0.138
MMD 2.5	0.451	0.216

Figure 52. Results of a Spearman's rank-order correlation (rho-value) for skin hydration and hardness.

As anticipated due to their superficial measurement depths, the MoistureMeter SC™ and Corneometer® CM825 generate similar correlations with data collected using the SATRA STD 226 Digital Durometer across all measurement sites. The MoistureMeter D® data demonstrate the same pattern of relationship direction (negative on the plantar foot, positive on non-plantar tissues) on the plantar heel and the plantar 3rd metatarsal head, however this is not reflected at the medial arch.

6.4.2.1.1. Further results: Skin hardness

Data collected using the SATRA STD 226 Digital Durometer was anticipated to be higher at measurement sites with thin soft-tissue overlying bone (i.e., at the dorsal 3rd metatarsal

head) than at sites with a thick layer of soft-tissue (i.e., the heel) (Kelikian & Sarrafian, 2011). This is not the case however (See Figure 53). This relevance of this is discussed in Section 6.5.3.

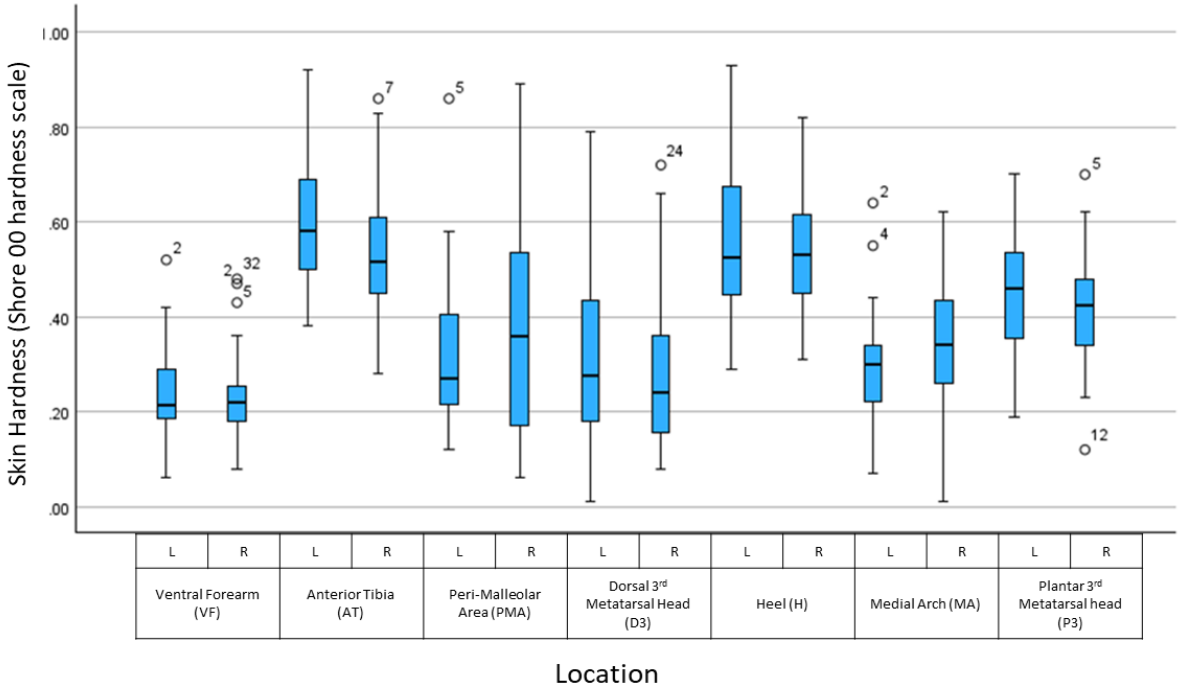


Figure 53. SATRA STD 226 Digital Durometer data collected from each measurement site.

6.4.2.1.2. Variation in correlation strength between body sites

Variation in correlation strength between equivalent comparisons is evident between different sides of the body (See Figure 54 and Figure 55). Rho-values are given to highlight how these are influences by outlier data-points. This is discussed in section 6.5.4.

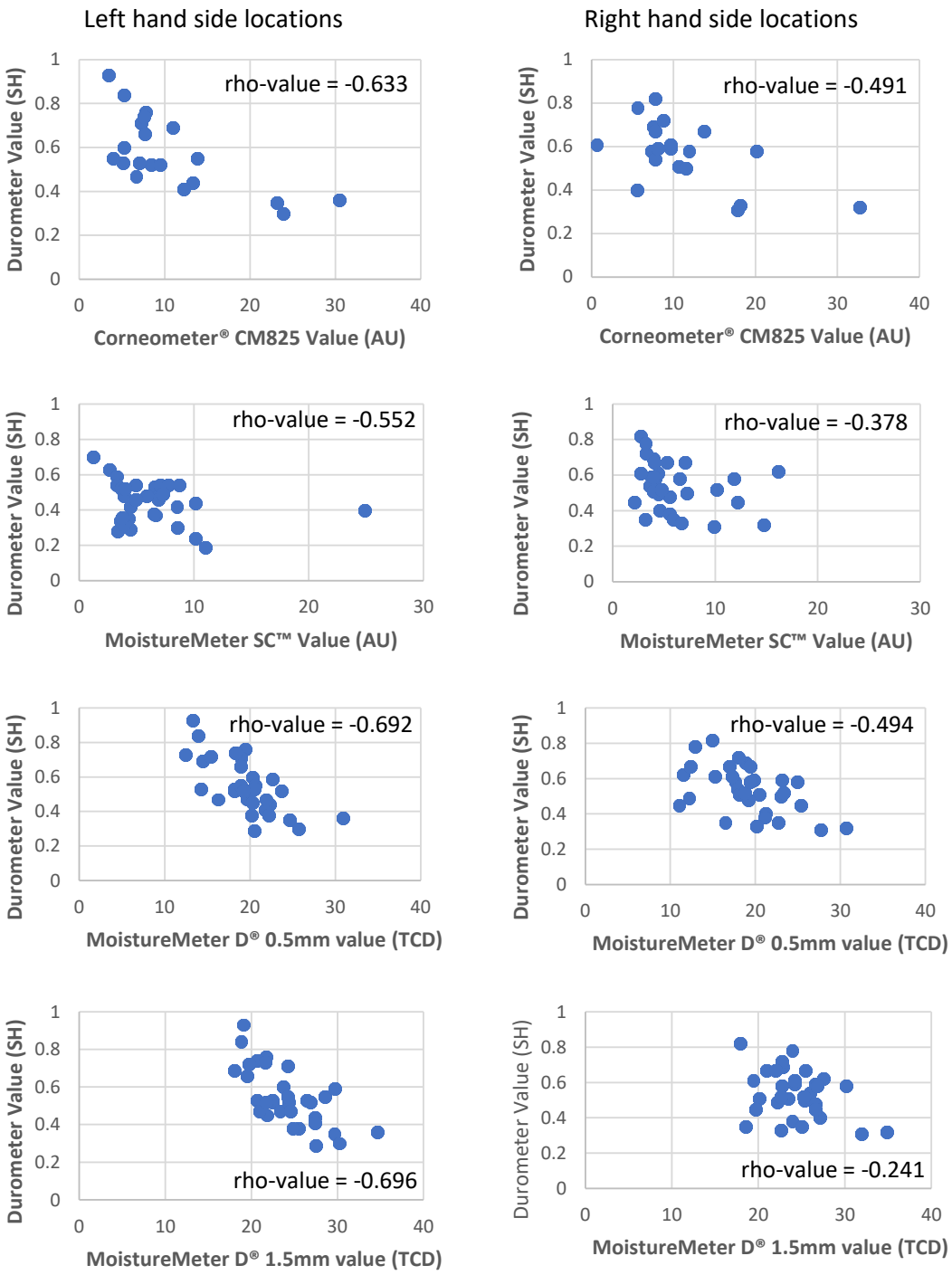


Figure 54. Scatter graphs of SATRA STD 226 Digital Durometer data and data obtained using the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® at the plantar heel.

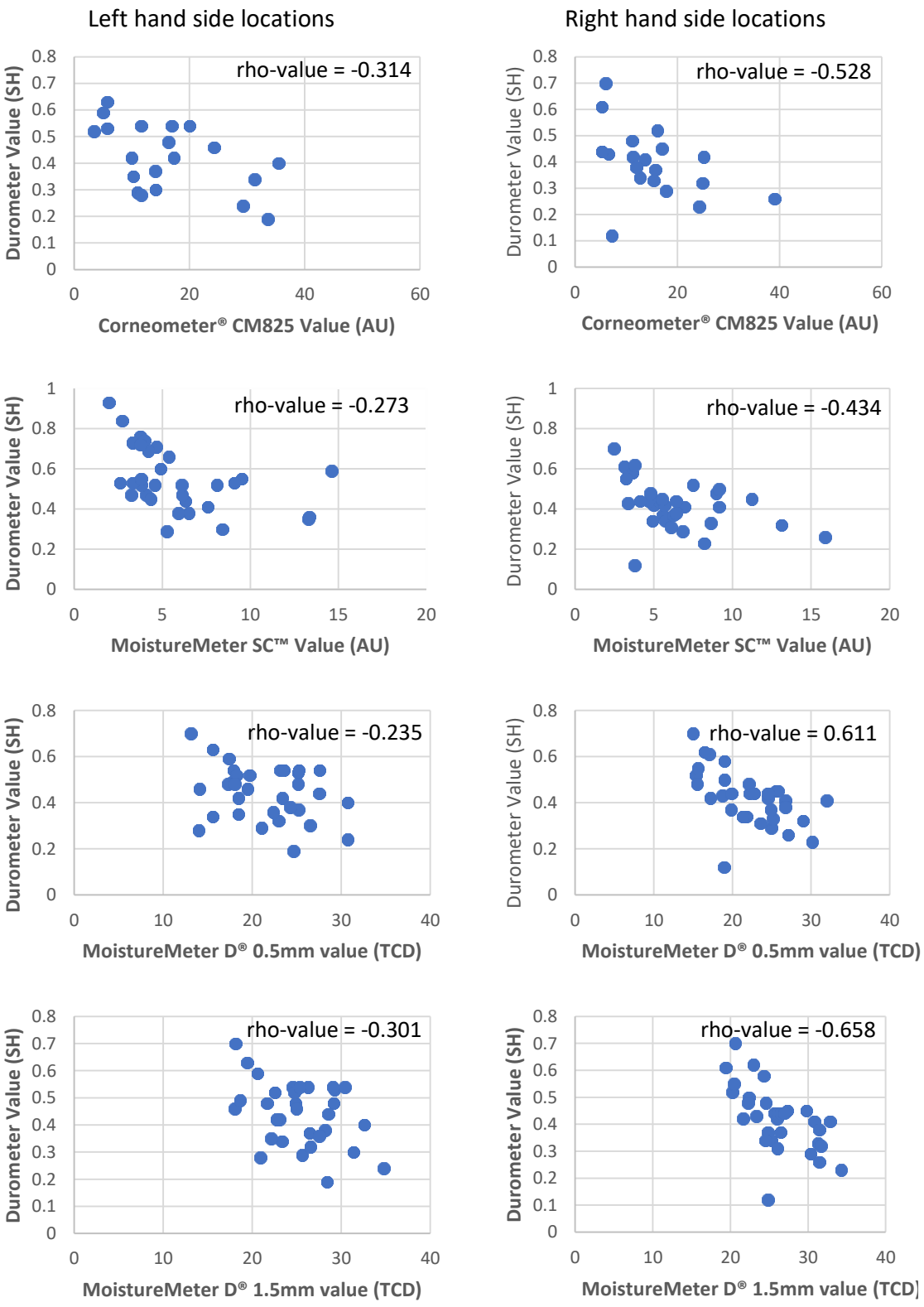


Figure 55. Scatter graphs of SATRA STD 226 Digital Durometer data and data obtained using the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® at the plantar 3rd metatarsal head.

6.4.2.2. Skin retraction speed and hydration

A single statistically significant (p-value: 0.002) correlation is demonstrated between the retraction speed of the skin following suction and the hydration of the skin overlying the 3rd metatarsal head, as measured using the 0.5 mm probe of the MoistureMeter D® (See Figure 56).

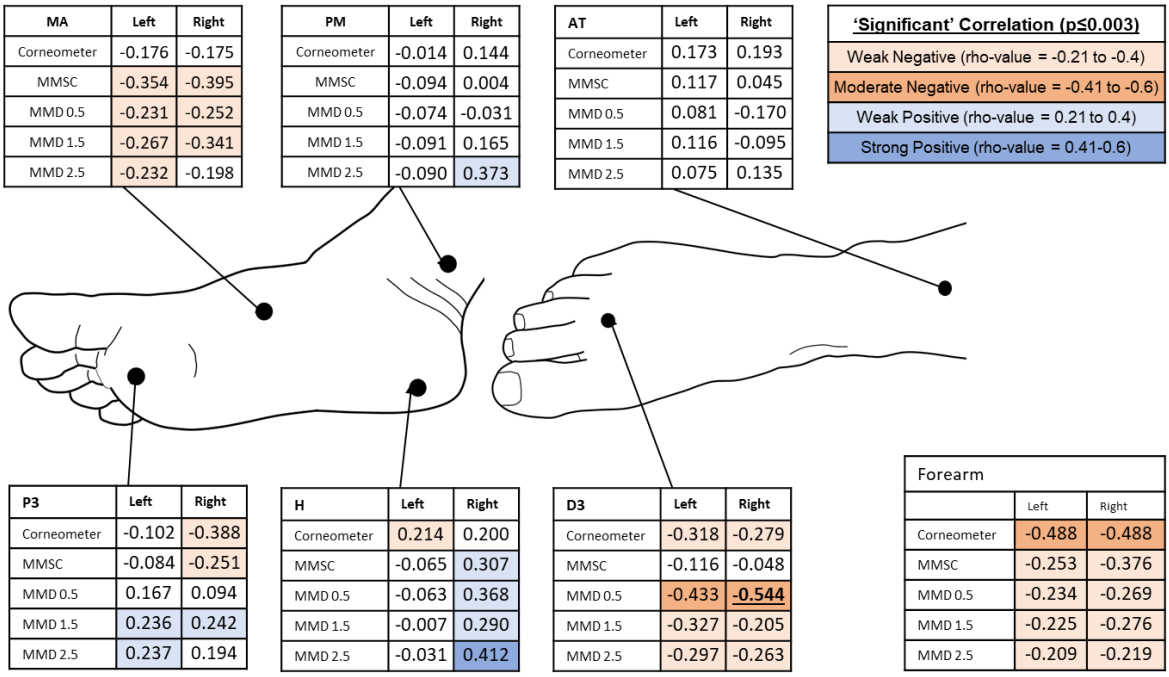


Figure 56. Results of a Spearman's rank-order correlation (rho-value) for skin hydration and elasticity (speed of skin retraction).

There is no distinct pattern demonstrated within this correlation analysis. Where a relationship is demonstrated between hydration and the retraction speed of skin, this is

weak and non-significant. In the dispersion of retraction-speed data itself, however, some difference is evident between plantar and non-plantar skin (See Figure 57).

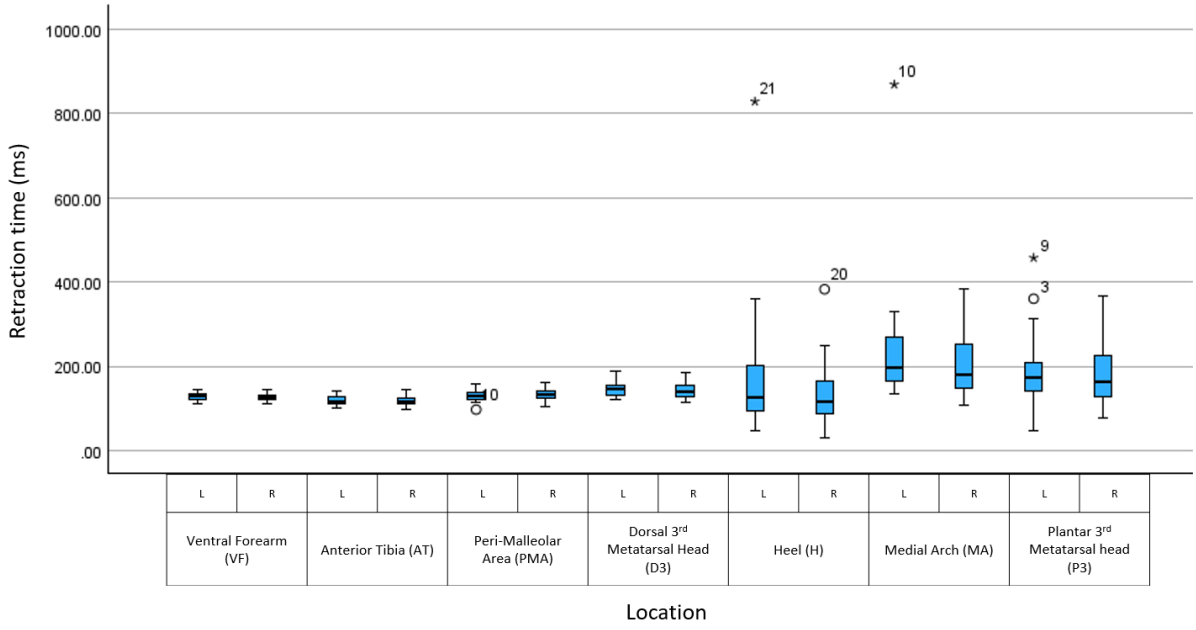


Figure 57. Retraction time of skin at different sites.

6.4.2.3. Roughness and hydration

The roughness of the skin surface and hydration data collected using the MoistureMeter SC™ demonstrated a moderate negative correlation with at the medial arch (p-value left: 0.0005, right: 0.0001), the peri-malleolar area (p-value left: 0.0024, right: 0.0006), the dorsal 3rd metatarsal head (p-value (left side only): 0.0027), and a moderate-strong negative correlation on the plantar 3rd metatarsal head (p-value left: <0.000, right: 0.002).

Data obtained using the MoistureMeter D® probes also showed a statistically significant moderate-strong negative correlation with skin roughness across several skin sites: the medial arch (0.5 mm probe p-value left: 0.0022, right: 0.0006), the heel (0.5 mm probe p-value left: 0.0003, right: 0.0004; 1.5 mm probe p-value (left only): 0.0005; 1.5 mm probe (left only): 0.0014) and the plantar 3rd metatarsal head (0.5 mm probe p-value left: <0.000, right: 0.002; 1.5 and 2.5 mm probe left and right all p-values <0.000).

No significant correlation was demonstrated between the roughness data and data collected using the Corneometer® CM825 at any skin site, or between the roughness data

and any hydration data collected at the anterior tibia, or ventral forearm skin sites (See Figure 58).

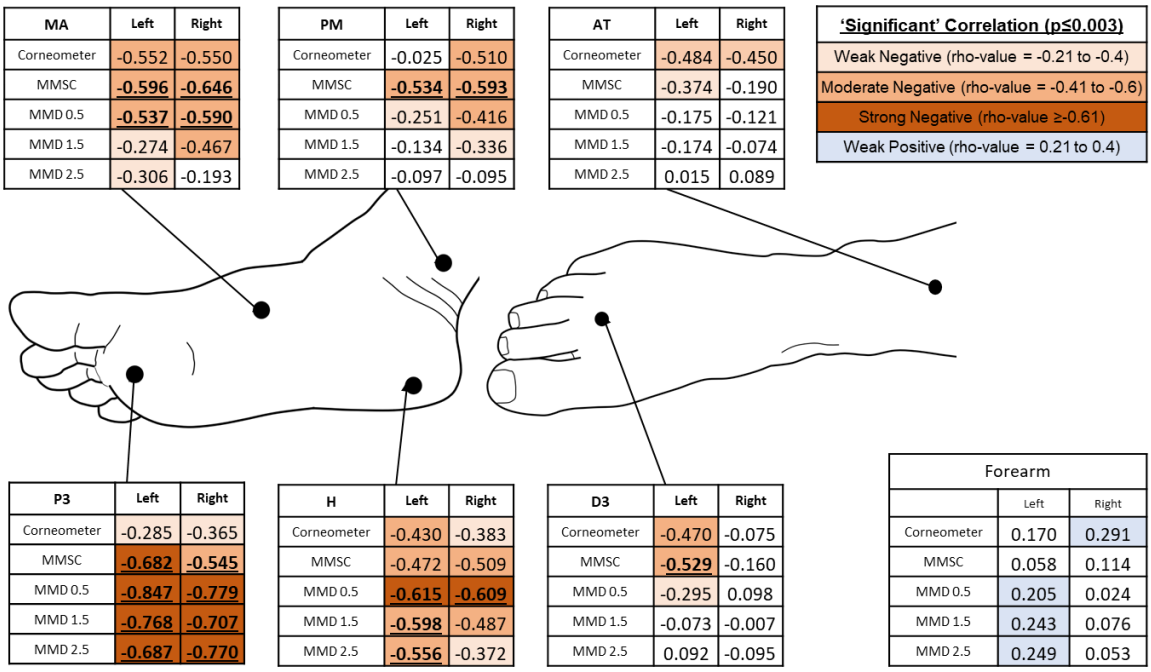


Figure 58. Results of a Spearman's rank-order correlation (rho-value) for skin hydration and roughness.

A negative correlation is anticipated on the plantar skin – low hydration values are associated with xerosis, generating a characteristically flaky skin surface, represented by a high contrast value when observed using the Visioscan® VC98.

6.4.2.4. Participant perception of foot skin features and hydration

6.4.2.4.1. Perception of foot skin dryness

In the FSkHQ, participants were asked whether they thought the skin on their feet was dry. In Figure 59 the hydration data collected from the feet of individuals who answered 'yes' (n=9) or 'no' (n=22) are displayed for each hydration measurement device (Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D®).

Data collected from foot skin (dorsal 3rd metatarsal head, plantar 3rd metatarsal head, medial arch, and heel) on the right side of the body were used for analysis. All datasets, apart from the MoistureMeter SC™ data, were normally distributed (tested using the Shapiro-Wilks (test $p>0.05$) and supported by reviewing Q-Q plots, central tendency, and histograms), as such, a t-test was used to investigate differences between groups for

Corneometer® CM825 data and all MoistureMeter D® data (0.5 mm, 1.5 mm and 2.5 mm probes) and the Spearman's rank order correlation was applied to MoistureMeter SC™ data.

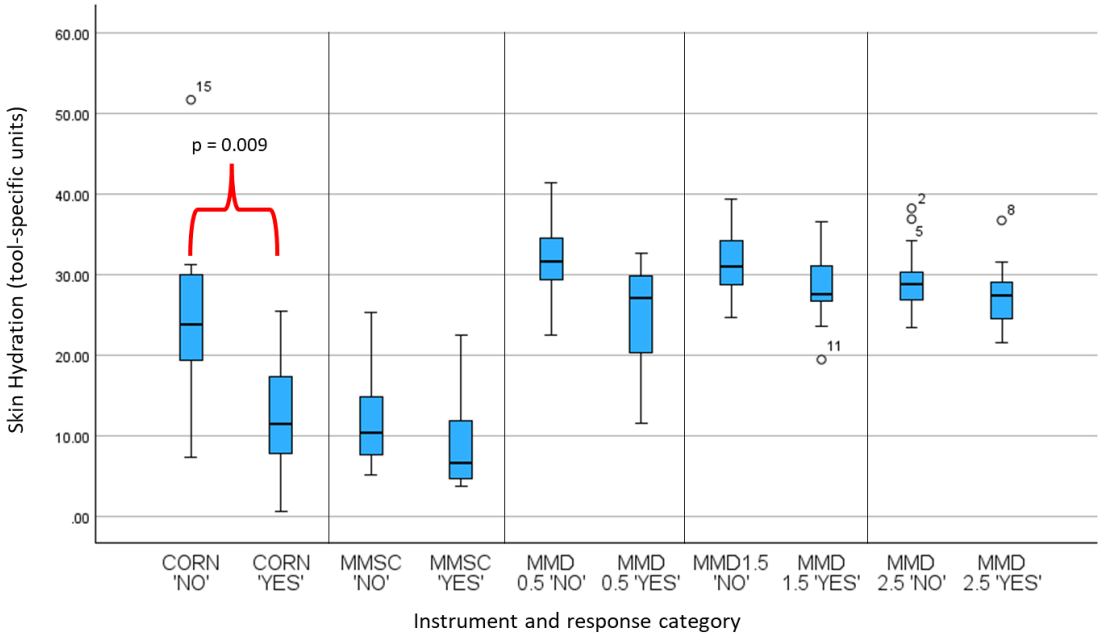


Figure 59. Skin hydration for participants who perceive their foot skin to be dry 'Yes' or not dry 'No', as measured using the Corneometer® CM825 (CORN), MoistureMeter SC™ (MMSC), MoistureMeter D® probes 0.5 mm, 1.5 mm and 2.5 mm (MMD 0.5, MMD 1.5, 2.5).

Although for each device, the participants who answered, 'yes' have visibly lower hydration values than participants that answered 'no', only data collected using the Corneometer® CM825 demonstrate a statistically significant difference (p-value: 0.009) between these two groups.

6.4.2.4.1. Skin hydration and composite score for scaliness, cracking, roughness and hardness (FSkHQ) from FSkHQ

The hydration data collected using each device has been examined for a relationship with the FSkHQ skin score. This comparison was conducted for each foot-skin site individually, and all sites combined. To prevent the confounding effect of individual's data appearing twice within analysis (Menz, 2004), only data from the one foot has been used for data analysis and presented below (the right foot). FSkHQ scores between the left and right foot

were the same for 96.4% of skin site and characteristic-specific scores (i.e, out of 496 only 18 scores were different for the left and right foot).

Multiple datasets used for this analysis were found to be non-parametric in distribution (tested via the Shapiro-Wilks test and review of Q-Q plots, measures of central tendency, and histograms) therefore Spearman's rank order correlation was applied to these data. The results of this analysis are displayed in Figure 60.

The Bonferroni correction was applied to reduce the risk of a type 1 error arising as a result of the multiple comparisons undertaken within this analysis, generating a threshold p-value of ≤0.007 for statistical significance.

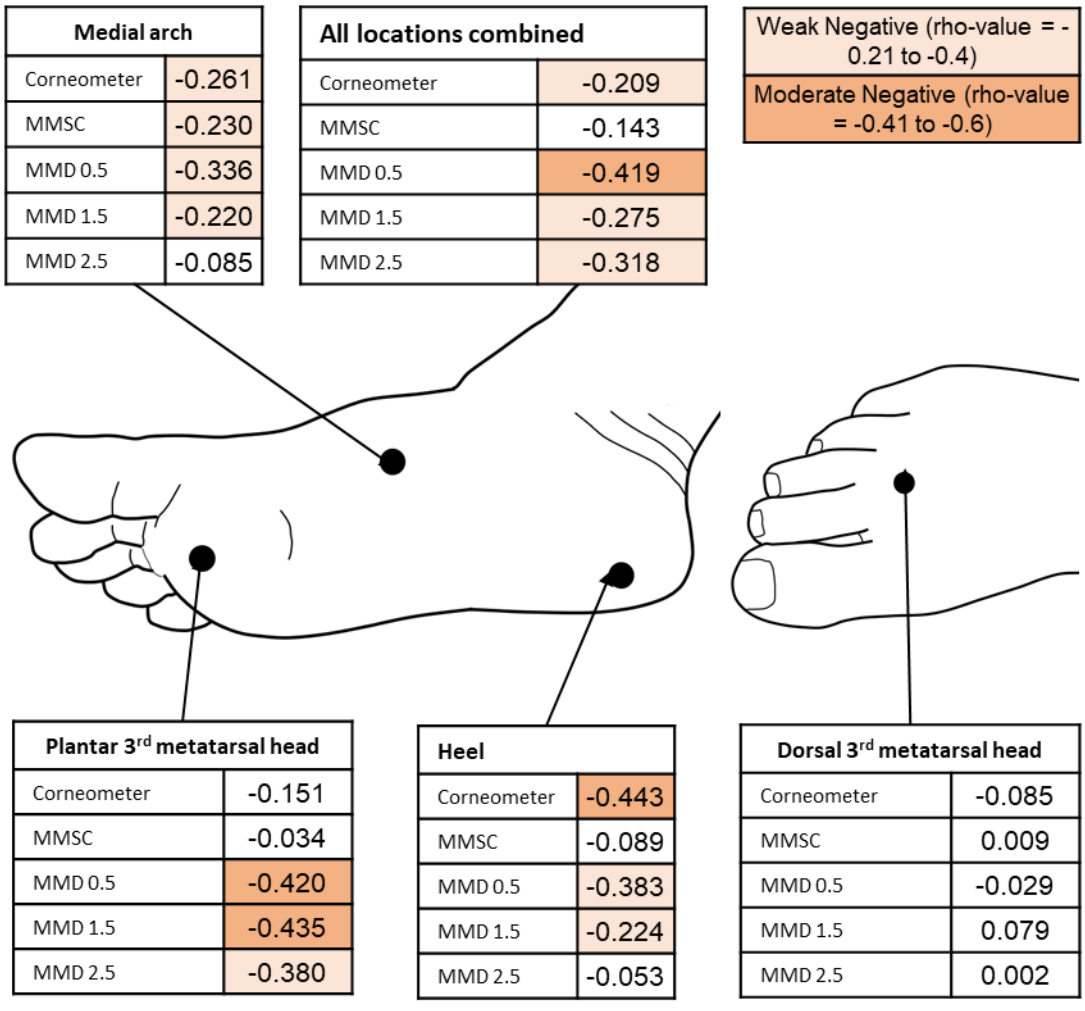


Figure 60. Results of a Spearman's rank-order correlation (rho-value) for skin hydration and FSkHQ scores.

No statistically significant (p≤0.003) correlations were identified between the composite FSkHQ scores and data collected using any hydration measurement device.

For all foot locations combined, the Corneometer® CM825 and the two deepest measurement probes of the MoistureMeter D® (1.5 cm and 2.5 cm) demonstrate a weak negative correlation with FSkHQ scores and the MoistureMeter D® 0.5 mm has a moderate negative correlation with FSkHQ score however none of these achieve statistical significance. The MoistureMeter SC™ shows no correlation with FSkHQ scores.

The MoistureMeter SC™ also shows no or weak negative correlation with the FSkHQ score from each foot skin location. The MoistureMeter D® probes show a weak-moderate correlation consistently across all plantar measurement sites, whereas the Corneometer® CM825 correlation with FSkHQ scores at individual skin sites varies considerably.

The absence of a correlation between skin hydration and FSkHQ score at the dorsal foot measurement site is not unexpected, as participants overwhelmingly gave a low FSkHQ score for the dorsal site. Out of 31 responses, 25 participants gave a score of '0' for this location (See Figure 61). The descriptors associated with the scoring categories for the FSkHQ are primarily associated with the characteristics of xerotic plantar skin, reflected in the higher FSkHQ scores for plantar skin sites.

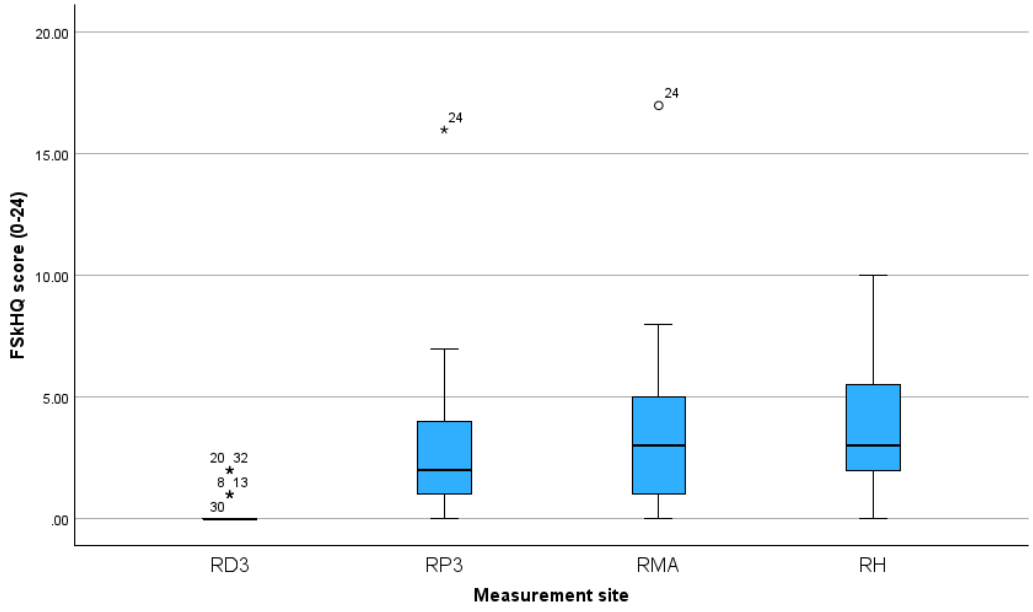


Figure 61. FSkHQ scores for four measurement sites on the foot: Right dorsal 3rd metatarsal head (RD3), right plantar 3rd metatarsal head (RP3), right median arch (RMA) and right plantar heel RH).

6.4.3. Correlation between data collected using commercially available hydration measurement devices.

Of the 70 data sets analysed (hydration data for each location for each device), 20 were non-normally distributed according to the results of the Shapiro-Wilks test (p values <0.05). In most instances, Q-Q plots, measures of central tendencies, and histograms were reviewed and found to support the results of the Shapiro-wilks test. For this reason, non-parametric statistical methods were employed for this data analysis.

The Bonferroni correction was applied to reduce the risk of a type 1 error arising as a result of the multiple comparisons undertaken within this analysis, generating a threshold p-value of ≤0.003 for statistical significance.

6.4.3.1. The Corneometer® CM825

Data collected using the Corneometer® CM825 has a strong, positive, and statistically significant correlation with data obtained using the MoistureMeter SC™ at most locations tested (See Table 43). The correlation between the Corneometer® CM825 data and MoistureMeter D® 0.5 cm depth probe ranges from weak to very strong and is highly variable between skin sites, as the measurement depth of the probe increases (1.5 cm and 2.5 cm) the strength of the correlations decreases and becomes less consistent across locations.

Table 43. Spearman's rank order correlation for Corneometer® CM825 data with the MoistureMeter SC™ and MoistureMeter D® (n=20)[††].

		LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
MMSC	rho-value	.901**	.799**	.719**	.671**	.675**	.860**	.770**	.803**	.812**	.734**	.852**	.869**	.723**	.715**
	p-value	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	rho-value	0.528	0.47	0.362	0.418	.821**	.796**	0.426	.642**	.739**	0.59	.776**	.689**	0.361	0.45
	p-value	0.017	0.035	0.117	0.067	0.000	0.000	0.061	0.002	0.000	0.006	0.000	0.001	0.117	0.047
1.5	rho-value	0.307	0.47	0.219	0.269	.816**	0.306	0.45	0.380	.647**	0.235	.633**	0.57	0.364	0.46
	p-value	0.189	0.038	0.354	0.251	0.000	0.189	0.047	0.098	0.002	0.318	0.003	0.009	0.115	0.040
2.5	rho-value	0.426	0.422	0.168	0.274	0.53	0.183	0.275	0.207	.643**	0.099	0.55	0.439	0.293	0.46
	p-value	0.061	0.064	0.480	0.243	0.016	0.440	0.240	0.382	0.002	0.677	0.012	0.053	0.210	0.040

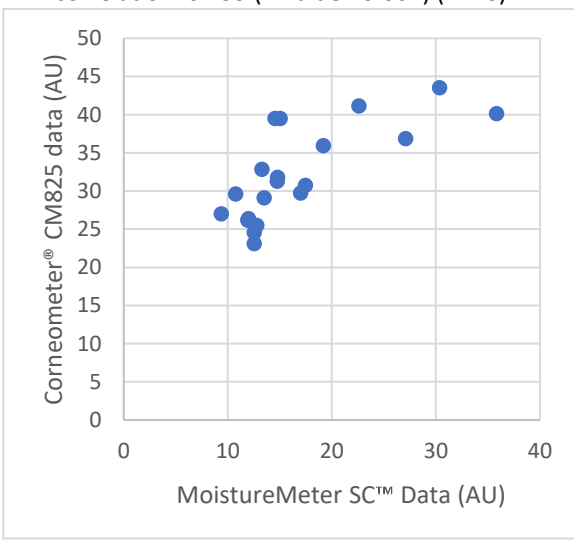
A strong positive correlation was anticipated between the Corneometer® CM825 and MoistureMeter SC™ data as they both collect data from within the SC. Due to the increased

[††] Indication of relationship strength: red = very strong relationship, orange = strong relationship, yellow = moderate relationship. Double asterisk indicates significance (p-value <0.003).

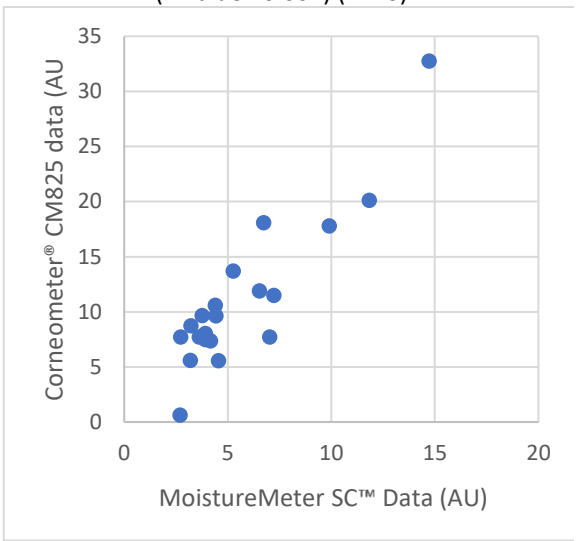
thickness of the SC on the plantar skin, data collected using the MoistureMeter D® 0.5 cm probe was also anticipated to correlate more closely with these superficial measures of SC hydration at the plantar foot locations. This is not the case, however.

Scatter-graphs are provided for several of these correlations in Figure 62 for later comparison with equivalent published data (see Section 6.5.3.).

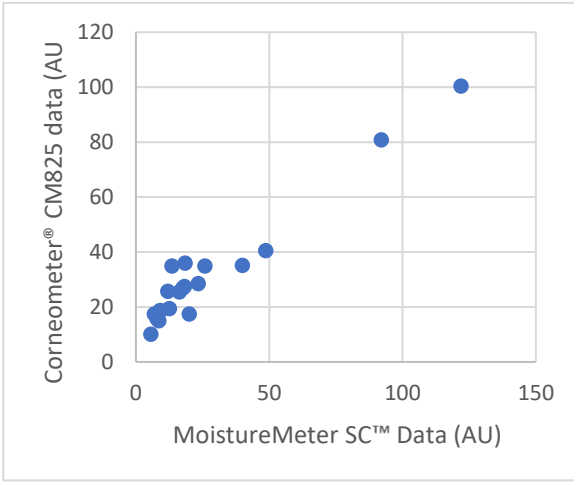
a. Ventral forearm – Spearman's rank order correlation: 0.799 (P-value <0.001) (n=20)



b. Heel – Spearman's rank order correlation: 0.734 (P-value <0.001) (n=20)



c. Medial arch – Spearman's rank order correlation: 0.869 (P-value <0.001) (n=20)



d. Plantar 3rd metatarsal head – Spearman's rank order correlation: 0.715 (P-value <0.001) (n=20)

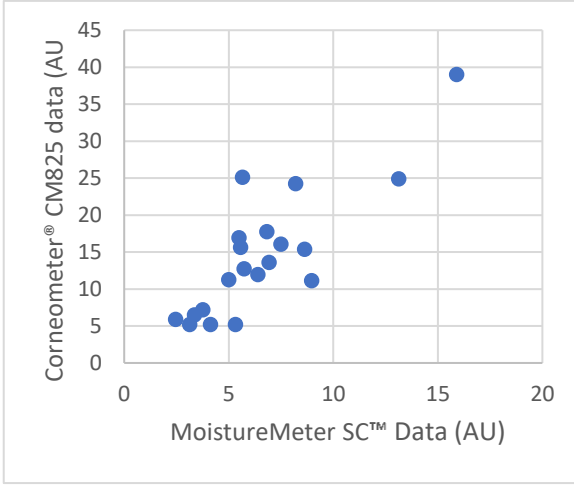


Figure 62. Results of a Spearman's rank-order correlation for data collected using the Corneometer® CM825 and MoistureMeter SC™ across several skin sites.

6.4.3.2. The MoistureMeter SC™

Data collected using the MoistureMeter SC™ demonstrates a moderate–strong, statistically significant, positive correlation with data collected using the MoistureMeter D® 0.5 cm measurement depth probe across all locations (See Table 44). The strength of these positive correlations reduces across all locations with the increased measurement depth (excluding the right heel and left ventral forearm), however the correlation remains strong at all sites for 1.5 cm probe data (excluding the left heel which is very strong), and moderate at all but 6 sites for the 2.5 cm probe (left anterior tibia, right heel and right medial arch display a moderate positive correlation and right peri-malleolar area, left and right dorsal third metatarsal head have a weak positive correlation). No distinct pattern in correlation strength is demonstrated between plantar and non-plantar skin sites.

Table 44. Spearman's rank order correlation for MoistureMeter SC™ data with the MoistureMeter D® (n=32).[‡‡]

		LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
0.5	rho-value	.590**	.708**	.574**	.687**	.709**	.678**	.626**	.583**	.891**	.593**	.714**	.739**	.754**	.680**
	p-value	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.5	rho-value	0.47	.612**	0.46	.622**	.644**	.528**	0.43	0.51	.826**	.601**	0.5	.614**	.581**	.614**
	p-value	0.007	0.000	0.008	0.000	0.000	0.002	0.014	0.003	0.000	0.000	0.004	0.000	0.000	0.000
2.5	rho-value	.531**	.569**	0.37	.557**	0.47	0.242	0.212	0.255	.654**	0.39	0.43	0.313	.508**	.553**
	p-value	0.002	0.001	0.037	0.001	0.006	0.183	0.243	0.159	0.000	0.028	0.015	0.081	0.003	0.001

6.4.3.3. The MoistureMeter D®

Data collected using the 0.5 cm probe of the MoistureMeter D® correlates very strongly with data collected using the 1.5 cm depth probe from most skin sites measured within this study (excluding the left and right dorsal 3rd metatarsal head sites and the right heel) (See Table 45). This is true, to a lesser degree, for the 2.5 cm depth probe which demonstrated a strong-very strong correlation with all measurement sites.

[‡‡] Indication of relationship strength: red = very strong relationship, orange = strong relationship, yellow = moderate relationship. Double asterisk indicates significance (p-value <0.003).

Table 45. Spearman's rank order correlation for MoistureMeter D® 0.5 mm depth probe data (n=32) with the MoistureMeter D® 1.5 mm and 2.5 mm depth probe.[§§]

		LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
1.5	rho-value	.943**	.921**	.791**	.887**	.904**	.742**	.660**	.681**	.898**	.633**	.856**	.901**	.909**	.959**
	p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.5	rho-value	.956**	.902**	.770**	.687**	.712**	0.47	.557**	.519**	.752**	.711**	.803**	.642**	.862**	.932**
	p-value	0.000	0.000	0.000	0.000	0.000	0.006	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000

Data collected using the MoistureMeter D® 1.5 cm probe shows a very strong positive correlation with the data collected using the 2.5 cm depth probe at all locations apart from the left anterior tibia, which shows a strong relationship (See Table 46).

Table 46. Spearmans rank order correlation for MoistureMeter D® 1.5 mm depth probe data (n=32) with the MoistureMeter D® 2.5 mm depth probe.

		LVF	RVF	LAT	RAT	LPM	RPM	LD3	RD3	LH	RH	LMA	RMA	LP3	RP3
2.5	rho-value	.962**	.941**	.626**	.766**	.787**	.763**	.832**	.701**	.771**	.719**	.921**	.799**	.938**	.922**
	p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Despite no clear pattern emerging between the data captured using commercially available hydration measurement devices at plantar and non-plantar skin, hydration data collected using probes of varied depth (0.5 cm, 1.5 cm, and 2.5 cm) show a difference in the water content of tissues across their depth at the plantar and non-plantar skin sites (See Figure 63).

6.4.3.3.1. Hydration gradient as captured using the MoistureMeter D® probes

On most of the non-plantar skin sites measured within this study, a reduction in tissue water is observed as the measurement depth of the MoistureMeter D® probe used is increased (Figure 63). On the heel sites and the plantar 3rd metatarsal head a reverse pattern is found, where the tissue water increases with increased measurement depth. The data obtained from the medial arch doesn't appear to increase or decrease with measurement depth change uniformly.

[§§] Indication of relationship strength: red = very strong relationship, orange = strong relationship. Double asterisk indicates significance (p-value <0.003).

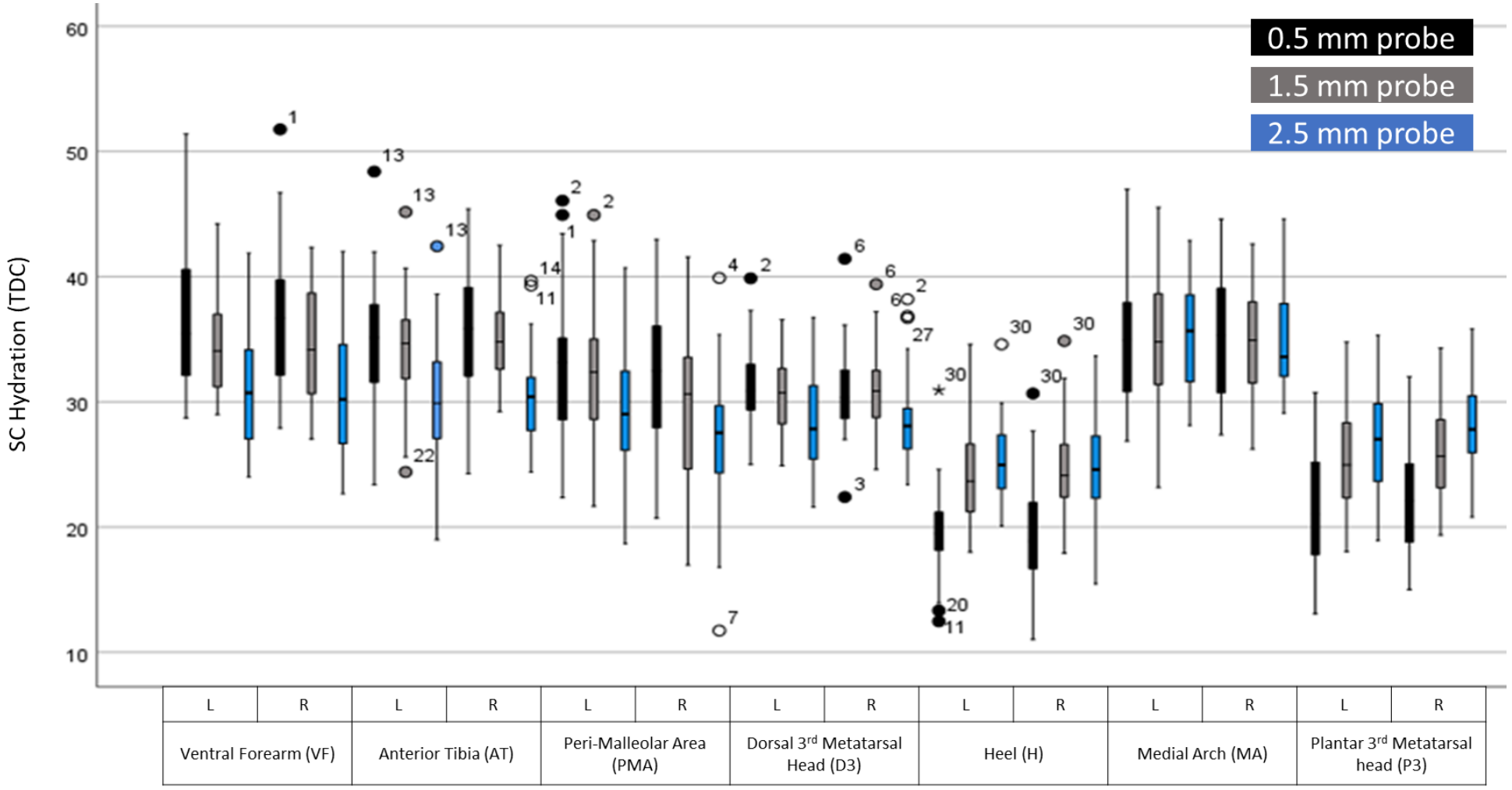


Figure 63. MoistureMeter D® data collected using 0.5 mm (0.5), 1.5 mm (1.5), and 2.5 mm (2.5) probes.

6.4.4. Summary of study outcomes

Through the conduction of this study, several conclusions can be made about the use of the Corneometer® CM825, MoistureMeter SC™ and the MoistureMeter D® on the foot:

- Skin hardness correlates more closely with the hydration data collected using the Corneometer® CM825 and MoistureMeter SC™ across the whole landscape of the foot. However on the plantar 3rd metatarsal head and heel skin the MoistureMeter D® generates data that correlates most strongly with hardness.
- The MoistureMeter SC™ collects data that most correlates most strongly and consistently with the roughness of the skin across the whole landscape of the foot, closely followed by the Corneometer® CM825. Data collected using the MoistureMeter D® correlates the strongest with skin roughness on the plantar 3rd metatarsal head and heel skin.
- The data collected using the Corneometer® CM825 is most closely related to the participants perception of their foot skin dryness.
- Finally, though this study, the correlation between different commercially available hydration measurement devices was investigated: The Corneometer® CM825 and MoistureMeter SC™ demonstrated a strong, positive, and statistically significant correlation across all skin sites. No pattern was evident between plantar and non-plantar correlations between different measures of skin hydration at the skin.

In the section below, the data collected within this study are considered in relation to equivalent published data.

6.5. Discussion

6.5.1. The Corneometer® CM825

Each published instance in which the hydration of the foot skin has been measured (Hashmi et al., 2016; Kirkham et al., 2014) has been undertaken at the University of Salford. The Corneometer® CM825 device used in the studies conducted by Kirkham et al (2014) and Hashmi et al (2016), was also used in this study. Any variation in the data collected across these works, therefore, cannot be attributed to inconsistencies in the equipment used.

Kirkham et al (2014) reported data collected from the medial-peri-malleolar skin using the Corneometer® CM825 (See Figure 10). These data display a very similar median and IQR to the data collected within this study (See Table 47).

Table 47. Medial peri-malleolar skin hydration measured using the Corneometer® CM825, as reported by Kirkham et al (2014) and collected within the current study.

	Kirkham et al (2014) (AU)		Current study (n=32) (AU)	
	Group 1 (n=10)	Group 2 (n=10)	Left	Right
Median	24.09	24.47	21.92	21.65
IQR	17.54	17.26	16.27	14.40

Hashmi et al (2016) also collected data using the Corneometer® CM825 from the plantar skin at the base of the 5th metatarsal. Although data were not collected from this location within this study, plantar measures were collected at two other plantar sites: the 3rd metatarsal head (P3) and the heel (H).

The hydration at the 5th metatarsal base collected by Hashmi et al (2016), align well with the plantar measures collected within this study. However, they are uniformly higher than those collected at the heel and lower than those collected at the 3rd metatarsal head (See Table 48).

Table 48. Plantar skin hydration data collected using the Corneometer® CM825 by Hashmi et al (2016) and the current study.

	Hashmi et al (2016) (AU)			Current study (n=32) (AU)			
	5th metatarsal			3rd metatarsal head		Heel	
	Group 1 (n=21)	Group 2 (n=20)	Group 3 (n=20)	Left	Right	Left	Right
Median	8.67	9.05	10.35	14.08	13.17	7.72	9.18
IQR	7.98	8.10	10.02	10.93	10.11	6.19	4.68

As there is no equivalent data available from the 5th metatarsal base from this study, it is not possible to establish whether these differences are the result of natural variation in the SC

hydration across the plantar surface, or a result of study design: In the study by Hashmi et al (2016) all participants had a callus on the plantar foot. It is not known whether the presence of callus on the foot is associated with lower skin-hydration in general on the foot. In future, it would be worthwhile to collect further data on equivalent skin sites between people with and without callus to investigate this.

6.5.2. The MoistureMeter SC™ and MoistureMeter D®

Mayrovitz et al (2013) published data collected using the MoistureMeter SC™ and MoistureMeter D® from various skin sites across the body. Data obtained using the MoistureMeter SC™ within this study is lower than the data collected by Mayrovitz et al (2013), particularly on the plantar skin. Mayrovitz et al (2013) reported a mean SC hydration of 30.1 AU on the plantar hallux, the only plantar skin site they measured. This was higher than the mean hydration on the dorsal foot (1-2 toe: 15.8 AU, 4-5 toe: 13.6 AU) and the peri-malleolar area (10 AU), but lower than the ventral forearm (39.2 AU). However, the data obtained from the plantar locations within this study were consistently lower (except for the medial arch) than all other locations (See Table 49).

Table 49. Data obtained using the MoistureMeter SC™ by Mayrovitz et al (2012) and from the current study.

	N	Ventral forearm (AU)	Anterior Tibia (AU)	Peri-Malleolar (AU)	*D3* – Dorsal 3rd metatarsal head *1-2 toe*- Dorsal skin between 1st and 2nd, *4-5 toe* – 4th and 5th metatarsals (AU)	*P3* – Plantar 3rd metatarsal head *H* – Heel *PH* – Plantar hallux (AU)
Mayrovitz et al (2012): Mean ± SD	32	39.2 ± 15.10	24.8 ± 13.1	10.0 ± 8.9	*1-2 toe*: 15.8 ± 14.4 *4-5 toe*:13.6 ± 11.6	*PH*: 30.1 ± 17.8
Our study: Median (IQR) left	32	15.10 (4.96)	11.02 (7.93)	8.97 (7.68)	*D3*: 10.13 (11.87)	*P3*: 5.42 (3.53) *H*: 4.78 (2.98)
Our study: Median (IQR) right	32	14.22 (5.13)	9.83 (7.24)	7.98 (7.03)	*D3*: 10.13 (7.23)	*P3*: 5.70 (3.17) *H*: 4.50 (2.92)

It is possible that this variation could be attributed to physiological differences in the SC hydration or structure between the plantar hallux, forefoot, and heel. However, the limited data available on the thickness of the plantar epidermis at these locations indicates that (albeit reported with great variability) epidermal thickness does not differ greatly between plantar sites (See Table 9).

The differences observed could also be attributed to an aspect of study design. Mayrovitz et al (2013) requested that participants abstain from using protects on their skin on the day of data-collection. However within the current study, product use was banned in the 7 days prior to data-collection. It is possible that the data obtained by Mayrovitz et al (2013) could be artificially raised due to application of skincare products to the feet. If this was the case, however, a similarly high SC hydration would be anticipated from the measured obtained using the MoistureMeter D® which is not the case.

The data from this study collected using the MoistureMeter D® 0.5 cm probe aligns closely with that collected by Mayrovitz et al (2013) on the forearm, anterior tibia, peri-malleolar area, and dorsal forefoot (See Table 50). The SC hydration data reported from the plantar hallux (mean: 31.6 AU) is higher than that of the plantar forefoot (median LP3: 21.70, RP3: 22.15) or heel (LH: 19.60, RH: 19.05). However, to a much smaller degree than the difference between the MoistureMeter SC™ data collected within this study and by Mayrovitz et al (2013). This could be attributed to the increased measurement depth of the MoistureMeter D reducing the readiness by which data are influenced by factors such as application of skincare products.

Table 50. Data obtained using the MoistureMeter D® by Mayrovitz et al (2012) and from the current study.

	N	Ventral forearm (TDC)	Anterior Tibia (TDC)	Peri-Malleolar (TDC)	*D3* – Dorsal 3rd metatarsal head *1-2 toe*- Dorsal skin between 1st and 2nd, *4-5 toe* – 4th and 5th metatarsals (AU)	*P3* – Plantar 3rd metatarsal head *H* – Heel *PH* – Plantar hallux (AU)
Mayrovitz et al (2012): Mean ± SD (TDC)	32	29.5 ± 4.0	34.7 ± 4.6	27.1 ± 4.6	*1-2 toe*:27.9 ± 4.1 *4-5 toe*: 27.7 ± 3.6	*PH*: 31.6 ± 5.0
Our study: Median (IQR) left (TDC)	32	35.63 (8.63)	35.04 (6.20)	33.33 (6.70)	*D3*: 30.97 (3.61)	*P3*: 21.70 (7.33) *H*: 19.60 (3.63)
Our study: Median (IQR) right (TDC)	32	36.68 (7.83)	35.33 (6.70)	32.48 (8.28)	*D3*: 30.57 (4.09)	*P3*: 22.15 (6.36) *H*: 19.05 (4.78)

6.5.3. Discussion of outliers

In Figures 49, 50 and 51, several outliers are visible within each of the datasets representing the hydration data collected using the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D®. As per the data analysis protocol (See Section 6.3.6.1.), these outliers were included within statistical analysis due to them being consistent across each instance of tool use (i.e., each of the three Corneometer® CM825 measures is of a similarly high value). This does not, however, preclude the demographics of these individuals being explored to identify whether these high values are reflective of a particular patient characteristic that may be related.

Table 51. Demographic of outlier participants and all participants.

	All participants	P11	P28	P30	P32
Age	27.9 (4.8) (mean (SD))	28	27	31	21
Sex	52% female	F	F	F	M
Time since feet were washed (hours)	All >6	23	6	15	6
Primary footwear	31/32 Close-toed shoes with socks or hosiery	Close-toed shoes with socks or hosiery			

The outlier data visible in these figures can be primarily attributed to four participants: P11, P28, P30, and P32 (labelled as P20 in Figure 49 due to the lower participant numbers for the Corneometer® CM825 data). As a cohort, these individuals do not have any uniform characteristics that would explain their skin hydration being higher than the rest of the participant group (See Table 51). They are mixed sex (1 male, 3 female), have not washed their feet in the proceeding 6 hours (time since washed spans from 6-23 hours), display typical footwear and hosiery patterns for the wider cohort (primarily wearing closed-toed footwear with hosiery), and are not consistently older or younger than the average participant (outlier participants are 21-31 years of age). From these data, it is not possible to hypothesise why these individuals may have a higher skin hydration than the rest of the participant group. It may only be presumed that these data are a reflection of the high variation in skin hydration. This phenomenon would be more clearly demonstrated in a study with larger participant numbers.

6.5.4. Relationship between skin hardness and hydration

The correlation analysis undertaken as part of this study highlights an interesting phenomenon. On the plantar skin (primarily the heel and plantar 3rd metatarsal head), the skin hydration data demonstrate a weak-strong negative correlation with skin hardness, with several statistically significant relationships identified (See Figure 52). Conversely, at the non-plantar skin sites, few correlations are indicated, none are statistically significant, and these are generally weak and positive.

The inequivalent correlations demonstrated between plantar and non-plantar skin sites is presumed to stem from the thickness and structure of the soft tissues at the measurement locations used in this study. As indicated in section 4.4.1, the SATRA STD 226 Digital Durometer is intended for use on materials of at least 6 mm thickness. The thickness of skin and soft-tissues across the body is variable. At skin sites such as the dorsal 3rd metatarsal head skin hardness data was anticipated to be high due to the thin soft tissues and superficial bony structures (Figure 64) (Kelikian & Sarrafian, 2011). Figure 53 shows, however, that skin sites overlying superficial bone (such as the anterior tibia (overlying the anterior crest) and peri-malleolus (overlying the medial malleolus)) do not produce higher durometer data than areas with thicker soft tissues (such as the heel).

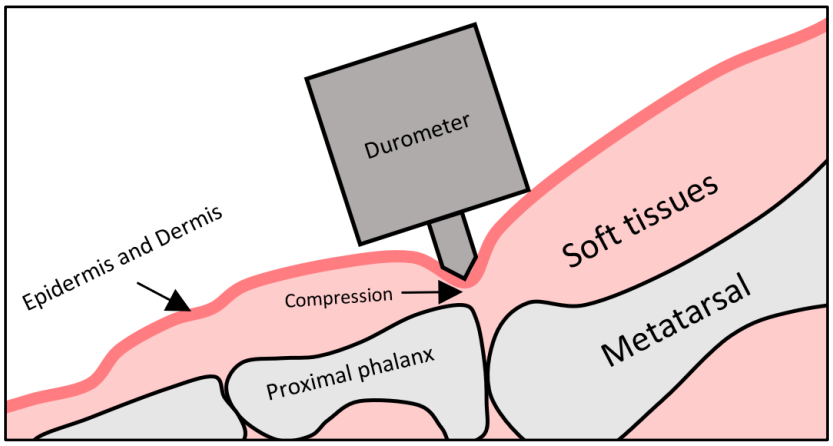


Figure 64. A diagram demonstrating the compression of thin soft-tissue during use of the Durometer on the dorsal 3rd metatarsal measurement site.

The soft tissue of the plantar foot at the measurement sites used within this study are highly likely to be at least 6 mm in depth (due to a thick SC, dermis, and plantar fat layer (See Table 9)), meaning that the data collected using the SATRA STD 226 Digital Durometer are

influenced by the deformation of the soft tissues alone. The plantar epidermis is thick and hard which raises durometer readings (Chatzistergos et al., 2022), generating similar values to non-plantar skin sites with superficial bone.

From this, it is suggested that plantar skin durometer readings are more indicative of actual skin characteristics than non-plantar durometer readings. This is supported by the increased strength of correlation between skin hardness and hydration on plantar skin sites, particularly as this demonstrates a pattern that is anticipated: the less hydrated the skin, the harder the skin.

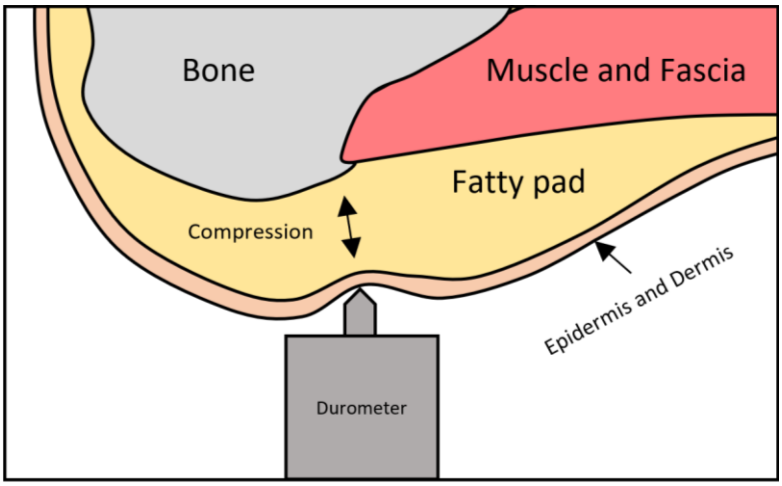


Figure 65. A diagram demonstrating the compression of thick plantar soft tissue during use of the SATRA STD 226 Digital Durometer on the plantar heel measurement site.

Confidence in this finding is undermined, however, by the variability between strength of correlation between the same skin sites on different sides of the body. For example, the durometer data has a statistically significant ($p \leq 0.05$), strong negative correlation with Corneometer® CM825 data on the plantar 3rd metatarsal head site (rho-value =-0.611), but a weak, non-significant negative correlation on the right plantar 3rd metatarsal head site (rho-value =-0.235). When plotted as a scatter graph (See Figure 55), these data sets both have a similar dispersion, however a small number of data-points reduce the strength of the correlation significantly for the right 3rd metatarsal head site. This pattern is reflected across several measurement locations (but primarily the plantar 3rd metatarsal head and the plantar heel site) and in the comparisons between SATRA STD 226 Digital Durometer data and all three hydration measurement tools (including each probe of the MoistureMeter D®). This is demonstrated visually for both of these skin sites in Figure 54 and Figure 55.

Despite the correlation coefficient output by Spearman's rank order test being influenced by a small number of data points at these sites, these data points do not appear to sit outside of the typical dispersion of the data (i.e., they are not visibly outliers in the context of the data) and do not consistently originate from one of two individuals. This would indicate that the resulting correlation coefficients were being reduced in strength by data that were not reflective of natural variance, however this is not the case, therefore the correlations identified within Figure 52 are considered valid.

In future, these data could be used to support the use of MoistureMeter D® to collect hydration data from the plantar skin that is a known reflection of the physical characteristics of the skin. This could also be extended to the Corneometer® CM825 and MoistureMeter SC™, however the correlations they displayed with skin hardness data was less strong and consistent than that displayed by the MoistureMeter D®.

6.5.5. Relationship between skin elasticity and hydration.

In the current study, only a single statistically significant correlation was identified between the retraction speed of skin and skin hydration; a strong negative correlation between retraction and hydration measured using the MoistureMeter D® 0.5 mm probe on the right dorsal 3rd metatarsal head (r-value: -0.544 (p-value: 0.002). Weak-moderate negative correlations were also found between skin retraction speed and hydration data at the medial arch, dorsal 3rd metatarsal head and forearm skin sites, and weak-moderate positive correlations were found at the plantar skin for the MoistureMeter D® at the plantar 3rd metatarsal head and the heel, albeit these were inconsistent and non-significant.

The retraction speed of the skin is anticipated to be decreased in skin that is elastic. Elastic skin is associated with increased hydration (Hashmi, Nester, et al., 2015). A negative correlation between skin hydration and retraction time is therefore anticipated (i.e., as skin hydration increases, retraction speed reduces).

The positive correlation between skin hydration and retraction speed (using the MoistureMeter D®) found at the plantar heel and 3rd metatarsal head is unexpected and inconsistent between the left and right sides of the body so the meaning that can be taken from these findings is limited. The only instance in which a similar comparison has been

untaken on the foot was completed by Hashmi et al in 2015. Data collected using the Cutometer® 580 MPA was correlated with Corneometer® CM825 data using the Spearman's rank order correlation test, primarily on hyperkeratotic lesions, but also on the healthy plantar skin at the 5th metatarsal base and the plantar metatarsal area. Hashmi et al (2015) use the Cutometer® 580 MPA to measure the skins response to the application of negative pressure. As within this work, the research team did not measure skin thickness they also could not directly calculate skin stiffness. Instead, the elasticity of the skin was quantified using the following equation:

$$\text{Elasticity} = \frac{deformation\ of\ the\ tissue\ (mm)}{force\ applied\ (mbar)}$$

The 5th metatarsal base showed a statistically significant weak positive correlation between skin hydration and elasticity (See Table 52). No correlation was found for the plantar metatarsal area. Stronger correlations were found at hyperkeratotic lesions, such as callus and fissures (r-values of 0.56 and 0.65 respectively).

Table 52. Spearman's rank order correlation for data collected using the Corneometer® CM825 and Cutometer from normal skin by Hashmi et al (2015) (n=93).

Skin site	5th Metatarsal base	Plantar metatarsal area
r-value	0.25	0.13
p-value	0.01	None provided – not significant

This positive correlation between skin hydration and the elasticity output of the work by Hashmi et al (2015) is anticipated, as in this instance elasticity is expressly being calculated, whereas within the current study retraction was used (which negatively correlates with elasticity) (Langton et al., 2017). This does support the findings of this study, however in future it would be beneficial to re-analyse the data collected using the Dermalab Elasticity probe using the same method employed by Hashmi et al (2015) to generate semi-equivalent data.

These data would not be entirely equivalent due to the different devices used to assess skin elasticity: The Cutometer® 580 MPA used by Hashmi et al (2015) has an aperture size of 2 mm Ø, this instrument only collects data from the epidermis, papillary dermis, and only partly the deeper layers of the dermis and subcutaneous tissues (Cua et al., 1990). The Dermalab

Elasticity probe, however, has an aperture of 10 mm Ø. With a larger aperture size, deeper tissues are subject to suction force. Data collected using this device are more likely to be influenced by the subcutaneous tissues than data collected using the Cutometer® 580 MPA.

Plantar soft tissues demonstrate high viscoelastic properties, meaning that when they are loaded, they initially respond in an elastic manner (i.e., a linear relationship between force and displacement), however they continue to deform over time (Parker & Hashmi, 2021). Although all skin displays viscoelastic properties, this is very pronounced on the foot as a protective mechanism for the recurrent high forces the plantar skin is exposed to during walking (De Clercq et al., 1994; Gefen, 2003; Gefen et al., 2001).

When considered in relation to the action of the Dermalab Elasticity probe, it is possible that this biomechanical tissue characteristic is responsible for the increased retraction time displayed at the plantar tissues: i.e., when the suction is ceased, the skin retracts in a linear fashion, but slows due to its viscoelastic properties prior to the skin returning to 33% of the depth of its original deformation (as per the retraction output). In future, if only the biomechanical characteristics of the uppermost soft tissues are intended to be observed, a device with a smaller aperture would be more suitable for use on the foot.

6.5.6. Relationship between skin roughness and hydration

Hashmi et al (2015) collected data using the same devices used in our study (Corneometer® CM825 and Visioscan® VC98 (contrast parameter)) to collect data from the foot. A correlation analysis (Spearmans rank order) was applied to these data, which generated findings markedly different from the current research (See Table 53).

Table 53. Spearman's rank order correlation for Corneometer® CM825 data and skin roughness from Hashmi et al (2015) and the current study. No statistically significant (p-value≥0.05) correlations identified.

	Hashmi et al (2015) (n=93)		Current study (n=32)			
Skin site	5th Metatarsal base	Plantar metatarsal area	Plantar 3rd metatarsal head		Plantar heel	
rho-value	0.00	0.09	Left	Right	Left	Right
			-0.285	-0.365	-0.430	-0.383

Although not statistically significant, the correlations identified on the plantar foot locations within the current data are consistently negative and range from weak to moderate. However, at the plantar locations tested by Hashmi et al (2015), no such correlation is

identified. Hashmi et al (2015) had a larger number of participants (n=93), meaning their investigation was not underpowered, testing protocols were very similar between these two studies, and although not entirely equivalent, testing locations were very similar.

One possible source of variation is the population used within these studies: Hashmi et al (2015) recruited participants with hyperkeratosis in order to examine the characteristics of hyperkeratotic lesions as well as healthy skin areas. Whereas within this study, only individuals with healthy skin were recruited. This has resulted in Hashmi et al (2015) having a much older population (mean:47.8 (range: 20-78) years) than was involved in this study (mean: 27.88 (range 21-40) years) and also generating different hydration and texture data.

At the plantar metatarsal area, Hashmi et al (2015) report a mean hydration value of around 8 AU (CI: approximately 6, 11) (values not provided), whereas the mean hydration for the current study at the plantar metatarsal area is 13.2 AU (IQR: 10.9) (left) and 14.1 AU (IQR 10.1) (right). Similarly, Hashmi et al (2015) report higher levels of contrast at the plantar metatarsal area of around 2.5 AU (mean) (CI: approximately 2, 2.6) than within this study: 0.73 AU (IQR: 0.75) (left) and 0.61 AU (IQR 0.6) (right).

It is possible that the relationship between skin hydration and contrast is less evident in the dryer, rougher skin tested by Hashmi et al (2015). In future, repeating this kind of study with individuals with foot skin with varied levels of hydration would facilitate exploration of this phenomenon further: i.e., is there a correlation between skin hydration and elasticity for individuals with no xerosis, mild xerosis, evident hyperkeratosis, for example.

Another area for consideration from this aspect of the study is the varying strength of correlations between skin sites from the same different hydration measurement devices. This is proposed to be due to tissue thickness. At the sites where plantar epidermis is reported to be at its thickest – the heel and plantar forefoot (See Table 9) – the devices with the deepest hydration measurement depth (MoistureMeter D® probes), or no set measurement depth (MoistureMeter SC™) have the highest correlation coefficient at these locations. Conversely, at locations where the epidermis is thinner – such as the medial arch and non-plantar skin sites (See Table 9) – the Corneometer® CM825 (which has a shallow measurement depth) demonstrates consistent weak-moderate negative correlations.

This finding can be used to guide the application of these devices in future. Where skin hydration that is known to be associated with skin roughness needs to be quantified (for example, in the case where an emollient for reducing foot skin roughness is being assessed), the MoistureMeter SC™ or Corneometer® CM825 would be best employed when the whole foot surface is being examined (due to their consistent correlations across all sites). Alternately, if only the plantar regions are being examined, the MoistureMeter D® 0.5 cm probe would be preferable for use as this demonstrates the strongest correlations at these sites.

6.5.7. Corneometer® CM825 and MoistureMeter SC™ Correlation

Alanen et al (2004) used the MoistureMeter SC™ and the Corneometer® CM825 on skin sites across the body. The authors found a strong correlation (R=0.75) between data collected from the ventral forearm by both devices, however, the range of the hydration values collected by the MoistureMeter SC™ was approximately 1.5 times that of the Corneometer® CM825 (MoistureMeter SC™ range: ≈15 to 75 AU; Corneometer® CM825 range: ≈65 to 105 AU).

As described in section 6.4.3., correlation tests demonstrated that Corneometer® CM825 and MoistureMeter SC™ data have a strong or very strong positive correlation (See Table 43) irrespective of measurement site (plantar or non-plantar). When plotted in a scatter graph, these data demonstrate a similar correlation to the data collected by Alanen et al (2004), however these do not reflect the same difference in data-ranges (See Figure 62).

6.5.8. MoistureMeter SC™ and MoistureMeter D® Correlation

Mayrovitz et al (2013) undertook a correlation analysis between data collected simultaneously using the MoistureMeter SC™ and MoistureMeter D® (0.5 mm,1.5 mm, and 2.5 mm probes). This has been presented as a correlation coefficient for all skin sites combined for MoistureMeter SC™ and each MoistureMeter D® probe separately (i.e. MoistureMeter SC™V MoistureMeter D® 0.5 mm, MoistureMeter SC™ V MoistureMeter D® 1.5 mm, MoistureMeter SC™ V MoistureMeter D® 2.5 mm) and as a correlation coefficient for each measurement site for only the MoistureMeter SC™ and 1.5 mm MoistureMeter D®

probe (i.e. MoistureMeter SC™ V 1.5 mm for ventral forearm data only, MoistureMeter SC™ V 1.5 mm for anterior gaiter data only etc.).

Data from only the right side of the body has been used for analysis to prevent the confounding effect of having duplicate data from one individual within the data (Menz, 2004). In Table 54, the correlation between data collected from all skin sites combined are presented for the MoistureMeter SC™ and MoistureMeter D® (0.5 mm, 1.5 mm and 2.5 mm probes) followed by the correlation from separate skin sites in table 51 for the MoistureMeter SC™ and MoistureMeter D® 1.5 mm probe.

Table 54. MoistureMeter SC™ and MoistureMeter D® 0.5 mm, 1.5 mm and 2.5 mm probe correlations for all sites combined from Mayrovitz et al (2013) and the current study.

Study	0.5mm	1.5mm	2.5mm
Mayrovitz et al (2013) (544 data points) (p-value):	0.604 (<0.001)	0.568 (<0.001)	0.424 (<0.001)
Current study (right side) (224 data points) (p-value):	0.749 (<0.001)	0.715 (<0.001)	0.534 (<0.001)

The correlation coefficient between MoistureMeter SC™ and MoistureMeter D® data from all probes are consistently higher within this study than those found by Mayrovitz et al (2013), who also combined the data in this way. This could be due in-part to the anatomical locations tested in each of these studies.

Mayrovitz et al (2013) collected data from 17 skin sites: Great toe plantar Hand palm (center), thumb pulp, medial peri-malleolus Hand palm (thenar), great toe dorsum, cheek (middle), foot dorsum (4–5 toe), foot dorsum (1–2 toe), hand dorsum (mid), forehead (middle), forearm anterior, hand dorsum (web), forearm dorsum, anterior gaiter (shin), lateral gaiter and medial gaiter.

From the 7 measurement sites used within this study, 6 are directly equivalent to sites measured by Mayrovitz et al (2013), or at least in such close proximity that comparison is possible (our measurement site: *equivalent site from Mayrovitz et al (2013)*): ventral forearm: *forearm dorsum*, anterior tibia: *anterior gaiter*, peri-malleolar: *medial peri-malleolus***,** dorsal 3rd metatarsal head: *foot dorsum (4–5 toe) and foot dorsum (1–2 toe),* plantar 3rd metatarsal head and heel: *plantar hallux.*

Although several of these sites demonstrate stronger correlations than their equivalent in the work of Mayrovitz et al (2013) (ventral forearm and anterior tibia) (See Table 55) these are not uniformly higher across all sites. However, Mayrovitz et al (2013) published a list of the MoistureMeter SC™ and MoistureMeter D® 1.5 mm probe correlations for all sites separately. The measurement sites identified as 'equivalent sites' within this study represent a large proportion of the skin locations that display the strongest correlation between the probes. It may be the case that the overall strength of correlations for all sites is lower within the study conducted by Mayrovitz et al (2013), as this is reduced by the inclusion of other skin sites with lower correlations, such as the lateral and medial gaiter sites.

Table 55. MoistureMeter SC™ and MoistureMeter D™ 1.5 mm probe correlations by site from Mayrovitz et al (2013) and the current study.

	Ventral forearm	Anterior Tibia	Peri-Malleolar	*D3* – Dorsal 3rd metatarsal head *1-2 toe*- Dorsal skin between 1st and 2nd, *4-5 toe* – 4th and 5th metatarsals	*P3* – Plantar 3rd metatarsal head *H* – Heel *PH* – Plantar hallux
Mayrovitz et al (2013) r-value (p-value)	0.374 (0.035)	0.322 (0.072)	0.724 (0.001)	*1-2 toe*: 0.439 (0.009) *4-5 toe*: 0.529 (0.002)	*PH*: 0.789 (0.001)
Our study (left) r-value (p-value)	0.467 (0.007)	0.460 (0.008)	0.644 (0.000)	*D3:* 0.430 (0.014)	*P3:* 0.581 (0.000) *H*: 0.826 (0.000)
Our study (right) r-value (p-value)	0.612 (0.000)	0.622 (0.000)	0.528 (0.002)	*D3:* 0.508 (0.003)	*P3*: 0.614 (0.000) *H*: 0.601 (0.000)

6.6. Conclusion

The data collected within this study will be used to inform the use of the commercially available hydration measurement devices:

The MoistureMeter SC™ and Corneometer® CM825 demonstrate a very strong positive correlation and the hydration data they collect correlates similarly with skin hardness and roughness across the foot skin surfaces. In the instance of assessing skin hydration with the intention of influencing consumer perception of foot skin dryness. However, the Corneometer® CM825 is most suitable for use.

The MoistureMeter D® probes, primarily, the 0.5 mm probe, collect data on the heel and plantar 3rd metatarsal head that strongly correlate with the physical characteristics of the

tissue (hardness and roughness). However their use is not supported for assessing skin features on non-plantar skin.

Within the next study, data have been collected that are indicative of the measurement depth of these three devices. These data are explored in Chapter 8 and are considered in relation to the physical characteristics of the skin they correlate with in Chapter 9.

Chapter 7: An evaluation of the biochemical composition of the foot skin using CRS.

7.1. Collaborator Statement

Due to the high rental cost of a Confocal Raman Spectroscope, this project is part of a collaboration between two other academic institutions within the EPSRC Centre for Doctoral Training in Prosthetics and Orthotics: The University of Southampton (Faculty of Health Sciences) and Imperial College London (Department of Mechanical Engineering). This collaboration necessitates the inclusion of protocol features not directly applicable to the aims and objectives of this PhD:

The University of Southampton - Sebum collection and storage using Sebutape® (Evalulab, Montreal, Quebec, Canada) from the heel and forearm of each individual within participant groups: older people with diabetes and older people without diabetes.

Imperial College London (ICL) – The use of ICL manufactured devices designed to measure the physical characteristics of the skin. Data was required from older people with diabetes and older people without diabetes from the plantar heel and arch.

These will not be discussed in detail in this document. However, incorporating these into the protocol will be addressed in section 7.3.8.

7.2. Introduction

This chapter describes a study in which the biochemical composition of the foot skin is measured in three groups of people using several instruments. This study generates a large volume of data that fulfils two distinct sets of objectives. The analysis and discussion of these data is split between this chapter and Chapter 8 to facilitate the exploration of these distinct objectives in full.

The broad application of the data collected in this study was facilitated through several study design factors, firstly, the inclusion of three participant groups:

As described in Chapter 2, people of advanced age and those with diabetes have an increased risk of developing foot-skin pathology (Boulton, 2014; Burzykowski et al., 2003). Unfortunately, little data exists on the composition of the skin of older people and people with diabetes to explain why this is the case and what steps may be taken to remedy or prevent this. Where data are available, they generate conflicting results – possibly due to the method used to compare SC composition between participant groups – and no data are collected from the foot skin (see Section 2.4.)

To examine the effect of age and diabetes status on skin composition, young people, older people with diabetes, and older people without diabetes were recruited for this study. The older people without diabetes group were used as a comparator for both young people and older people with diabetes groups. i.e., young people and older people without diabetes were compared to examine the impact of age on skin composition, and older people without diabetes and older people with diabetes were compared to examine the influence of diabetes on skin composition.

This aspect of the study design was incorporated to fulfil objective 2 (See Figure 66). Collecting CRS data from the foot skin of people of these different foot-risk makes it possible to ascertain whether increased foot risk is associated with a change in foot skin composition.

For the purposes of this work, individuals with type 1 or 2 diabetes were recruited to the group of participants with diabetes as foot health risk is associated with both conditions similarly (Boulton, 2004).

The young people group recruited for this study were also selected to be the participants for an emollient trial. These individuals were anticipated to be the participant group from whom it would be easiest and fastest to collect CRS data from (facilitating collection of twice the volume of data within the same data-collection period). This was predicted as they were anticipated to have the highest skin hydration, resulting in a smooth skin surface (enhancing contact with the scope), and an increased ability to remain stationary for extended periods.

This study component was included to fulfil objective 3 (See Figure 66). Participants within the young people group were given an emollient to apply to one foot prior to data collection. Data were collected from both feet for this participant group, generating data on the treated and untreated skin composition.

The volume of physiological skin composites water and NMF were measured in treated and untreated skin as well as one emollient ingredient: urea. Urea is an active ingredient widely used in emollients on the foot skin (Cobos-Moreno et al., 2021; Piquero-Casals et al., 2021). Urea is a keratolytic agent, meaning that it enhances the action of the enzymes within the skin that digest the bonds between keratinocytes (Berardesca & Cameli, 2020). In dry skin, this reduces the build-up of denucleated skin cells at the superficial SC (Piquero-Casals et al., 2021). Application of urea to the foot skin has been shown to increase skin hydration, increase skin elasticity, and reduce callus growth (Piquero-Casals et al., 2021). Despite its wide use, it is not known how far urea penetrates the foot skin, its impact on the volume of water in the skin, and the volume of NMFs produced and retained.

By comparing the composition of treated and untreated plantar foot skin, it was possible to elucidate how far into the plantar skin emollient ingredients can penetrate, and the impact this has upon other physiological skin composites indicative of skin barrier function.

As alluded to, despite the novelty of the data generated by the Gen2-SCA, there are practical implications of its use. In particular, the time taken to measure each skin site. The skin sites examined using this tool were therefore limited to the below:

- The ventral forearm. This skin site is easily accessible and is widely used for dermatological research.

- The heel. This large flat surface of this site makes it the most accessible of all plantar skin sites for CRS testing. This location represents the thickest portion of plantar skin, and a major site for loading (Hessert et al., 2005; Kelikian & Sarrafian, 2011).
- The medial arch. Although the foot shape poses a challenge for CRS analysis, it is poorly characterised in the literature and is of interest as it sits at the border of plantar and non-plantar skin.

The hydration measurement devices used within this study were the DermaStat®, Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D®. These have been used simultaneously alongside CRS to provide insight into their measurement depth. This aspect of the study is explored in Chapter 9.

7.2.1. Novelty statement

Despite the commonality of foot-skin pathology, and the established link between skin composition and pathology, the composition of the plantar skin has not previously been investigated in-vivo in such detail. Data collected from the plantar foot using CRS represents the first data-set available on the hydration gradient and the volume of NMF and urea within the plantar SC. These data will inform the formulation of emollients designed for use on the plantar foot.

Further, in this study, the composition of the foot skin is measured in individuals with varied foot-skin risk. Although skin-differences between people of different ages and diabetes status have been examined previously, this has never been achieved on the plantar skin. This work uses CRS to generate novel data on the differences in plantar skin composition between these population groups, generating an understanding of the underlying causes of foot-skin pathology in these populations.

In addition, this study represents the first instance in which the penetration of an emollient into the plantar skin is examined using CRS. This provides valuable insight into the gradation of water, NMF, and urea in the plantar skin resulting from emollient application.

7.2.2. Objectives and hypotheses

Three objectives have been established for this study (See Figure 66).

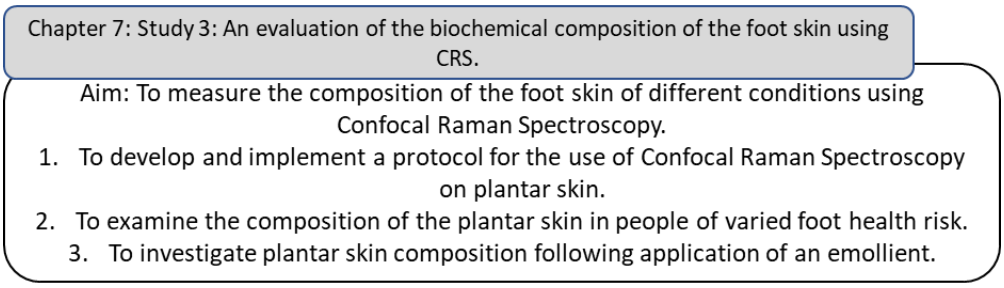


Figure 66. Objectives for 'An evaluation of the biochemical composition of the foot skin using CRS'

Hypothesis for objectives 2 and 3 are given below. These have been generated following a review of literature (see Section 2.4). No hypotheses are given for objective 1, as this does not generate an output that requires assessment using statistical analysis.

7.2.2.1. Objective 2 hypotheses:

6. The water content in the deeper layers superficial SC is significantly lower in the plantar skin compared to non-plantar skin.
7. The water content in the plantar SC of older people will be significantly lower than that of younger people at the same measurement depths.
8. The NMF content in the plantar SC of older people will be significantly greater than that of younger people at the same measurement depths.
9. The water content in the plantar SC of people with diabetes will be significantly lower than that of age-matched non-diabetics.
10. The NMF content in the plantar SC of people with diabetes will be significantly greater than that of age-matched non-diabetics.

7.2.2.2. Objective 3 hypotheses:

4. The water content of emollient-treated SC will be significantly greater than untreated SC at the same measurement depths.
5. The NMF content of emollient-treated SC will be significantly greater than untreated SC at the same measurement depths.

6. The urea content of emollient-treated SC will be significantly greater than untreated SC at the same measurement depths.

A note on terminology used within this chapter:

For conciseness, the biochemical composition of the skin will be described using the term 'composition'.

7.3. Method

Data was collected from September to December 2022. This was a cross sectional design with a single 3-hour data-collection session. Data collection was undertaken in the Skin laboratory at the University of Salford. The study protocol was reviewed and approved by The University of Salford Ethics Panel (reference number: 6751).

7.3.1. Participant groups and recruitment

Three participant groups were recruited: young people, older people without diabetes, and older people with diabetes. The inclusion and exclusion criteria are given in Table 56.

Table 56. Participant group demographics and inclusion and exclusion criteria.

Group name	Description	Inclusion criteria	Exclusion criteria
Young people	People without diabetes aged 18-35	Individuals aged 18+ of any sex or race.	Diabetes diagnosis, current skin pathology on the foot (excluding corns and callus), inability to refrain from using topical applicants to examination areas for 7 days prior to data-collection, lower limb loss.
Older people without diabetes	People without diabetes age-matched to 'older people with diabetes'		
Older people with diabetes	People with diabetes	Individuals aged 18+ with Diabetes (any type), of any sex or race.	Current skin pathology on the foot (corns and callus, for example), inability to refrain from using topical applicants to examination areas for 7 days prior to data-collection, lower limb loss.

Participants were recruited via convenience sampling from individuals accessible to the researcher in the local area. Older participants with and without diabetes were recruited from the University of Salford Podiatry clinic. This is a Podiatry teaching clinic based at The University of Salford.

Recruitment of participants with diabetes was carried out first. The researcher searched records of patients and dispatched recruitment materials via post to people fitting the exclusion and inclusion criteria for older people with diabetes. Recruitment material and participant information sheet can be found in Appendices 14 and 15. Upon receiving a positive response and booking a data-collection session for these individuals, the researcher dispatched recruitment materials to individuals fitting the exclusion and criteria for older

people without diabetes who were close in age (within a 5-year age bracket (+/- 2.5 years)) to the newly recruited participant.

As the older people without diabetes were recruited, the demographics of these and the older people with diabetes were compared and further purposeful recruitment was undertaken where required (i.e. where the average age of participants was higher in the older people with diabetes group, recruitment materials would be dispatched to individuals at the lower-end of the 5-year age bracket for newly recruited participants). Participants in the young people group were primarily students and staff from the University of Salford recruited via an internal message board. The age range for this cohort was determined following review of equivalent studies (age boundaries of young group: (lower) 18-23 and (upper) 28-40 years of age (Boireau-Adamezyk et al., 2021; Choe et al., 2018; dos Santos et al., 2019a; Egawa et al., 2007)).

Due to the limited time period for recruitment and data-collection, a specific age-bracket for older participants was not pre-determined as this could unnecessarily restrict recruitment as there is little consensus in literature around the age at which skin-composition changes. The demographics of the patients of the University of Salford Podiatry Clinic, and the requirement for participants to have diabetes (or be age-matched to an individual with diabetes) generated a participant group of advanced age.

As an incentive to participate in this study, individuals recruited from the University of Salford Podiatry clinic patients were offered Podiatry treatment by the researcher (HCPC registered Podiatrist) free of charge.

7.3.2. Emollient study

The emollient used within this study is the Intense Nourish formulation from Scholl, a mid-range product intended for use on moderately dry skin (See Figure 67). The full ingredient list for this product can be found in Appendix 12.

Figure 67. Scholl Intense Nourish emollient. Image extracted from https://www.scholl.dk/fodplejeprodukter/hard-hud/scholl-intense-nourish-fodcreme/ on 05/01/23.

7.3.3. Participant demographic data collection

Demographic data was collected that focussed on factors that might affect foot skin composition (See Appendix 11 for the data collection form).

Table 57. Participant demographic data captured and justification.

Factor	Justification
Sex	There is limited and conflicting evidence available on the impact of sex on skin hydration (Luebberding et al., 2013; Rogiers et al., 1990)
Time since skin sites washed	Capacitance measures should only be collected from a skin site 5hrs after cleansing (Rogiers et al., 1990)
Diabetes type (1 or 2), diagnosis date, most recent HbA1C level and date, diabetes medication, monofilament test	Indication of disease progression/severity which can impact skin characteristics (Lai et al., 2021)
Body Mass Index	The influence of body composition on tissue dielectric constant of skin is uncertain (Mayrovitz et al., 2020; Mayrovitz et al., 2017)
Ethnicity	There is limited and conflicting evidence available on the impact of ethnicity on skin characteristics (Du Plessis et al., 2013)

7.3.4. Laboratory conditions

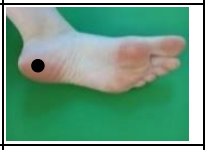
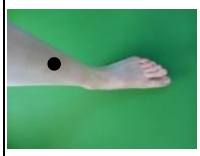
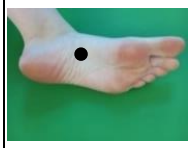
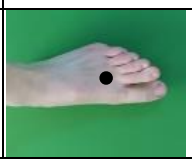
Data were collected in a laboratory at the University of Salford, which maintains a consistent temperature and humidity during the day. Further details on this space can be found in

Section 5.2.1. as the same laboratory was used for this study. Environmental conditions were recorded at the commencement and conclusion of each data collection period.

7.3.5. Measurement Locations

Data were collected at six sites on the body, four of which are on the foot. These sites are described, and their significance is explained in Table 58. Hydration measurement devices were applied to all measurement locations, CRS data was collected from three locations.

Table 58. Skin sites for measurement. CRS use indicated with an asterisk.

Site Name (abbreviation)	Anatomical Location	Figure	Site Name (abbreviation)	Anatomical Location	Figure
Ventral Forearm (VF)*	10 cm proximal to the antecubital crease		Heel (H)*	2 cm inwards from posterior centre of the heel	
Anterior Aspect of Tibia (AT)	Central anterior surface 10 cm proximal to the midpoint of the malleoli		Medial Arch (MA)*	Plantar aspect of base of 1st metatarsal	
Dorsal 3rd MPJ (D3)	Dorsal aspect of 3rd MTP joint		Plantar 3rd MPJ (P3)	Plantar aspect of 3rd MTP joint	

7.3.6. Emollient Intervention

Participants in the young people group were provided with a tube of Intense Nourish emollient (Scholl) (See Appendix 12) and instructions to apply one finger-tip-unit of the product to one foot (right/left side alternated by participant number) once per day in the 7-days before data-collection (See Appendix 13). During this period, participants were asked to abstain from using other topical applications on the feet.

7.3.7. Instruments

For details on the instruments listed, please refer to Chapter 4.

Commercially available hydration measurement devices:

DermaStat® (Arche Healthcare Ltd, Connecticut, United States)

Corneometer® CM825 (Courage and Khazaka electronic, Cologne, Germany)

MoistureMeter SC™ (Delfin Technologies, Kuopio, Finland)

MoistureMeter D® 0.5 mm probe- (Delfin Technologies, Kuopio, Finland)

Confocal Raman Spectroscopy:

Gen2-SCA (RiverD International, Rotterdam, The Netherlands)

7.3.7.1. Order of instrument use

The order of instrument use was designed to minimise the impact one activity could have on subsequent measurements. Benchtop hydration measures were conducted first due to their high sensitivity to changes in superficial skin hydration, as confirmed by work in Chapter 5. The use order was: DermaStat®, Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D®. The DermaStat® was used first as it has a very short contact time and therefore a minimal occlusive effect and the manufacturer does not indicate the need for an acclimatisation period (Arche Healthcare, 2018).

CRS hydration testing occludes the skin surface by the scope (RiverD International BV, 2020).

7.3.7.2. Hydration measurement protocol

Each device was used three times on each skin site and the mean value of these used for statistical analysis, in line with the manufacturer's instructions in the case of Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D® (Alanen et al., 2004; Courage & Khazaka electronics GmbH, 2010; Delfin Technologies, 2016; EvaluLab, 2018), and for consistency with the other measures in the case of the DermaStat®. The use of the DermaStat® and Corneometer® CM825 is shown in Figure 68. A full description of the use of these devices can be found in Section 4.2.

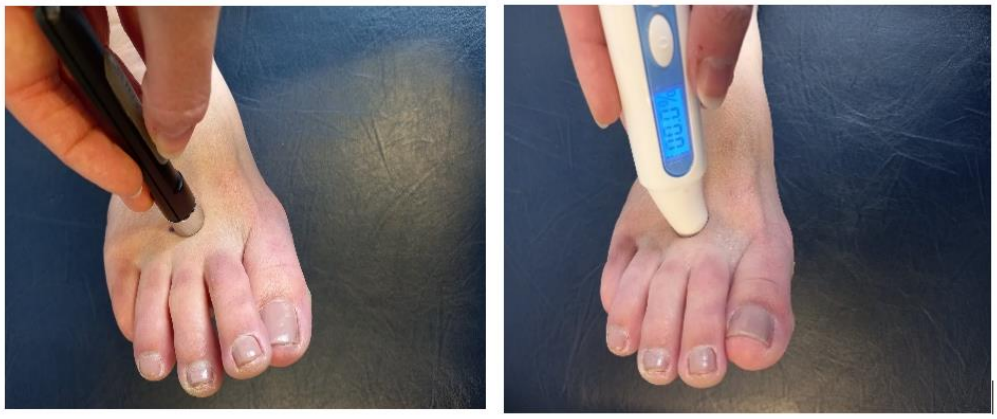


Figure 68. Demonstration of the use of the DermaStat® (left) and Corneometer® CM825 (right) on the dorsal 3rd metatarsal head measurement site. Image source: Personal collection.

7.3.7.3. CRS data collection

As this is the first instance in which CRS has been applied to the plantar skin, a protocol was developed by the author to facilitate data-collection. The positioning of the device and participant are demonstrated below.

The Gen2-SCA comes with two measurement platforms See Figure 69. These are fixed to the top of the device and support the weight of the body part, whilst an aperture in the centre of these allows the skin to contact a scope on the uppermost surface of the instrument. A flat measurement platform as used for heel and ventral forearm measures, and a convex stage for arch measures (See Figure 70).

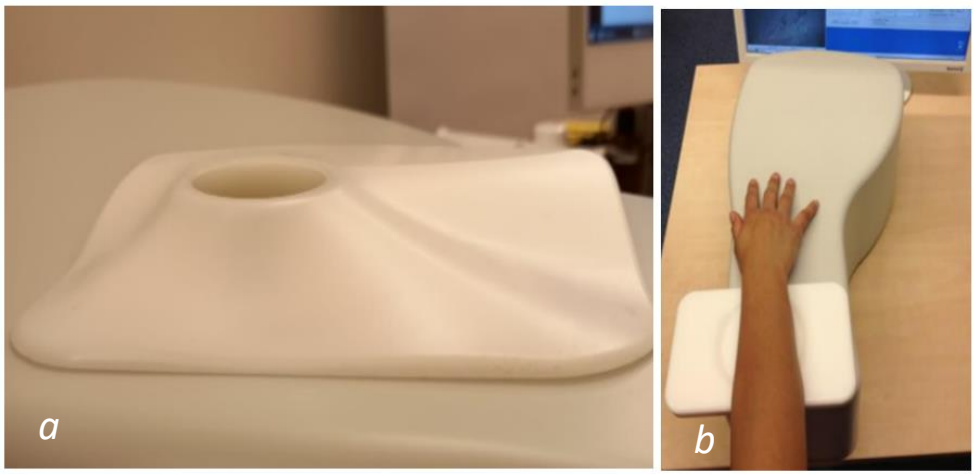


Figure 69. Flat (a) and convex (b) measurement platforms. Images extracted from the 'Gen2-SCA brochure' accessible at: https://www.riverd.com/.

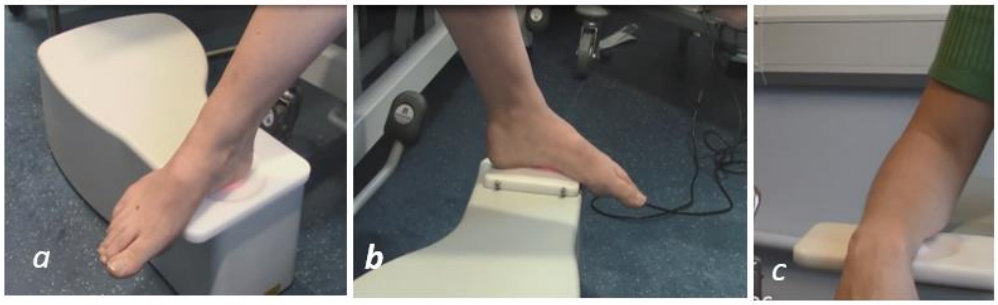


Figure 70. Positioning of skin sites on Gen2-SCA. Skin sites being measured: a. heel, b. medial arch, c. ventral forearm.

7.3.8. Data collection protocol

Upon entering the laboratory, participants were asked to remove their shoes, socks, and hosiery and sit on a plinth for a 15-minute acclimatisation period. During this period, the participant completed a consent form (See Appendix 16), and a demographic form (See Appendix 11). The researcher checked the foot skin for signs of pathology (data-collection ceased if any pathology beyond mild-moderate xerosis was observed) and identified and marked testing sites using a surgical marker.

The DermaStat® was used first, then the Corneometer® CM825, MoistureMeter SC™, and MoistureMeter D® (after 20 minutes of acclimatisation). This was followed by CRS data-collection and supplementary testing necessitated by the collaborative aspect of this work. The full testing schedule may be seen in Table 59.

Table 59. Data-collection schedule.

Stage	Activity (time-point)
Commencement	Participant arrives (00:00) Shoes and hosiery removed
Acclimatisation period	Participant rests seated and completed paperwork Temperature and humidity recorded
Commercially available hydration measurement devices 'Young people': both feet 'Older people' and 'Older people with diabetes': one foot	Use of: DermaStat® (00:15) Corneometer® CM825 (00:20) MoistureMeter D® MoistureMeter SC™
CRS Use 'Young people': both feet 'Older people' and 'Older people with diabetes': one foot	CRS Applied to: Ventral forearm (High wavelength - Fingerprint region) Heel (High wavelength – Fingerprint region) Arch (High wavelength – Fingerprint region)
Sebutape collection	Application, removal, and storage of Sebutape on dry ice
Physical testing	Handheld tribometer (Heel only) Indenter (Heel only)
Podiatry Treatment	'Older people' and 'Older people with diabetes'
Finish	Data collection complete. Temperature and humidity recorded

Participants sat with their legs raised on the plinth for all testing apart from during CRS work when they sat with their legs over the edge of the bed, with either the plantar foot or the volar forearm resting on the top of the CRS platform (See Figure 70).

Following data-collection, podiatry care was provided to participants within the older people without diabetes and older people with diabetes group.

7.3.9. Data processing

7.3.9.1. Commercially available hydration measurement devices

Where any of the three repeated measures were not collected due to instrument failure (most commonly due to hydration levels being too low) any other values collected at the same site were excluded from analysis. The rate at which this occurred is detailed and discussed in section 8.4.2.

7.3.9.2. CRS data

The output from CRS data-collection is a spectrum from each measurement depth, for each location, from both the 'fingerprint' and 'high-wavelength' spectral ranges. The Skintools 3 software fits the shape of these spectra (i.e. the location and height of the peaks) to reference spectra for materials known to exist within the skin: NMF and urea reference spectra have characteristic peaks within the fingerprint spectral range, and water has characteristic peaks within the high-wavelength spectral range. This is explained in more detail in section 4.3.1.

Due to the difficulties associated with collecting CRS data on the plantar skin (irregular architecture of the foot, inhomogeneity of skin surface, difficulty stabilising the skin surface/limb), data quality was inconsistent (see Section 7.3.9.) so it was not permissible to simply submit all CRS data into SkinTools 3 and presume the output was accurate.

Historically, methods used to ensure data-quality have not been well defined in literature concerning the use of CRS. For transparency and to aid others in applying CRS to challenging skin surfaces, a 3-stage protocol has been developed for the assessment of data quality.

Stage 1 – Data capture

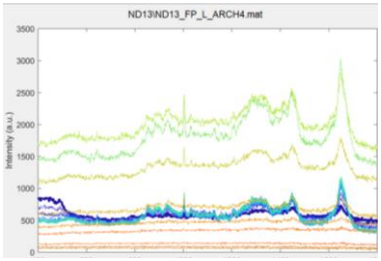
During data-collection, the researcher reviewed the spectra in real-time. Following training with manufacturer and familiarisation with spectra, the researcher was able to identify signs of high noise in the data or signal saturation. These are demonstrated in Figure 71.

Signal saturation occurs when the scope is not entirely covered by the skin surface, allowing light from the environment to reach the scope, or (in the instance where only the superficial measures are saturated) when the skin surface is not entirely pressed against the scope and causes some reflection of the emitted light. This high signal obscures the 'peaks' within the spectra indicative of material composition, making them unsuitable for analysis. Where the signal was consistently saturated, data-collection was ceased, skin placement adjusted, and measurement was re-started.

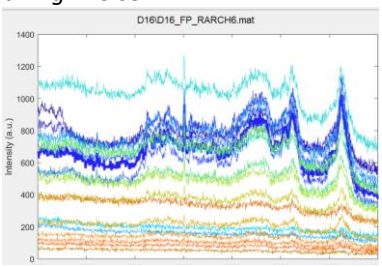
High spectra noise (background signal not indicative of material composition) is visible in the spectra as high variability in signal strength between anticipated peaks. High noise reduces the ability of the software to differentiate between artifacts in the signal and peaks indicative of skin composition, impeding data analysis. Where spectra were extremely noisy, data-collection was ceased and re-commenced elsewhere.

Data were saved where some signal saturation was evident, or the signal was moderately noisy, but the researcher recorded data-quality inconsistency in their logbook for later assessment (demonstrated in stage 2, Figure 73).

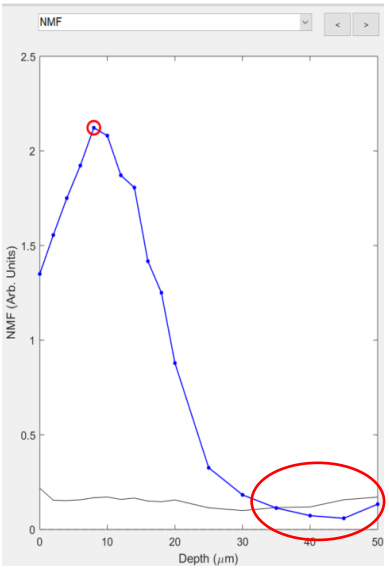
a. Low noise



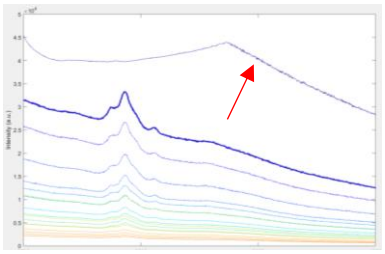
b. High noise



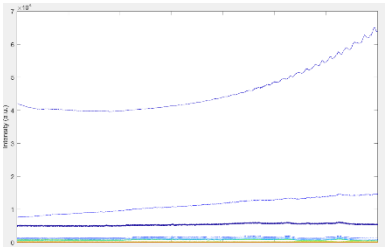
c. Sigma line above NMF value



d. Single spectrum 'Saturated'



e. All spectrum 'Saturated'



f. Single anomalous value

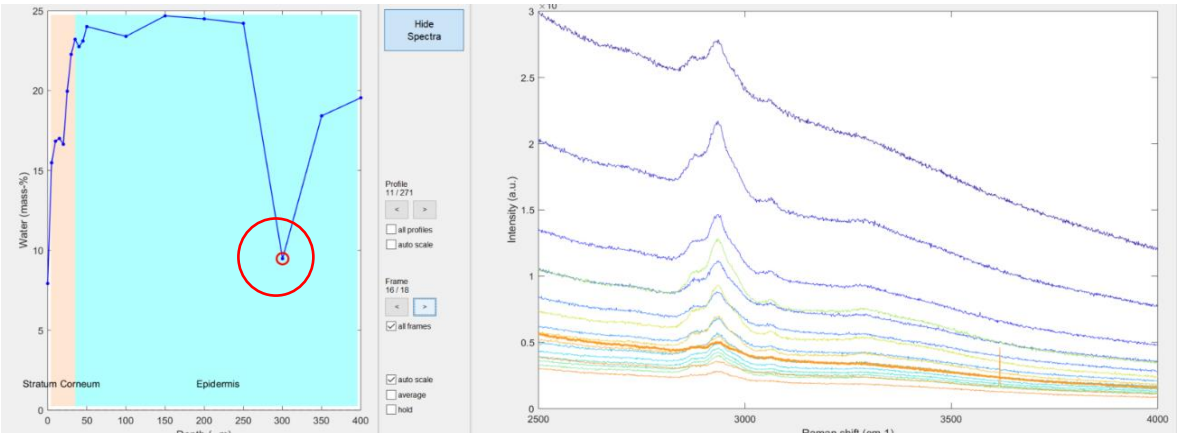


Figure 71. A demonstration of issues identified within Raman spectra.

Stage 2 – Data transfer

Following data collection, all data were transferred to SkinTools 3 for review. Incomplete files were deleted (partial completion indicated saturation or high noise) and files with uncertain quality were assessed individually. A single file contained several spectra (forearm - 17 spectra, heel - 18 spectra, arch - 20 spectra): where these were consistently of poor quality, the file was deleted. However where only one or two spectra were of poor quality, the file was retained.

Stage 3 – Data processing

All files were processed in SkinTools 3. This programme calculated the volume of water, NMF, or urea within the tissue according to the measurement region. These data were then extracted into Excel software. Fingerprint region data were provided alongside two data quality indicators: signal-to-noise ratio and a sigma value.

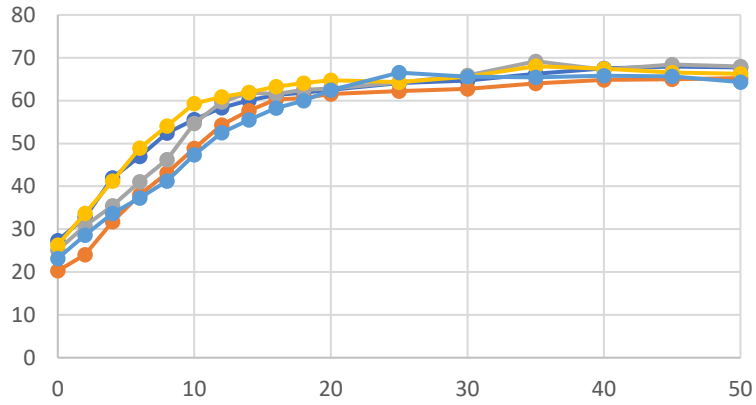
The signal-to-noise ratio is described by manufacturer as 'a quality metric of system performance'. It is calculated as the signal-to-noise ratio of the calibration measurement of fused silica (RiverD International BV, 2020). Following simulations of applying various thresholds of signal to noise ratio to data, RiverD advised that a threshold of <10 should be applied when assessing the quality of data obtained using the Gen 2 SCA. As such, data with a signal to noise ratio of below 10 were deleted.

The sigma value is an indicator of statistical significance, its calculation is described by RiverD as: "The value of the sigma line represents the concentration of the selected component at which its Raman signal contribution equals the standard deviation of the residual spectrum (the spectrum resulting from the subtraction of the fitted spectrum from the measured spectrum)" (RiverD International BV, 2020). Where the value of the material is below the sigma value (at the equivalent measurement depth) (See Figure 71 where the blue compound volume line (blue) drops below the sigma value line (black) in image *c*), these data are not of assured significance. As such, data with a value lower than their sigma value were deleted.

No such indicators of quality are available for high-wavelength data. Instead, these are assessed subjectively by the researcher according to their consistency with equivalent data. All data were plotted as concentration gradients (i.e. material volume versus skin thickness), and where data were anomalous, these were removed (See Figure 71, image *f*). For consistency, this same process was undertaken for fingerprint region data. However, very few anomalous data remained following the removal of data with poor indicators of quality.

Finally, all gradients from each repeated measure were plotted onto the same graph, and instances where a single gradient was markedly different to the others this were removed (See Figure 72).

a. ND20 Ventral Forearm Hydration Gradient



b. ND02 Right Arch Hydration Gradient

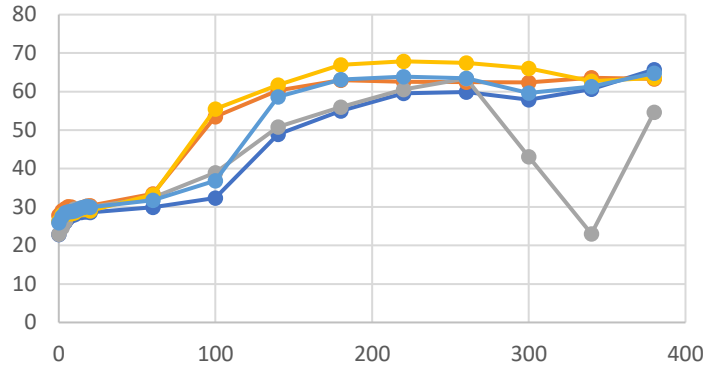


Figure 72. Example data demonstrating multiple hydration gradients collected at the same location from a single individual: a. consistent data, b. grey line inconsistent with dataset.

This process resulted in the generation of a single gradient for each material, at each skin site for each individual. These data were used for analysis.

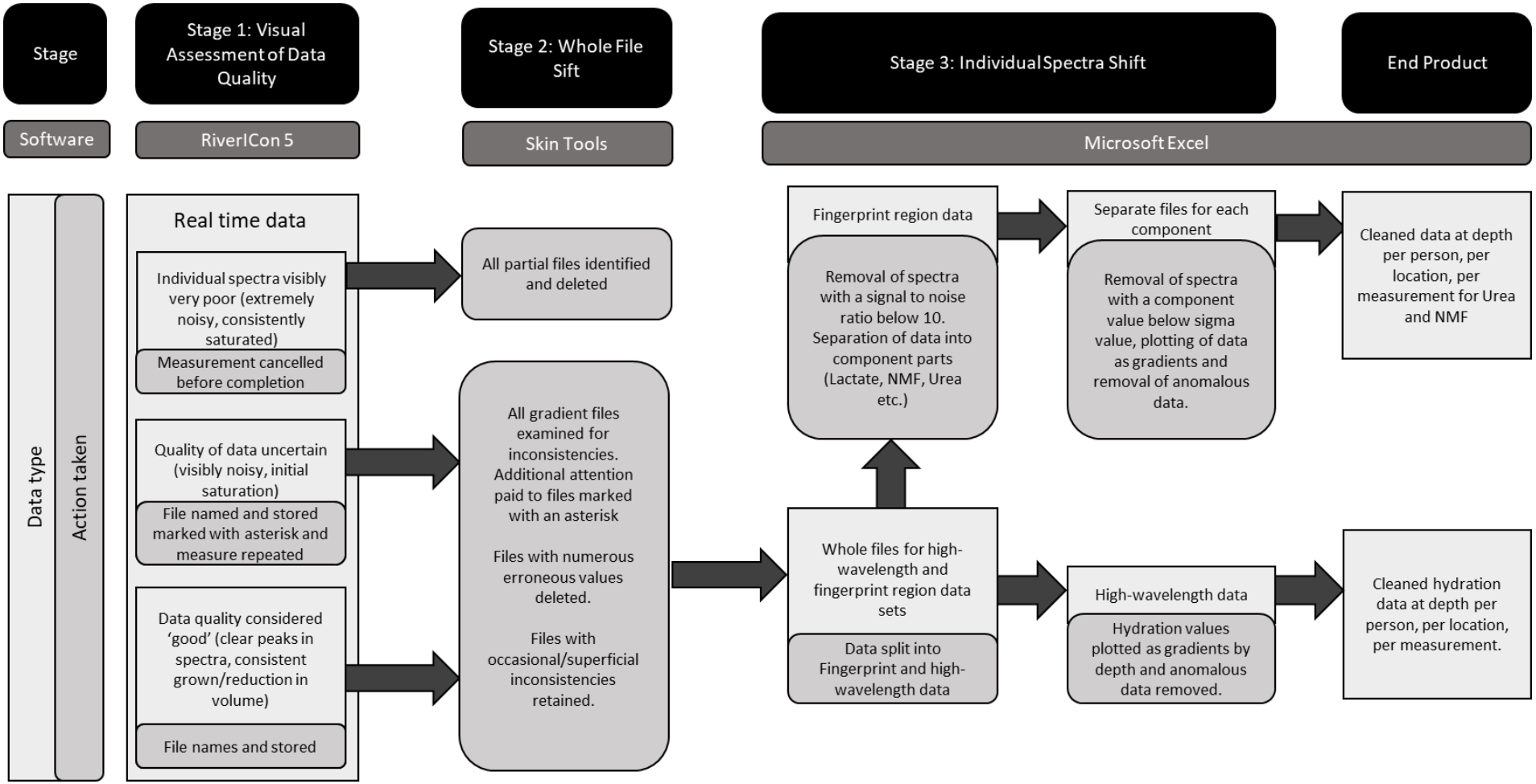


Figure 73. CRS data quality assurance flow chart.

7.3.10. Statistical Testing

The statistical tests used to test the hypotheses relevant to this study are presented in table 58.

Table 60. Statistical analysis plan for each hypothesis.

Study design	Parametric Testing	Non-Parametric Testing
Between-groups (i.e. young people and older people or older people without diabetes and older people with diabetes)	T-Test	Mann Whitney-U
Within-group (i.e., treated and untreated skin)	Paired T-Test	Wilcoxon test or Sign test, dependence on distribution of differences.
Normality testing: The Shapiro-Wilks test was applied to determine normality of data-distribution. Where this indicated data were non-parametric, Q-Q plots, measures of central tendency and histograms were be reviewed to ascertain whether this was correct.		

This analysis plan was developed to reflect the methods used in the literature. Within equivalent comparisons on the non-plantar skin, authors chose to compare groups by conducting T-Tests separately at each measurement depth (Egawa & Tagami, 2008a). However, this method does have some limitations:

1. This method requires many comparisons to be applied to the same data sets, which increases the likelihood of a Type I error arising. The Bonferroni correction can compensate for this risk (Binder et al., 2017). In this work, the outcome of these analyses was published without application of the Bonferroni correction, to aid their comparison with previous work, followed by a review of the results with the p-value modified using the Bonferroni correction.
2. Separate T-tests were applied to data sets at each measurement depth and interpreted separately, treating them as discrete from one another, where in reality, they are part of a continuum and inherently linked. Boireau-Adamezyk et al (2014) and Choe et al (2018) compared the total volume of materials within the SC as a comparator between participants, negating this issue. However, the method used by Boireau-Adamezyk et al (2014) and Choe et al (2018) was unsuitable for this study due to a paucity of SC thickness data from plantar skin. It was considered pertinent, instead, to make use of a statistical model in which the measurement depth could be input as a variable and factored into analysis such as a mixed effect model.

   A mixed effects model was used with material volume as the dependant variable, a fixed effect for the group, and a random intercept at the patient level (McCulloch &

Searle, 2001). Maximum likelihood estimation and an autoregressive covariance structure was used as a steady decay in the correlation between observations is anticipated with increasing depth. This was applied to each dataset (for each material at each location for the age, diabetes status, and treated and untreated skin comparisons) to identify whether there were any statistically significant differences in material volume between the two participant groups being investigated.

7.4. Results

Thirty-four participants participated in the study, as described in Table 61. Although participant numbers differ between participant groups, the impact of participant number on data volume per group was somewhat limited for several reasons. Firstly, data collection from older people with diabetes and young healthy people was completed first so initial issues with instrument faults and unavailability disproportionately affected these groups. Secondly, the quality of data obtained from the young people group were consistently higher, resulting in less data-loss during analysis. For transparency, the n-number for each set of results will be displayed in the following sections.

Table 61. Participant group demographics.

	Young people (n=8)	Older people without diabetes (n=11)	Older people with diabetes (n=15)
Age (years) (Mean (SD))	27.75 (3.23)	62 (10.92)	61 (11)
Sex (% female (n))	37% (3)	45% (5)	40% (6)
BMI ($kg.m^{-2)}$	27.04	26.87	32.63
Ethnicity (% of participants (n))	75% (6) White British 12.5% (1) Black Caribbean 12.5% (1) Pakistani	81.8% (9) White British 9.1% (1) White Irish 9.1% (1) Indian	93% (14) White British 7% (1) Indian

7.4.1. Data loss and impact on analysis across measurement depth

After removing data of low quality, some data sets were reduced in number at deeper measurement depths. This was most evident in fingerprint-region data the arch and heel skin beyond 100 µm and 150 µm depth (respectively), and beyond 15 µm at the ventral forearm.

The author anticipated data loss due to poor quality to from deep layers of the plantar skin due to the large distance the laser light and the returning signal have to travel through the tissues. However, data loss due to poor quality has also been observed at the ventral forearm for the fingerprint-region data. Following discussion of this issue with RiverD, it became apparent that this is due to the inhomogeneous structure of the epidermis at the ventral forearm between 0-50 µm. Due to the thin SC at the ventral forearm, the laser light passes into the viable epidermis, where nucleated cells, melanosomes, and various other structures increase scattering of light and therefore signal loss. At measurement depths beyond the SC, therefore, the exposure time of the fingerprint measurements should be extended. At the

heel and medial arch, the SC is much thicker, so it is much deeper into the tissue that this issue occurs.

For the purposes of this work, fingerprint-region individual data will not be displayed for the ventral forearm due to their limited number. Arch and heel data will be displayed up to 250 µm and 100 µm measurement depth (respectively) however statistical analysis will only be completed up to 100 µm for the arch and 150 µm for the heel.

High-wavelength region data are not impacted by this level of data loss and as such will be displayed and analysed for the full measurement depth. Future investigations would benefit from extending the exposure time used during fingerprint-region data collection.

7.4.2. Comparison between participant groups – young people and older people without diabetes

Data were collected on the volume of water and NMF in the skin of the ventral forearm, medial arch, and heel of people of different ages. Each dataset (split into depth, skin site, and participant group) was assessed for normality using the Shapiro-Wilks test. Most datasets were normal in their distribution. However, several from each skin location were non-normally distributed, so all data analysis was completed using non-parametric tests.

These data address hypotheses 1-3 relating to objective 2 (see Section 7.2.2.1.).

7.4.2.1. Hydration gradients

The hydration gradient observed in the skin is different at the ventral forearm, heel, and arch, and a statistically significant difference between the hydration of the skin is evident between the young and older participants within the SC of the ventral forearm (depth (p-value): 2 µm (0.034), 4 µm (0.012), 6 µm (0.006), 8 µm (0.021), 10 µm (0.021), 12 µm (0.016), 14 µm (0.027), 16 µm (0.043) (See Figure 74).

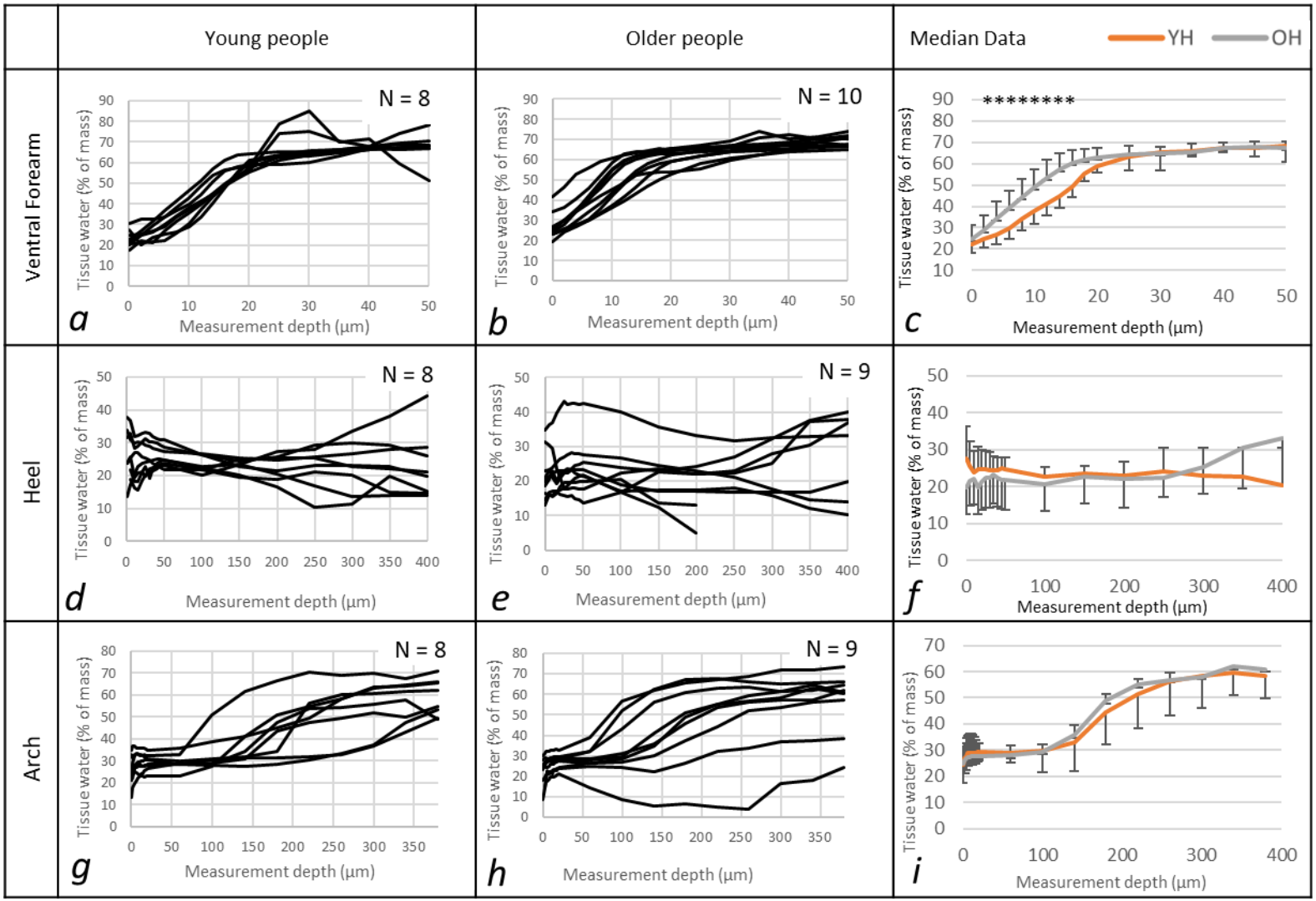


Figure 74. Water content of skin for young and older healthy participants. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants. Significant differences (p-value <0.05) in water content identified by a Mann-Whitney U test are indicated by an asterisk.

7.4.2.1.1. Anatomical site differences in hydration gradient (Hypothesis 1)

The hydration gradient at the ventral forearm and arch follow a similar pattern. At the SC surface, water constitutes approximately 20-30% of the mass of the tissue increasing to 60-70% of tissue mass at the top of the viable epidermis. On the ventral forearm, this increase begins at the skin surface, occurs over a relatively small tissue depth, and is consistent between individuals (from surface to 15-20 μm in young people, and from the surface to 10-20 μm in older people). On the arch, the water content remains low up to a depth of 50-100 μm into the skin where it rises gradually, stabilising at approximately 200 μm depth.

The heel skin displays a different pattern. At the surface, the skin water represents 20-30% of the tissue mass, as at the other skin sites, however this does not display a uniform increase over depth. For some individuals, a slight increase or decrease in water content can be observed across tissue depth, however these do not follow the same pattern observed at the ventral forearm or arch skin (i.e., a defined period of increase and plateau as the viable epidermis is reached).

These data support the hypothesis that the low water content of the superficial SC extends further into the plantar skin than in non-plantar skin.

7.4.2.1.2. Differences between groups (Hypothesis 2)

There were no statistically significant differences in water content between the groups for arch (p-value range: 0.067-1) and heel SC (p-value range: 0.139-1). At the ventral forearm, however, older healthy participants had a significantly higher water content between measurement depths between 2 μm and 16 μm (p-value range: 0.006-0.043).

These data indicate that the plantar SC of older people has higher water content than the plantar SC of younger people at the same measurement depths.

7.4.2.2. NMF gradient

A high signal-noise ratio was observed for data collected from the ventral forearm and at the deeper measurement depths captured on the heel and arch. Following the removal of poor-quality data, ventral forearm data were too limited for statistical analysis, and heel and arch data were restricted to more superficial measurement depths (no change in n resulted from this for the data presented).

NMF content of the skin was found to be significantly higher in the older participant group at 60 µm depth (p-value: 0.05). Otherwise, NMF content was very similar across skin depth for both populations (See Figure 75).

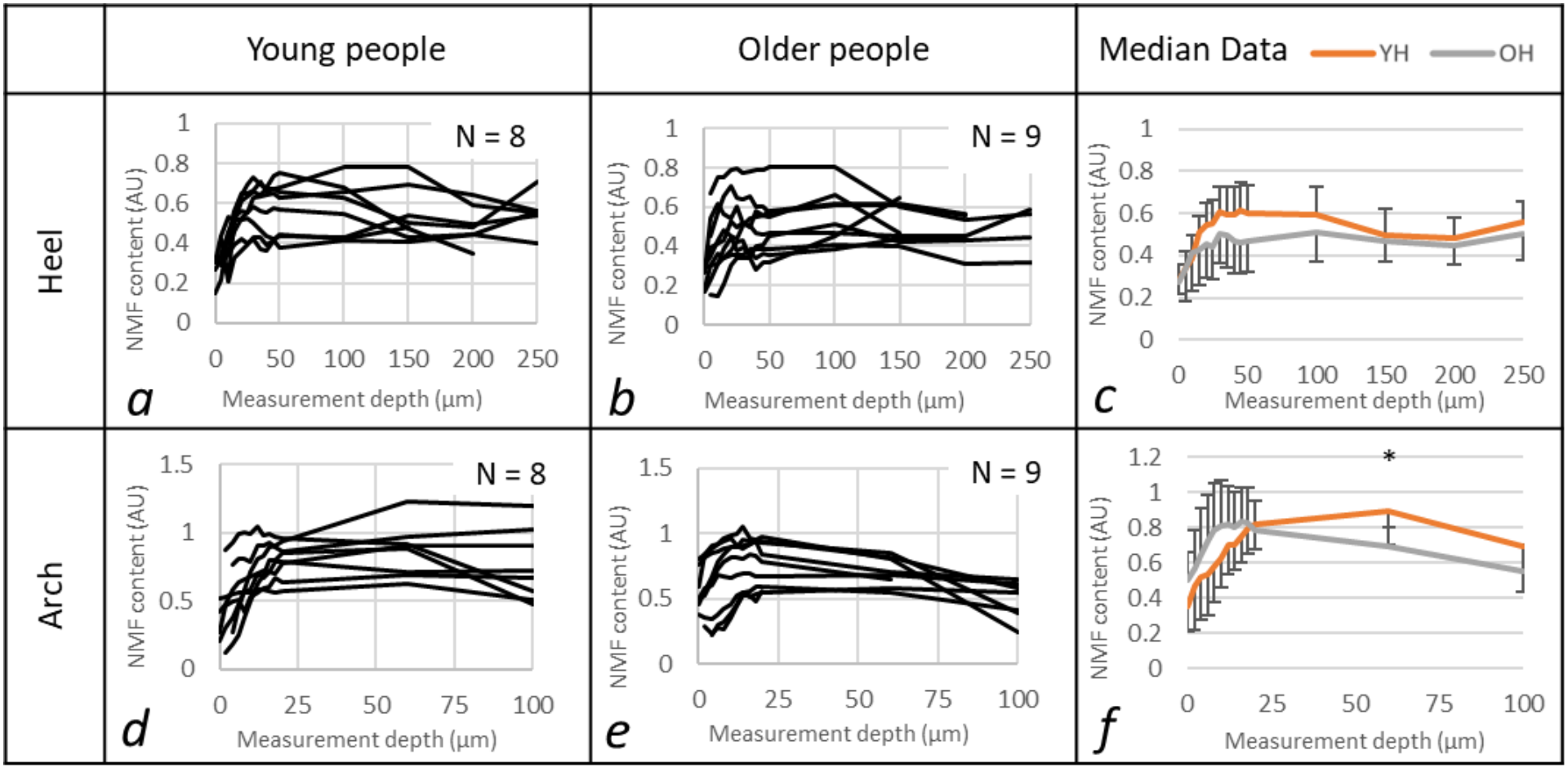


Figure 75. NMF Content of skin in young and older healthy participants. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants. Significant differences (p-value <0.05) in NMF content identified by a Mann-Whitney U test are indicated by an asterisk.

7.4.2.2.1. Anatomical site and group differences in NMF gradient (Hypothesis 1 and 2)

The NMF gradient is consistent between skin sites and participant groups: at the skin surface NMF content is low (0.2-0.5 AU), this rises in the superficial 25-35 µm (to 0.5-1 AU on the arch and 0.4-0.8 AU on the heel), and remains relatively stable for the remainder of the SC. In the arch data, there is some indication of the border of the viable epidermis, as the NMF content begins to reduce for some individuals at 60 µm measurement depth.

These data demonstrate that the plantar SC of older people does not have higher NMF content than the plantar SC of younger people at any measurement depth on the heel, or at any depth other than 60 µm on the arch skin.

7.4.3. Comparison between participant groups – People with Diabetes and age–matched non diabetics.

These data address hypotheses 4-5 relating to objective 2 (see Section 7.2.2.1.).

Data were collected on the volume of water and NMF in the skin of the ventral forearm, arch, and heel in age-matched people with and without diabetes. These datasets (data split into depth, skin site, and participant group) were found to be primarily parametric. However, several were non-parametric (tested using the Shapiro-Wilks test). Therefore, non-parametric statistical analysis methods were employed.

The Mann-Whitney U test has been used to identify any differences between the volume of water and NMF content of the SC at each measurement depth.

7.4.3.1. Hydration gradient

A statistically significant difference was identified between the water content of forearm skin between people of the same age with diabetes and or without diabetes, at the depths of 0-18 µm depth (p-values respectively for depths increasing in steps of 2 µm: 0.026, 0.013, 0.011, 0.022, 0.026, 0.022, 0.026, 0.019, 0.016, 0.048, 0.036 respectively) and 45 and 50 µm depth (p-values: 0.042 and 0.042) (See Figure 76).

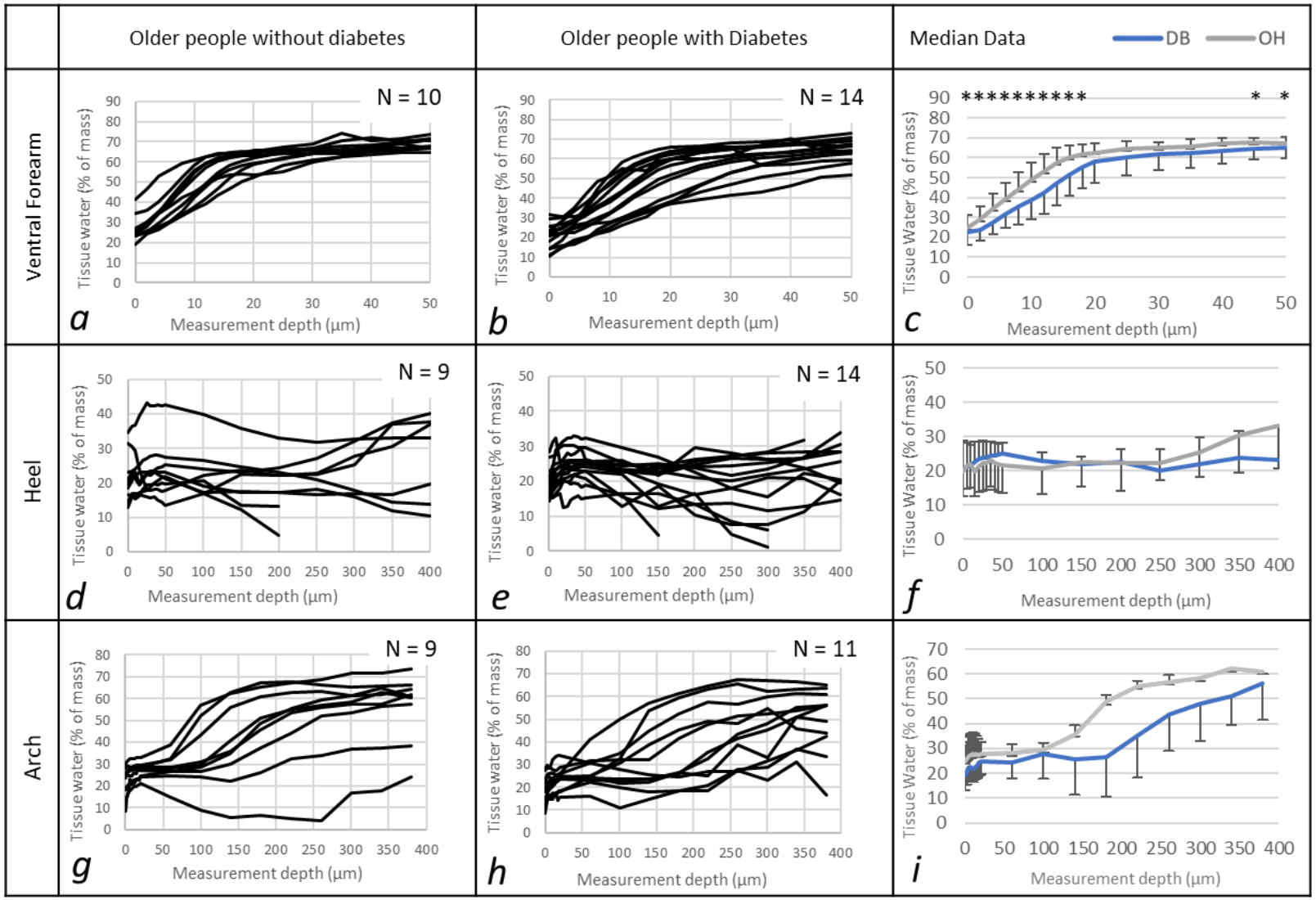


Figure 76. Water content of skin in age-matched Diabetic and Non-Diabetic ('Older Healthy') participants. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants. Significant differences (p-value <0.05) in water content identified by a Mann-Whitney U test are indicated by an asterisk.

At these measurement depths, people without diabetes had higher water content in their skin than people of the same age with diabetes. This is reflective of the increased variation between the hydration gradients of individuals within the diabetes group: Although most individuals had a similar hydration gradient to that demonstrated within the group of people without diabetes, four participants have a hydration gradient that increases more gradually over the skin depth, leading to a markedly lower SC hydration in the mid-portion of the gradient (10 µm – 20 µm) and a smaller difference in deeper tissues which only becomes statistically significant at the two deepest measures where the standard deviation is reduced.

Although there are no statistically significant differences between the water content of tissues at different measurement depths between people with and without diabetes at the plantar skin (heel and arch), people with diabetes have more variability in the depth where the water content of the skin begins to rise on the arch skin: Most people without diabetes have a marked increase in SC hydration at between 50 and 100 µm. This is except for two individuals who have an extended period of low hydration. For people with diabetes, however, this abrupt change can be observed between 100µm -250 µm. This suggests that the SC thickness is increased and more variable on the arch skin in people with diabetes than in people without diabetes.

Although these data demonstrate that the water content of the SC in people with diabetes is significantly lower than that of age-matched non-diabetics in the ventral forearm, this is not reflected in the data obtained from the plantar SC.

7.4.3.2. NMF gradient

The NMF gradient of the plantar and non-plantar skin of age-matched people with and without diabetes has also been observed for differences at each measurement site using the Mann-Whitney U test. Unfortunately, these data were also impacted by a high signal to noise ratio (as described in section 7.4.2.2.). Arch and heel data collected at superficial measurement depths less severely affected by this issue have been compared between groups and do not significantly differ at any measurement depth (See Figure 77).

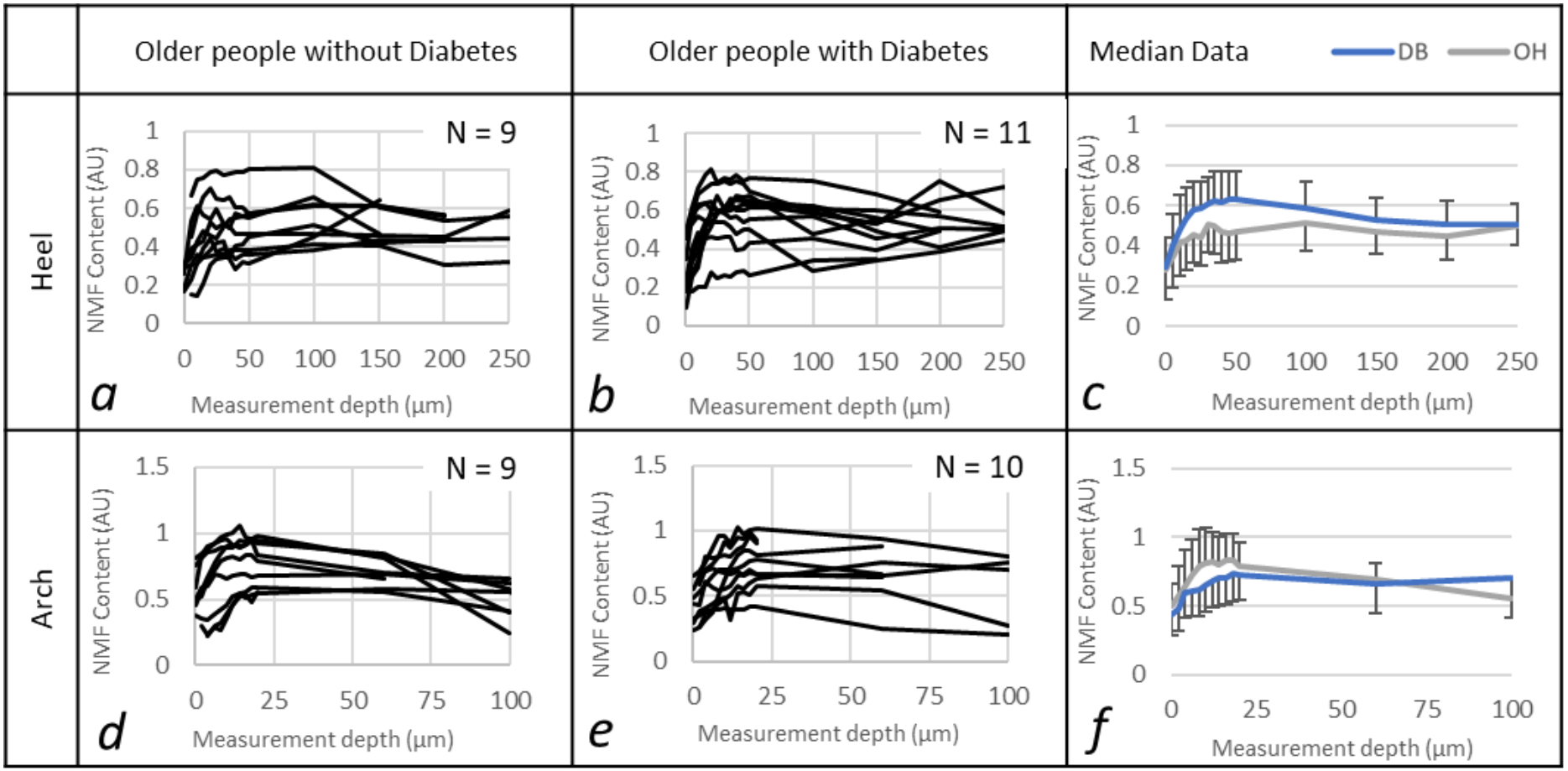


Figure 77. NMF content of skin in age-matched Diabetic and Non-Diabetic ('Older Healthy') participants. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants

The participants with diabetes do not appear to display the same increased variability in NMF gradient as they do water gradient. However, due to the small volume of data available, comparisons and conclusions should be made with caution.

The volume of NMF in the plantar SC of participants with diabetes is not significantly lower than that of age-matched non-diabetics at any depth.

7.4.4. Comparison between participant groups – Emollient treated and untreated young people.

Data were collected on the volume of water, NMF, and urea in treated and untreated plantar skin of healthy young people. Most datasets used in this study were parametric in distribution (tested using the Shapiro-Wilks test), however, several from each skin location were non-parametric. Therefore, all data analysis was completed using non-parametric tests.

These data provide the opportunity to address the hypothesis related to objective 3 (see Section 7.2.2.2. Objective 3 hypotheses).

7.4.4.1. Hydration gradient

The water content of treated plantar skin does not differ from the water content of untreated plantar skin at any measurement depth to a statistically significant level (See Figure 78).

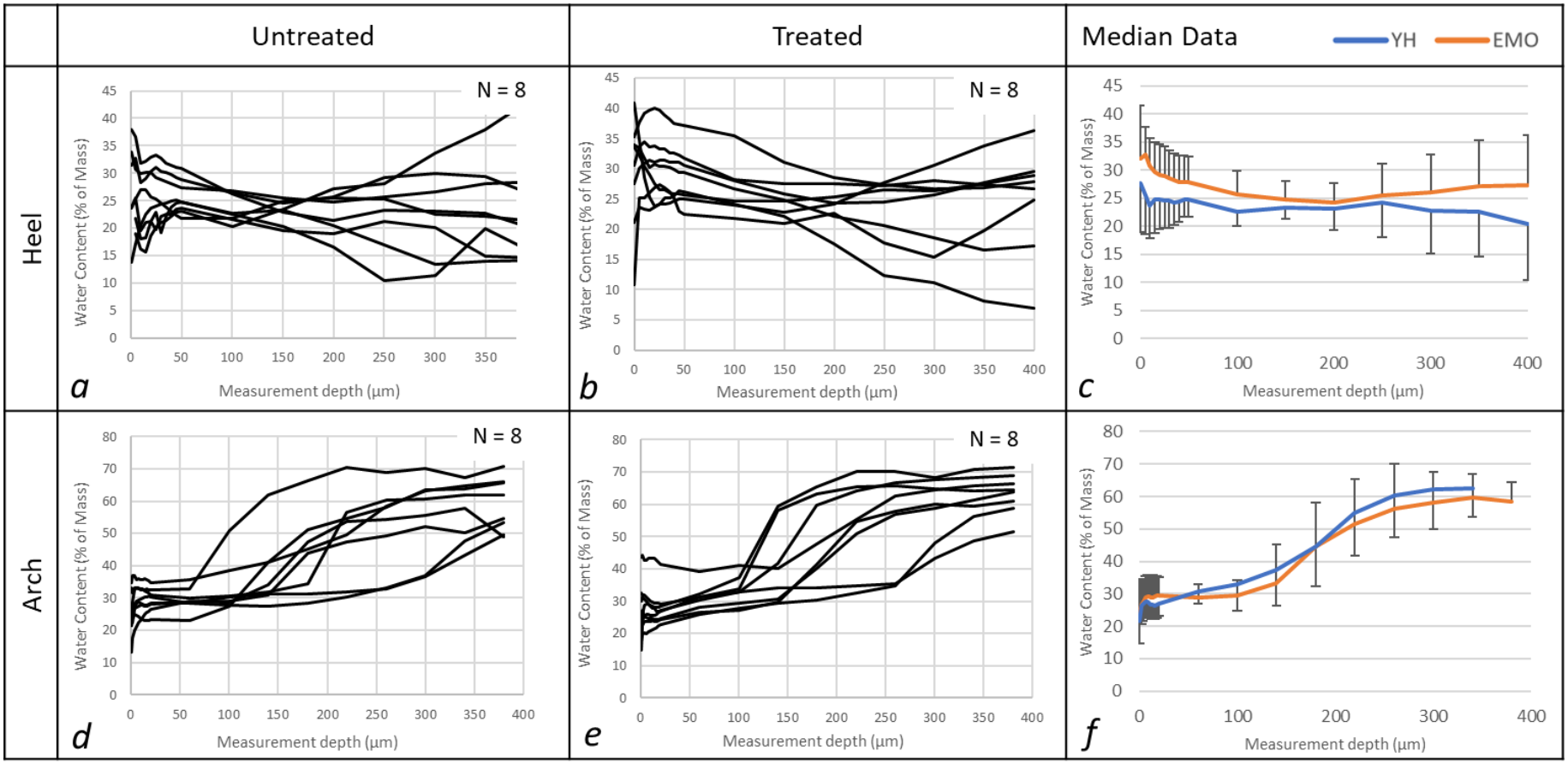


Figure 78. Water content of treated (EMO) and untreated (YH) skin. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants.

On the heel the median water content of the treated SC is approximately 5% higher (water as % of tissue mass) than untreated SC at the skin surface and down to 100 µm depth, however this difference is not statistically significant when tested using a sign test (p-value >0.05). No differences are visible between the hydration gradient of treated and untreated arch skin.

The data indicates that in a healthy, young population, the water content of the plantar SC is not influenced by emollient application across a depth of 0-400 µm.

7.4.4.2. NMF gradient

Similarly to the water gradient, the NMF gradient within the plantar tissues does not show any statistically significant differences between treated and untreated tissues at any depth (See Figure 79).

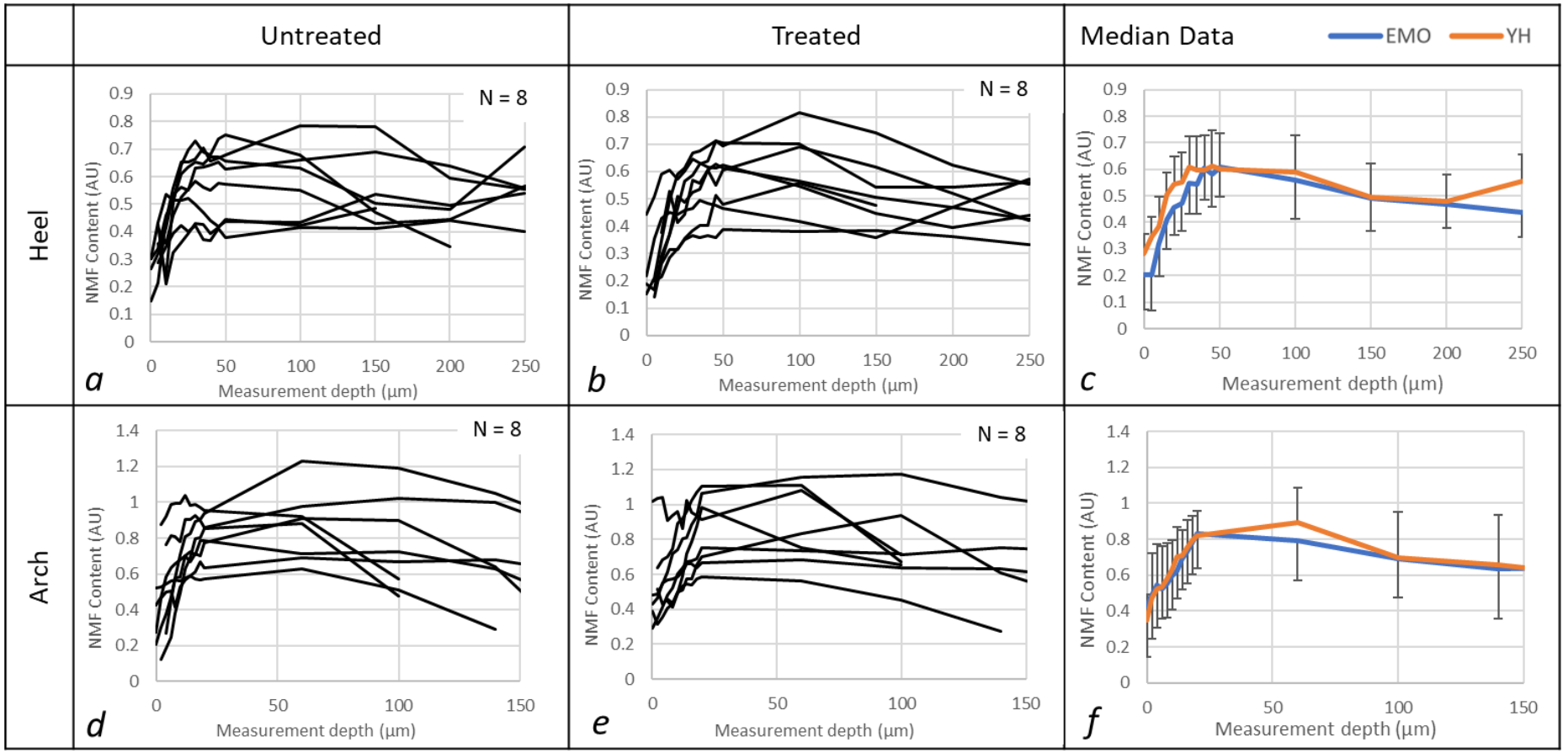


Figure 79. NMF content treated (EMO) and untreated (YH) skin. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants.

Both on the heel and arch sites the composition of NMF across tissue depth is consistent between treated and untreated tissues. No significant differences are identified using the sign test at any depth.

This finding indicates that in a healthy, young population, the NMF content of the plantar SC is not influenced by emollient application.

7.4.4.3. Urea gradient

A sign test found that urea content of treated heel SC was significantly higher than the urea content of untreated heel SC at measurement depths of 5-50 μm (p-values (respectively for depths increasing in steps of 5 μm): 0.031, 0.016, 0.016, 0.008, 0.016, 0.008, 0.016, 0.008, 0.008, 0.008) (See Figure 80).

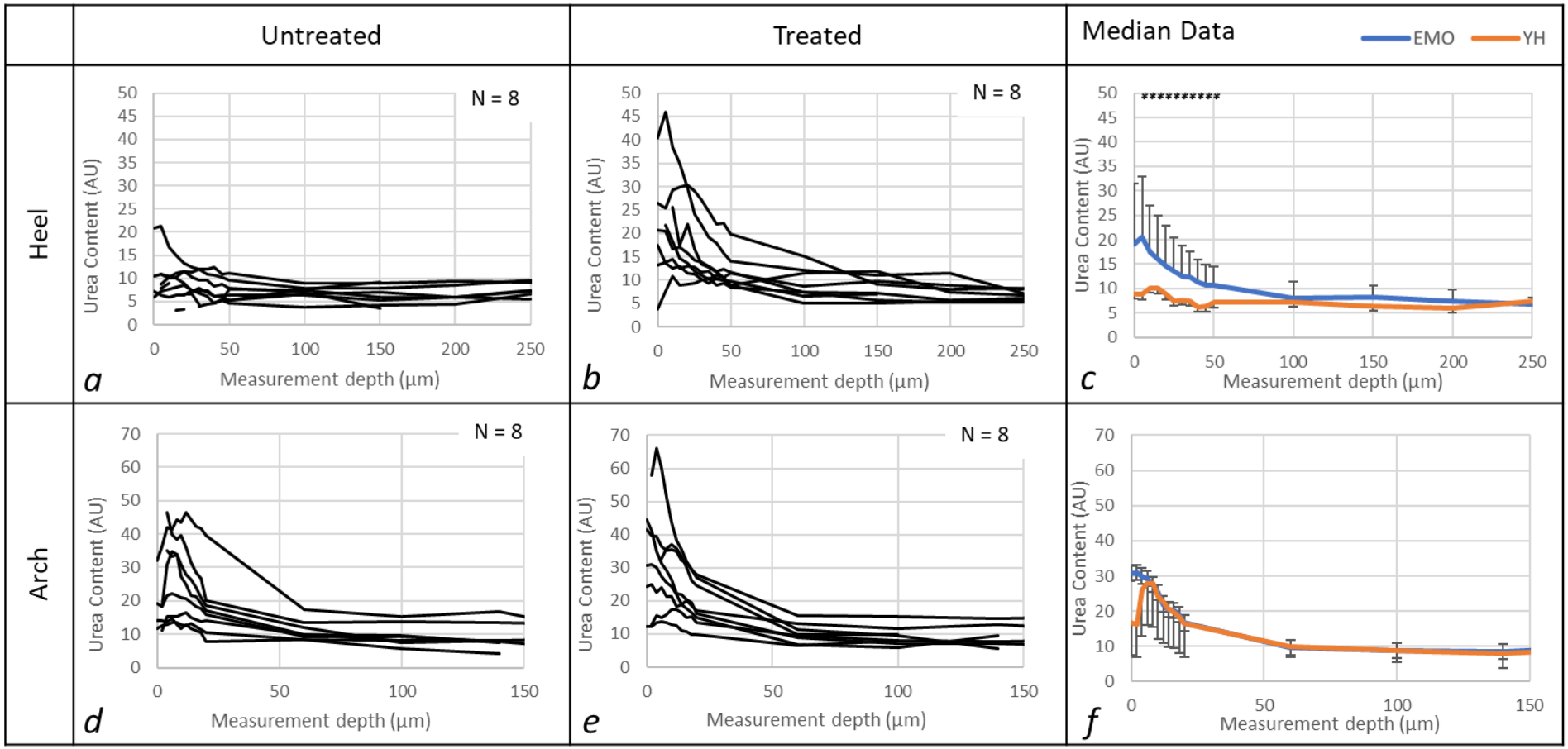


Figure 80. Urea content treated (EMO) and untreated (YH) skin. Leftmost two columns: individual data represented by a black line. Rightmost column: median values of all participants. Significant differences identified using a sign test are indicated by an asterisk.

No significant differences were found at any measurement depth within the arch skin, although the urea content of the arch skin does appear to be higher superficially in treated skin down to a depth of around 10-15 µm.

These data indicate that by applying emollient containing urea, the urea content of the plantar heel SC is significantly increased to a depth of 50 µm.

7.5.6. Further Statistical Analysis

7.5.6.1. Application of the Bonferroni Correction

The statistically significant differences identified in the between and within-group comparisons demonstrated above have been re-assessed with a p-value modified using the Bonferroni correction (See Table 62). Due to the varied number of measurement depths used within these comparisons (and therefore the number of comparisons being made) the modified p-value for the ventral forearm is different from that of the heel and arch. With the p-value corrected for multiple comparisons, no results reach statistical significance.

Table 62. Application of the Bonferroni Correction to statistical tests that originally produced 'significant' results.

Groups	Material	Location	Number of Comparisons	Modified significance level	Measurement Depth (µm)	*p*-value	Significant?
OH V YH	Water	VF	17	0.0029	2	0.034	No
					4	0.012	No
					6	0.006	No
					8	0.021	No
					10	0.021	No
					12	0.016	No
					14	0.027	No
					16	0.043	No
	NMF	ARCH	13	0.0038	60	0.5	No
Db V Non-Db	Water	VF	17	0.0029	0	0.026	No
					2	0.013	No
					4	0.011	No
					6	0.022	No
					8	0.026	No
					10	0.022	No
					12	0.026	No
					14	0.019	No
					16	0.016	No
					18	0.048	No
					45	0.036	No
					50	0.042	No
Treated V Untreated	Urea	Heel	13	0.0038	5	0.031	No
					10	0.016	No
					15	0.016	No
					20	0.008	No
					25	0.016	No
					30	0.008	No
					35	0.016	No
					40	0.008	No
					45	0.008	No
					50	0.008	No

7.5.6.2. Mixed Effects Model

A mixed effects model has also been applied to these comparisons to negate the statistical complexities associated with the application of repeated tests and the assumptions these tests make about the data (McCulloch & Searle, 2001).

No significant differences were identified between groups using this model, except for the volume of urea found in the heel between the treated and untreated skin groups (See Table 63).

Table 63. Mixed effects model results.

Comparison	Material	Location	p-value	Significant?
Treated and untreated skin of young people	Urea	Heel	0.046	Yes
		Arch	0.526	No
	NMF	Heel	0.507	No
		Arch	0.771	No
	Water	Heel	0.402	No
		Arch	0.79	No
Young people and older people without diabetes	Urea	Heel	0.518	No
		Arch	0.21	No
	NMF	Heel	0.728	No
		Arch	0.57	No
	Water	Heel	0.628	No
		Arch	0.762	No
		VF	0.774	No
Older people without diabetes and older people with diabetes	Urea	Heel	0.436	No
		Arch	0.123	No
	NMF	Heel	0.846	No
		Arch	0.861	No
	Water	Heel	0.512	No
		Arch	0.5	No
		VF	0.546	No

7.5.7. Additional results

To support the discussion of the results presented in Section 7.6, some additional results are presented below:

7.5.7.1. SC thickness

The thickness of the SC is highly relevant to the discussion of the hydration gradient. Below, the analysis results of the SC thickness of the ventral forearm are described. Unfortunately, due to limitations of the SkinTools 3 software, the thickness of the plantar SC has not been calculated. Therefore, this analysis has not been undertaken for the arch or heel data.

SC thickness data were parametric in distribution (Shapiro Wilks test: YH p-value = 0.899, OH p-value = 0.110), but the assumption of homogeneity of variances was violated, as assessed by Levene's test for equality of variances (p-value = 0.045). A Welch T-test was used, which showed that although the mean SC thickness of the young people group was 4.39 ± 2.34 µm (Mean ± standard error) higher than the SC thickness of the older people group, this difference was not statistically significant (p-value = 0.085) (See Figure 81).

Figure 81. A boxplot showing the thickness of the ventral forearm SC of young participants and older participants without diabetes.

A comparison was also carried out for the ventral forearm SC thickness of people with diabetes and people without diabetes (See Figure 82) SC thickness data were parametric in distribution (Shapiro Wilks test: OH p-value = 0.110, Db p-value = 0.992), and there was

homogeneity of variances, as assessed by Levene's test for equality of variances (p-value = 0.824). Therefore, an Independent Samples T Test was applied. This showed that the SC of the skin of people without diabetes was 4.11 ± 2.6 µm (Mean ± standard error) thinner than the SC of the diabetic population, although this difference was not statistically significant (p-value = 0.134) (See Figure 82).

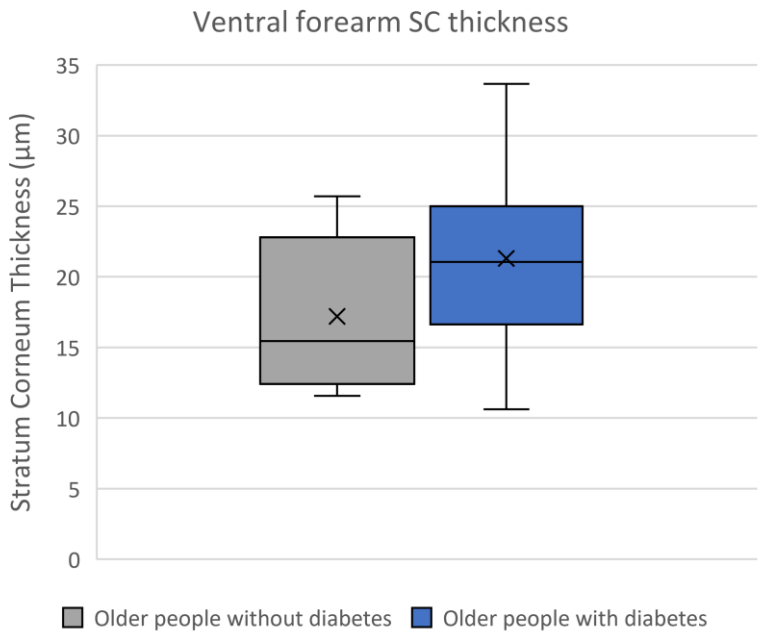


Figure 82. A boxplot showing the thickness of the ventral forearm SC of older people with diabetes and age-matched non-diabetics.

7.5.7.2. Water and NMF content of skin

Figure 83 demonstrates the volume of water and NMF within the plantar SC at the help and arch, plotted onto the same x-axis representing measurement depth. In most instances, as the water content increases, as does the NMF content of the tissue. This is particularly evident in graphs *b, c, d,* and *f.*

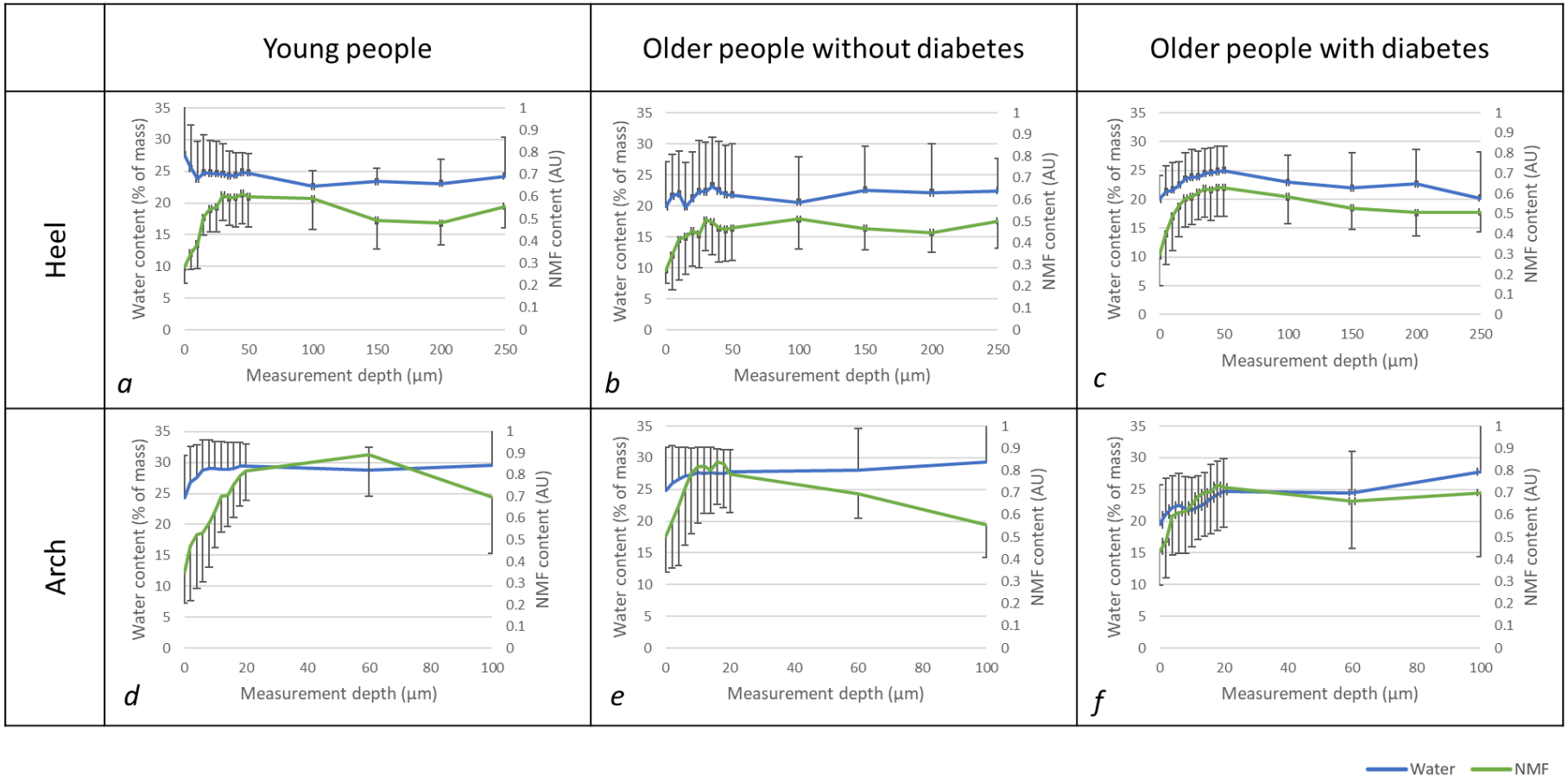


Figure 83. A demonstration of the water and NMF content of the heel and arch for three participant groups.

7.5.7.3. Hydration gradients of treated and untreated skin

In this study, the application of Scholl Intense Nourish was not shown to significantly increase the water content of the heel skin at any measurement depth when measured using CRS (See Figure 84).

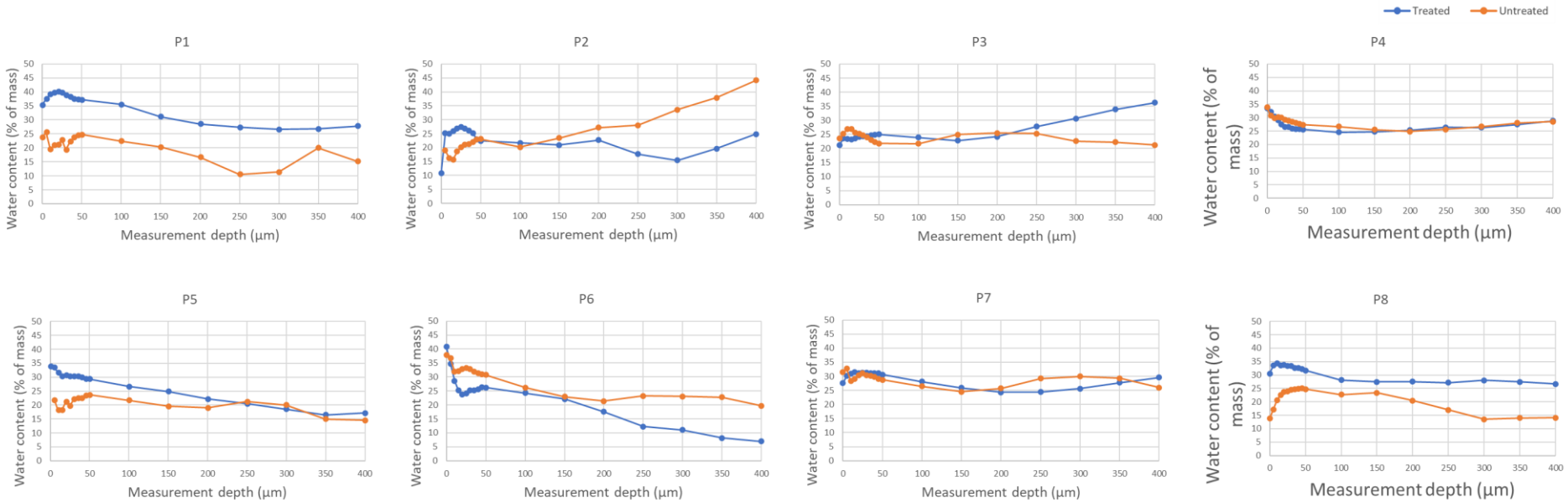


Figure 84. A graph for each participant showing the water content of the treated and untreated heel skin.

Some individuals do appear to have increased water content in the treated skin than the untreated skin (Participants P1, P5, and P8). These changes were not sufficient to generate a statistically significant difference between the median water content of treated and untreated skin across the full cohort.

This change is not reflected in the equivalent arch data (See Figure 85). These data do, however, demonstrate the similarity in the hydration gradient of tissues at the same skin site from different sides of the body.

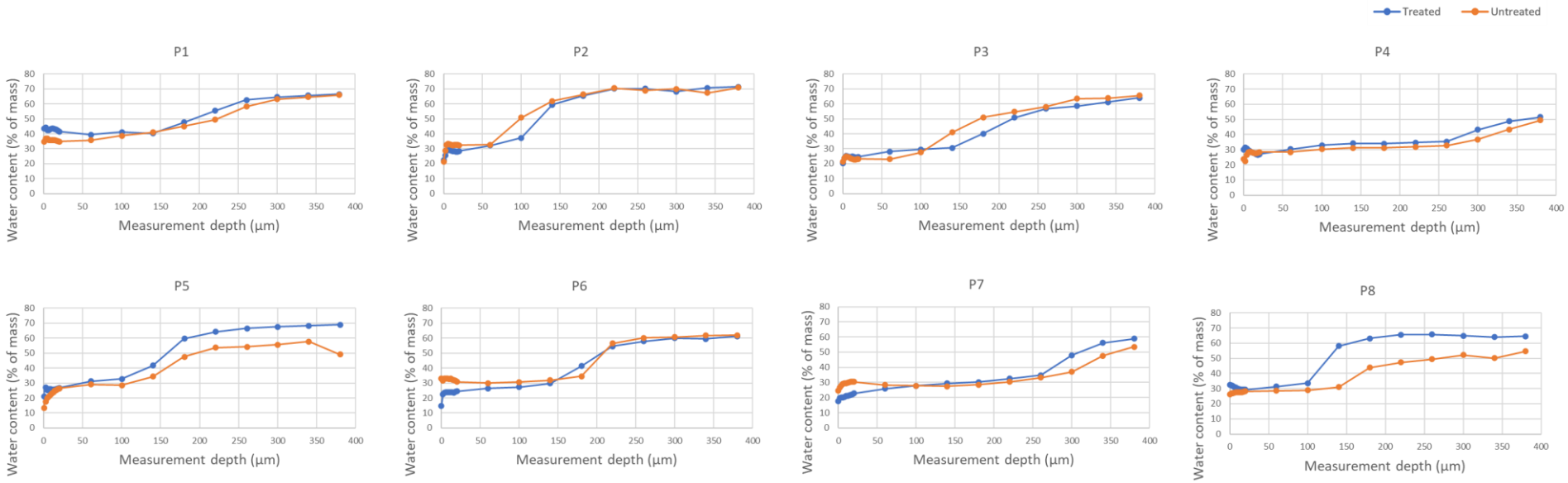


Figure 85. A graph for each participant showing the water content of the treated and untreated arch skin.

7.6. Discussion

This is the first time that data of this kind has been collected on foot skin. This provides a basis for comparison with measures taken in previous research using different methods. This section will begin with examining the gradation of water and NMF in the SC of young, healthy individuals compared to similar data collected using CRS previously from comparable skin sites. This will be followed by exploration of the outcomes of this study relating to differences between and within participant groups, alongside other instances in which this has been achieved using various non-invasive measurement techniques. Finally, the statistical analysis techniques, along with method design will be discussed. The merits and limitations of the methods will be outlined.

7.6.1. Hydration gradient

The hydration gradient in the SC to varies in shape between the ventral forearm, arch, and heel sites. The ventral forearm and arch SC both demonstrated an initial low water content (20-30% of tissue mass), followed by a rise and a plateau as the viable epidermis was reached (15-25 µm depth). The initial low hydration within the arch SC (20-35% of tissue mass), however, extended more deeply (from 50-200 µm) into the tissue than in the ventral forearm SC. On the heel, the initial low hydration (20-30% of tissue mass) extended deep into the tissue, seemingly beyond the maximum 400 µm measurement depth of the Gen2-SCA.

The hydration gradient of the ventral forearm in a healthy young population has previously been reported by Egawa et al (2007) and shows similar features to our equivalent young healthy data (See Figure 86), starting at 30-40% tissue mass within the superficial approximately 5 µm of the SC and rising to 60-70% at approximately 20 µm depth. These values and the characteristic shape of the gradient are reflected in the current research.

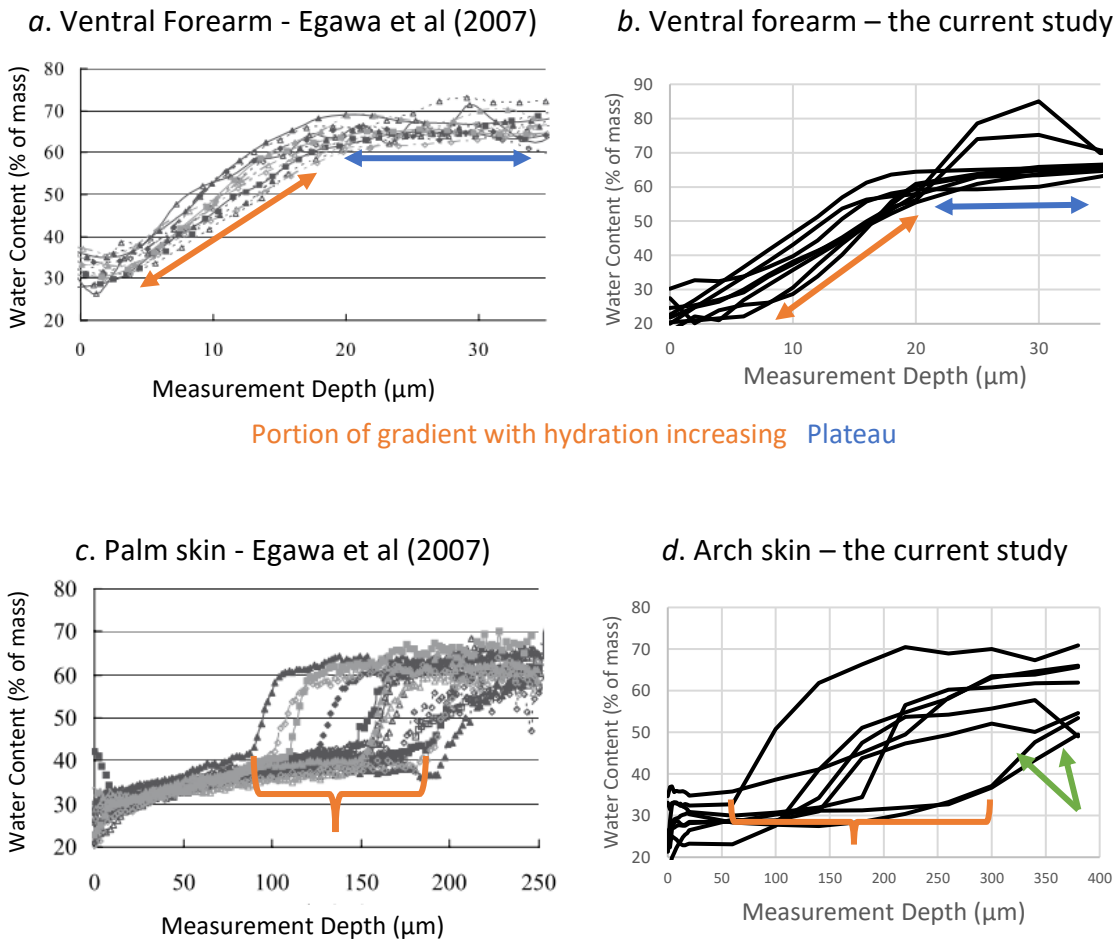


Figure 86. Comparison of data from 'In vivo Estimation of Stratum Corneum Thickness from Water Concentration Profiles Obtained with Raman Spectroscopy' (Egawa et al., 2007) (a and c) and equivalent data from this study (b and d).

There are no published data for the arch and heel SC hydration using CRS methods. However, some data are available on the palmar skin, which is the most similar skin surface characterised in this manner. The palmar hydration gradient is markedly different to other non-plantar and non-palmar sites. The superficial water content of the tissue is lower (20-30% of tissue mass) and extends much further into the tissue, rising very slowly for an extended period (between 80 and 190 µm depth) before increasing rapidly and plateauing at between 55-70% of tissue mass (between 100-200 µm depth (See Figure 86, image *c*). This extended region of low hydration represents the thick SC on the palmar skin (Egawa et al., 2007).

The hydration gradient of the arch skin is similar in shape to the palmar skin, starting with an extended superficial low hydration level (20-30% of tissue mass from the surface to 50-300 µm), eventually rising to a plateau at a higher water content (55-70% of tissue mass at 200-250 µm) (Figure 86, image *d*). This demonstrates that the arch skin has a similarly thickened superficial low-hydration portion to the palm. However, there is more variability between participants in the arch regarding the skin depth at which the initial rise occurs and the level at which water volume in tissue eventually plateaus.

This may be the result of the homogeneity of the skin sites used within these studies: Egawa et al (2007) collect palm data from the ball of the thumb. This area is well-defined, and the texture of the tissue is consistent across the finger pad. The arch site measured in this study, however, lies at the boundary between the plantar and non-plantar skin. This may account for some variability in the skin characteristics at this location (Boyle et al., 2019; Maceo, 2009; Uemura et al., 2016). Additionally, depending on the arch profile of individuals, and their propensity to pronate during walking, some individuals would be more prone to weight-bear on this area of skin. This would further introduce variability into the characteristics of the medial arch skin (Boyle et al., 2019; Dun Jack Fu, 2014; Swensson et al., 1998).

For two participants (labelled in Figure 86, image *d*), the water content of the arch tissue appears to be steadily increasing from 200-250 µm onwards. It does not plateau before the maximum measurement depth of 380 µm. This suggests that the full thickness of the SC extends beyond this measurement region. The increased SC thickness in these individuals may be reflective of the skin being measured more laterally in these people (reflecting more characteristics of plantar skin), skin changes resulting from weight-bearing on this tissue, or callus growth at this skin site resulting from shear (i.e., if an individual wears footwear or orthoses that rub on this skin site). In future, it would be beneficial to collect static and dynamic plantar pressures of the foot skin in weightbearing to contribute to the understanding of which plantar tissues are weightbearing. Additionally, data relating to footwear use and sporting activities would inform discussion around recurrent shear that could modify skin characteristics.

At the heel the water content of the heel skin remains relatively consistent across the full 400 µm measurement region. From this, it is assumed that the initial ‘low hydration’ portion of

the hydration gradient of the heel extends beyond 400 µm, which aligns with what we know of the thickness of the SC on the heel (Vela-Romera et al., 2019) (See Figure 74).

7.6.2. NMF concentration gradient

The concentration profile of NMF in the skin has not been widely explored and has never been examined on the human foot. The limited data available is from work conducted by Egawa et al (2008) which provides a demonstration of the volumes of various lipids and skin constituents the SC depth on the ventral forearm of 45 healthy Japanese volunteers.

Unfortunately, due to the low-quality of fingerprint-region spectra obtained from the ventral forearm within the current study, insufficient data were obtained on the volume of NMF within the SC at this location for comparison with the work of Egawa et al (2008).

However, data collected on the NMF volume of the arch and heel SC is available (See Figure 87). The data provided by Egawa et al (2008) is not provided alongside any indication of actual material volume (or equivalent arbitrary units from RiverD). Therefore, comparisons between our data and these are limited to discussion of the shape of the concentration profile across SC depth.

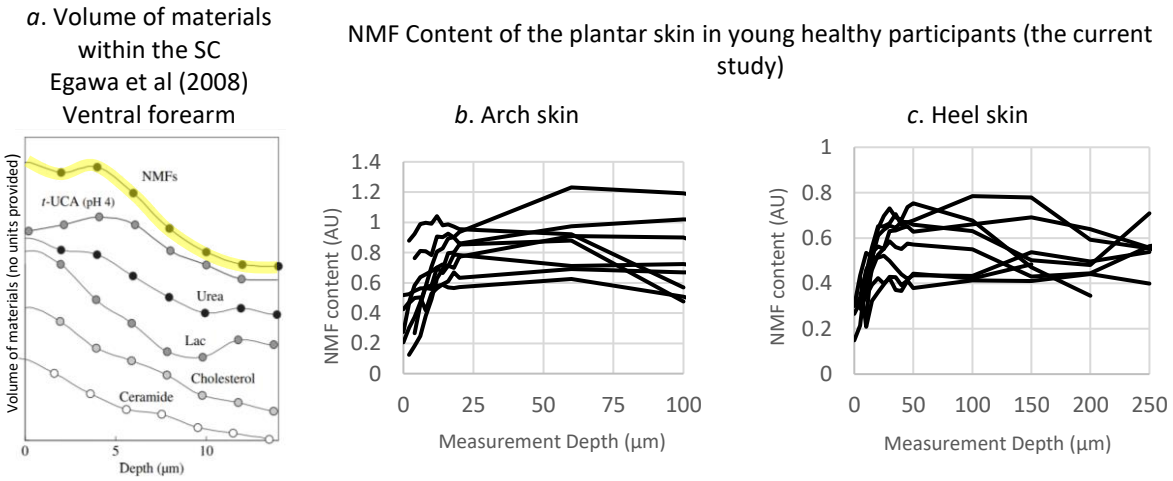


Figure 87. NMF concentration gradient data from Egawa et al (2008) (highlighted yellow) (a) and this study (b and c). Used with permission.

The data reported in this chapter reveals a low (relative to the overall magnitude of the gradient) concentration of NMF at the surface of the SC which increases rapidly in the superficial (arch: 0-12 µm, heel: 0-35 µm) SC. This is followed by a plateau or a decline in NMF

volume in the SC from this measurement depth onwards for most participants. Plotted data collected by Egawa et al (2008) does not have a similar shape at the superficial SC, instead, it is consistently flat in the uppermost 5 µm, and decreases gradually from 5 µm onwards.

The NMF concentration at the SC in the arch and heel may reflect different SC layers at these skin sites, not the SC thickness directly (as this extends beyond the measurement depth of the Gen2-SCA (Strzalkowski et al., 2015)), but structures within SC:

The stratum compactum and stratum disjunctum are layers within the stratum corneum that display different properties. The stratum compactum has well-ordered, highly compacted corneocytes, whereas the overlying stratum disjunctum has a looser arrangement of corneocytes with fewer corneodesmosomes (A. Rawlings et al., 1994). The stratum disjunctum has also been found to have reduced lipid content (Elias et al., 1988), increased susceptibility to influence by topical applicants (Fartasch et al., 1997), and larger, more hydrophobic and less fragile corneocytes (Harding et al., 2003).

It is possible that the different in the composition of the SC at its uppermost surface is reflective of the stratum disjunctum, with the loss of SC materials being a result of its irregular cell structure and propensity to be affected by topical applicants or surfactants.

The findings of this study support this theory. The increased thickness of the SC at the heel (greater than the arch) aligns with an increase in the 'wash out' depth of NMF and the penetration depth of urea:

- At the heel, the urea content of the treated SC is higher than the untreated SC down to a depth of 50-100 µm (albeit these are not statistically significant beyond 50 µm). In contrast, this effect is only observed on the arch to approximately 25 µm (no statistically significant differences).
- On the heel, the NMF 'wash out' region reached approximately 50 µm depth, whereas on the arch this is approximately 20-25 µm.

This speculative difference in the structure of the SC on the plantar foot is important to the interpretation of plantar skin data collected using measurement devices with a penetration depth superficial to the lower boundary of the stratum disjunctum. This theory will be discussed further in Chapter 8 in the context of commercially available hydration measurement devices that collect data in this region.

Irrespective of the conjecture of the structural differences between the plantar and non-plantar tissues relevant to the volumes of NMF within these tissues, there are other limitations applicable to this comparison. The data presented by Egawa et al (2008) represents the average NMF content of the skin from 45 participants, no data are provided on the individual profiles or the dispersion of data at each measurement depth. This removes the opportunity to draw comparisons between the data variability across SC depth. Similarly, the lack of material units inhibits direct comparison of material volumes between locations, which would provide useful insight into the differences in plantar and non-plantar NMF values that have not previously been observed.

In future, histological analysis of the stratum corneum on the plantar foot could confirm or dismiss the theory raised regarding the thickness of the stratum disjunctum on the plantar foot, and how this relates to the SCs ability to retain or uptake materials at this location.

It would be beneficial to examine the thickness of the stratum disjunctum and stratum compactum in hyperkeratinisation. Hyperkeratosis are formed due to an interruption into the physiological processes that facilitate desquamation (Kim et al., 2010; Rubin, 1949; Thomas et al., 1985). This may result in the thickening of both SC layers simultaneously or thickening of either layer independently. With further research into the penetration depth of emollient materials into the plantar SC, it may be possible to formulate a product that targets the SC layer responsible for hyperkeratosis.

7.6.3. Differences between groups: Young Healthy and Older Healthy

7.6.3.1. Water

Within the current study, the older participant group was found to have higher water content within the SC of the ventral forearm than the younger participant group at measurement depths 2-16 µm. This portion of the SC is where the water content is rapidly increasing, and these differences appear to result from a difference in the trajectory of these changes between the groups. i.e., the water content of the SC in the older healthy group appears to be increasing faster. These differences are reflective of the increased SC thickness in the younger participant group (although the difference between SC thickness in these two groups was not statistically significant) (See Figure 81).

This observation contradicts the findings of Egawa et al in 2007, in which data from two groups of women of different ages (n=31) (young: 22-40 years (mean 32) and old: 59-76 years (mean 67)) were compared. Younger participants were found to have higher SC water and a thinner SC (See Figure 88). Boireau-Adamezyk et al (2014) similarly reported increased thickness with age (n=40).

This disagreement could be presumed to be the result of sex-differences between studies, as Egawa et al (2007) and Boireau-Adamezyk et al (2014) only tested female participants, however Choe et al (2018) found 'modest' positive changes in ventral forearm SC thickness with age in a mixed-sex cohort.

Choe et al (2018) also identified these changes in a smaller participant group (n=11) than was involved in this study (n=19), limiting the speculation that these may be the result of small participant numbers.

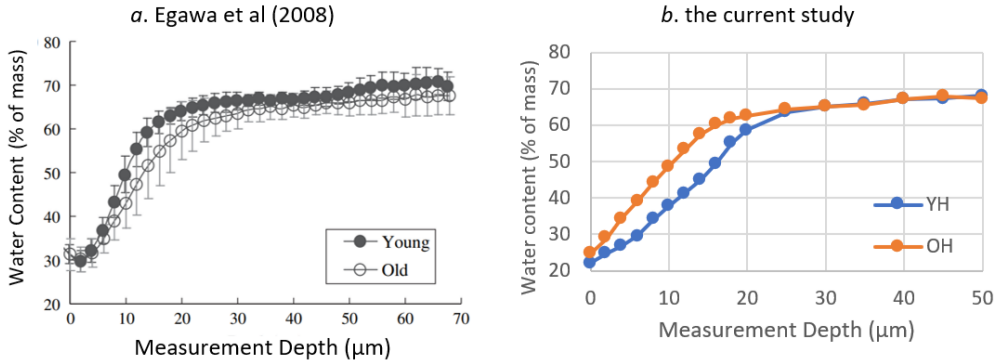


Figure 88. Hydration gradient of the ventral forearm skin from participants of two age groups from Egawa et al (2008) (a) and the current study (b) (YH: young people, OH: older people without diabetes).

Seasonal changes in skin thickness could also be considered concerning the findings of the current study (Nam et al., 2015), which was conducted in September-December. However, Boireau-Adamezyk et al collected data during a similar season: September-October in France; and Egawa et al (2008) studied the differences in skin hydration gradient across seasons (n=27) and found few differences between seasons. Choe et al (2018) did not publish the time of year when data-collection was undertaken.

This inconsistency with published literature is not entirely unexpected, as described in section 2.5., there is inconsistency in the differences identified between participant groups throughout the limited instances of CRS use. Additionally, the primary objective of this work

is not to study the SC thickness between groups on non-plantar skin, but rather to observe for any changes in plantar skin composition between groups. Nonetheless, this incongruency with published data is worthy of consideration when drawing conclusions from the data collected within this study.

7.6.3.2. NMF

Within this study, the NMF content of the plantar SC does not appear to be consistently higher or lower for one participant group. Egawa et al (2008), however, found that NMF content of the SC of the ventral forearm increased with age (See Figure 89).

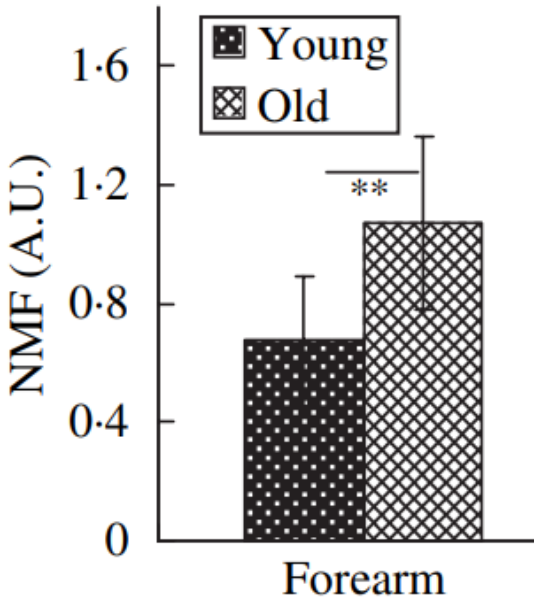


Figure 89. NMF content of the ventral forearm skin in a young and older participant group collected by Egawa et al (2008). Used with permission (n=31).

Unfortunately, these data are not entirely comparable to those obtained within this study:

Due to low signal-noise ratio for the fingerprint region of ventral forearm CRS measures, no directly equivalent data are available from this study. Only data from the plantar heel and medial arch are available. It is not known how the volume of NMFs in the plantar SC relate to NMF concentration elsewhere in the SC.

Also, the findings of Egawa et al (2008) are given as a value representing the total volume of NMF in the SC between the skin surface and 8 µm into the SC, not as a concentration gradient.

This method of data presentation prevents exploration of where within the SC differences emerge between the concentration gradient of NMF in the SC between participant groups. This information would provide insight into where within the SC changes occur to NMF volume which could be useful for formulating emollients that are intended to replenish NMF lost with age.

7.6.4. Differences between groups: older people with diabetes and age-matched non-Diabetics

Although some investigations have been conducted to investigate the use of CRS on the skin of people with diabetes previously, none have directly measured the skin composition and compared this to equivalent non-diabetic populations, either on the foot skin or on non-foot skin.

7.6.4.1. Water content

In the current study, people with diabetes were found to have significantly less water in the SC of their ventral forearm (p-value <0.05 for measurement depths 0-18 µm). These differences also extended into the viable epidermis (p-value <0.05 for measurement depths 45-50 µm). This indicates that these differences are not the result of a difference in SC thickness, but a reflection of a change in water content of the whole tissue associated with the disease process of diabetes.

These results are consistent with comparable investigations into skin hydration (of non-foot sites) and diabetes status conducted by Sakai et al (2005) and Park et al (2011). Albeit these only indicated the superficial skin hydration due to their use of conductance or capacitance-based devices to measure skin hydration at a superficial level (the Skicon 2000 and Corneometer® CM825), these found skin hydration reduced with poor glycaemic control.

Within the data collected in the current study, large variability in the water content of the skin was exhibited at the heel and plantar 3rd metatarsal head, in both people with and without diabetes. It is possible that, with a larger number of participants, a repeat of this study would generate a statistically significant difference in the water content of the plantar

SC, similar to that demonstrated on the ventral forearm. This would align with the results of investigations of Sakai et al (2005) and Park et al (2011), and what is known of the plantar SC in diabetes.

7.6.4.2. NMF

Within the current study no statistically significant differences were identified in the volume of NMF in the SC on the ventral forearm, heel, or arch skin between people with and without diabetes. There is some evidence available, through investigation using other measurement methods, that indicate NMF in the SC is influenced with diabetes:

Lechner et al (2019) reported slightly higher NMFs within the surface biomarkers extracted at the dorsal foot and the plantar heel, although these differences did not reach statistical significance: Dorsal foot (diabetic, non-diabetic (mean ± SD): 101.7 ± 70.4, 65.0 ± 37.1) and plantar heel (199.0 ± 113.2, 148.4 ± 86.06).

Due to the large variability in NMF data collected within the current study, it may be the case that a larger cohort would generate data more reflective of the small NMF changes observed by Lechner et al (2019).

The volume of NMF within the tissue is also linked to the structure of the epidermis (Nakagawa et al., 2004) which is of interest within this work. In Figure 77, the initial period in which NMF content rises ranges from 0 µm to approximately 50 µm in the heel, and 20 µm in the arch for individuals with diabetes. For those without diabetes, however, this extend to approximately 40 µm in the heel, and 10-15 µm in the arch. As discussed, this area of variability depth may be representative of the uppermost, disorganised layer of the SC, the stratum disjunctum. If this is the case, this layer is shown to be thicker in people with diabetes than people without diabetes.

7.6.5. Differences within groups: treated and untreated skin

Despite applying an emollient to one foot for seven days before testing, there were no statistically significant differences in the water or NMF content of the treated and untreated heel or medial arch skin. Application of the emollient was anticipated to result in a rise in SC

hydration and NMF content, as demonstrated by the hypotheses formulated for this investigation (See 7.2.1.) and purported by the manufacturer.

Emollients are typically used to restore materials lost from the skin due to a disruption in the skin barrier – i.e. following exposure to surfactants. NMF may be washed from the skin and reduce the skins ability to retain water (A. V. Rawlings et al., 1994). The resulting changes to the skin surface (increased flaking, disruption in the lipid matrix, for example) then allow for further penetration of external materials, which can worsen the problem. This is one aspect of the dry skin cycle (Rawlings & Matts, 2005).

In skin pathology, the dry skin cycle is broken through the application of an emollient which supplements the materials that have been lost from within the skin (water and NMFs for example), or (in the case of a more lipid-heavy emollient) occludes the skin surface to allow restoration of these from within (Rawlings & Matts, 2005).

When the skin barrier is functioning well, as it is within this healthy, young population, it will resist the ingress of external materials (Rawlings & Matts, 2005). As described, skin with pathology is less resistant to penetration. The healthy status of the foot skin within this population limits the transferability of the data obtained within this study to people with skin pathology.

In future, it would be useful to measure the penetration of emollients into the SC in people with varying degrees of xerosis. This would provide an opportunity to observe if or how emollient components penetrate the skin surface and replenish lost NMF and water because changes to the skin barrier.

The only difference in composition that is identified between the treated and untreated skin is urea volume. Within the superficial layers of the SC the urea content is increased on the treated skin, up to a depth of 10-15 μm in the arch skin and 50 μm in the heel skin (this difference is statistically significant at depths of 5-50 μm in the heel).

Although this has not resulted in increased water volume within the tissue (as demonstrated by the consistency in hydration gradient at these depths between the treated and untreated skin), this is a useful indicator of the penetration depth achievable for materials applied topically to the foot skin. The urea has penetrated further into the heel skin than the arch

skin, which has a thinner SC. This indicates that the penetration depth of urea is proportional to the thickness of the SC, possibly due to the structural and chemical alterations evident in the uppermost SC – characterised as the stratum disjunctum by Elias et al (Elias et al., 1988).

7.6.5.1. Examination of hydration gradient for treated and untreated skin for individuals

In Figure 84, the hydration gradient of treated heel skin has been broken down into the 8 individuals involved in this study. Water content in the superficial SC appears to be higher than that of untreated heel skin for participants 1, 5 and 8. These changes are not evident in the other participants, which has resulted in no statistically significant differences being identified between the water content of treated and untreated skin across the full group, as discussed in the previous section.

This may be the result of physiological differences between the water content of the heel skin prior to emollient therapy: the individuals who show a response to emollient therapy had lower superficial SC water content on the untreated heel skin (0-50 µm depth mean water (% of mass) 22.6, 21.4, 22.3) in most instances than individuals who did not show a response (0-50 µm depth mean water (% of mass) 20, 24.5, 29.5, 33, 30.1). If the hydration gradient of the heel skin is consistent between the left and right side of the body, this suggests that the individuals whose hydration gradient changed as a result of emollient application, did so as a result of a physiologically lower water content.

Similar data has been presented from the skin of the medial arch (See Figure 85). In these data, there are no clear instances in which the hydration gradient of the SC has been changed due to emollient therapy and there are no differences evident between the physiological skin hydration between individuals who did (0-60 µm depth mean water (% of mass) 35.8, 22.7, 27.5) didn't respond to emollient therapy (0-60 µm depth mean water (% of mass) 31.4, 23.4, 27.3, 32.1, 28.9). This data supports the theory discussed in the previous paragraph: consistency between hydration gradient of equivalent skin on different body sides is demonstrated, and when no physiological difference in the SC hydration is observed, no effect of emollient therapy is shown. Although these data do also raise the question: why does the physiological hydration of the heel skin differ between responder and non-responder, and not the arch?

The answer to this may lie in the structure of the SC at the arch and heel. As has been discussed, the superficial stratum disjunctum appears thicker on the heel skin. It is possible that this area of tissue is where physiological differences in SC hydration are observed, due to the altered lipid composition and cell structure.

These findings when individuals' response to emollient therapy are observed, further support the suggestions made at the conclusion of section 7.6.5., that future investigations should include individuals with varied baseline skin hydration.

7.6.6. A discussion of NMF and water content of the plantar skin across its depth

Due to the role of NMF in binding water in the skin, it was anticipated that higher volumes of NMF in the skin are associated with higher water content. Figure 83 shows the hydration gradient of the arch and heel skin for each participant group, with the NMF volume overlaid on a secondary axis. From the tissue measurement depths used within this study, the volume of these materials appears to change in the same way at equivalent depths i.e., increasing in volume between 0 and 50 µm depth, followed by a plateau.

This finding supports the validity of the data collected within this study, as it follows the pattern expected of these data. In future, it would be useful to examine the correlation between these data using statistical analysis. If the correlation between the volume of NMF and water content across tissue depth were proven to be consistent and strong, this would remove the need for both compounds to be quantified from the same tissue in some instances – i.e., the volume of NMF within the tissue could be approximated from water data, or vice versa.

7.6.7. Statistical Methods

Prior to the application of the Bonferroni correction, several between and within group statistical comparisons yielded significant results, however when the Bonferroni correction was applied no statistically significant results remained. This is not unexpected due to the small volume of data available for each group within this study, the high variability in data, and the large number of comparisons resulting in a high threshold for significance. In fact,

Binder et al (2017), applied Bonferroni correction under similar circumstances and also generated the same outcome.

The non-significant results of the Mixed Effects Model found within this study are also not unexpected. Where differences between participants have been observed when data are presented as gradients, the magnitude of these differences are small. The change that is most evident is in the urea data for treated and untreated skin comparisons at the heel, which are reflected in this being the single significant result from the mixed effect model analysis.

Unfortunately, this method of analysis does remove some of the nuance associated with data analysis when comparisons are made at each measurement depth. Using the mixed effect model, no information is provided on where across the skin surface differences are evident, only an indication of overall difference is provided. This restricts discussion of how these changes may indicate a difference in SC thickness, as opposed to a reflection in physiological SC hydration.

In instances where it is possible to calculate the thickness of the SC, it would be preferable to only apply the mixed effect method to data from equivalent structures (i.e. only data from the skin surface to the base of the SC), this would generate data that reflects differences in material from entirely comparable tissues. However on the plantar skin, where the SC depth is not quantifiable using CRS, the results are instead reflective of the differences between material composition across full measurement depth, which may not contain commensurate structures. In future, it may be useful to combine both a mixed effects model and multiple comparisons across depth in instances where SC thickened cannot be determined. This method would facilitate overall comparisons of material volume difference between groups, but with the additional insight into where these occur within the tissues, and how this is potentially influenced by the structures present.

7.7. Study design limitations and recommendations for future research

The design of this study was intended to generate data relevant to the wider problems facing foot health care (an ageing population and increased rates of diabetes), and to support the commercial interests of Scholl. However, the demographics of the participants recruited for this study limit the transferability of the data generated somewhat.

The health of the plantar soft tissues can become compromised in individuals with diabetes due to development of sensory, motor and autonomic neuropathy, leading to xerotic foot skin and high underfoot pressures, often contributing to the development of DFU (Boulton, 2014).

Within this study, the participants with diabetes were recruited from the University of Salford Podiatry clinic. This facility is a private teaching clinic. Treatment by students represents a small risk to the patients attending, and as such, any individual who is classed as being high-risk for foot-health problems (i.e., development of non-healing wounds, due to vascular disease or complications of diabetes, for example) is referred into a local NHS trust. The patients recruited for this study are therefore not representative of the population with diabetes who currently experience complications of diabetes in the foot.

Unfortunately, little information is available on the early-stages of diabetic foot complications and how these reflect future foot-health status. If more information were available, it may be possible to postulate from the data collected within this study how changes between people with and without diabetes (prior to the development of evident manifestations of diabetes in the foot) evolve over time.

In future, it would be pertinent to recruit individuals with various stages of foot complications associated with diabetes, i.e., people who have previously ulcerated, people who are at high-risk of ulceration, and individuals who have developed neuropathy but are not yet exhibiting soft tissue changes in the foot. This kind of study would require a thorough assessment of the vascular and nervous system, as well as long-term glycaemic control, to encompass other factors into analysis that may contribute to soft tissue changes in the foot.

As well as varying the participant demographics, it would also be interesting to collect data from areas of plantar skin that are vulnerable to ulceration, for example an area of tissue that has previously ulcerated (to examine SC remodelling), or an area of high-pressure (following pressure-mapping). This may provide insight into the changes in tissues immediately prior to ulceration and indicate what materials could be delivered into the tissue to prevent/reverse these changes.

Another participant group within this study that does not necessarily represent the population it is intended to reflect is the young healthy patients in the emollient study. As has

previously been discussed (see Section 7.6.5.), the demographics of this cohort (more specifically, the health of their foot skin), is not representative of the target market for the emollient Intense Nourish. Intense Nourish is intended for use on dry foot skin, whereas the participants involved in the emollient trial were required to have foot skin free from pathology.

In future, it would be beneficial to repeat this study with a range of individuals with varied levels of foot skin xerosis. This would generate data on how foot skin composition changes with xerosis, how penetration of emollient ingredients is influenced by changes in the skin barrier resulting from xerosis, and whether compositional changes in xerosis can be remedied using emollients. Such studies could benefit from being conducted longitudinally.

In such future studies, it would also be pertinent to introduce more standardisation around emollient application and foot-care during the emollient application period. Within this study, as no instruction was given for wearing hosiery during sleeping hours, it is possible that a small amount of the product applied to one foot could have been transferred to the other foot. It is also worthy of note that no record of compliance was kept by the participants, although all participants verbally confirmed their regular use of the emollient during testing with the researcher, in future it may be preferable to require participants to complete a usage diary to ensure compliance. Alternately, emollient containers could be weighed before and after the usage period to determine if some individuals had used more or less than advised.

Finally, as has been a common theme throughout the discussions within this chapter, each aspect of this study would benefit from increased participant numbers. Compared to other skin sites, the foot skin has much increased variability in composition between individuals. Although statistically significant findings were evident when the statistical method employed by some authors was undertaken, when the Bonferroni correction was applied to the p-value in the instances where multiple comparisons were used, these were all insignificant. Through increasing participant numbers, likelihood of retaining some statistically significant results in this instance are increased. Beyond achieving statistical significance, it would also be beneficial to identify what constitutes a meaningful difference in material volumes. This would assist the interpretation of results to go beyond simply whether differences between groups are statistically different, but whether these differences have any real implications on the behaviour of the skin itself.

7.8. Conclusion

The hydration, NMF, and urea composition of the plantar skin has been characterised in three participants groups, and with and without emollient application. These data have generated understanding that can be applied to the wider understanding of foot care for people of advanced age and people with diabetes, as well as providing valuable insight for the benefit of industries working to treat foot skin pathology.

The hydration gradient of the plantar tissues is markedly different to non-plantar tissues. This has implications on the use of (widely used) commercially available hydration measurement devices, discussed further in Chapter 8.

The plantar skin composition does not consistently differ between young and older people, or older people with diabetes and older people without diabetes, although some minor differences are identified in between-participant variability between groups that are worthy of further investigation.

Application of emollient to the plantar skin for a one-week period does not change the water or NMF composition of the tissue in a young healthy position, however urea does penetrate the plantar SC to varying degrees according to the SC thickness.

Despite the novelty of these data, the real impact of this work lies in its demonstration of the use of CRS on the plantar skin. CRS is primarily used within the cosmetics industry to assess the penetration of topical emollients, but it has also been employed within research to look for differences in skin composition resulting from a disease process (such as diabetes). Despite the commonality of skin disease on the foot, it has not been applied to the plantar foot due to issues anticipated in collecting data from such a hard, dry skin surface.

Through undertaking this study, a protocol for the application of CRS to the plantar foot, and analysis of the resulting data, has been successfully developed and demonstrated.

Chapter 8. Using CRS to investigate the validity of commercially available hydration measurement devices for use on the plantar skin.

8.1. Introduction

Historically, the study of foot skin composition in-vivo has been limited to measurement of its water content using devices that employ electrical methods to indicate skin hydration. The compact size, fast data-collection, and low cost of these instruments makes them convenient for use in a clinical setting. However, there is some uncertainty concerning the validity of the data these collect.

In this study, these 'surface' measures of skin hydration have been collected alongside CRS. As CRS provides water content of skin across its depth up to 400 μm (see Section 4.3.2.) it is possible to directly compare the data collected using the 'surface' devices to the actual water content of the skin at the depth they purport to measure.

This information is important as it informs the future use of these instruments, particularly on the foot. For example: If a skin complaint is found to occur because of hydration changes at a particular depth within the skin, then it would be pertinent to use a hydration measurement device that collects data at this location. i.e., if flaky skin occurs due to low hydration in the superficial skin layers of this skin, an instrument with a relatively shallow measurement depth should be used to assess the effectiveness of emollients intended to remedy this. Conversely, if a pathology hails from dryness within the deeper skin layers (for example, deep skin fissures), a hydration measurement device with a deeper measurement depth would be more suited to monitor the pathology with interventions.

In this chapter, the data collected in the study described in Chapter 7 are analysed to investigate the depth at which hydration measurement devices are collecting data within the skin. These data provide valuable context to the findings of the studies described in chapters 5 and 6, offering insight into why these devices represent tissue characteristics differently at different skin sites, which are discussed in Chapter 9.

8.1.1. Novelty statement

This study represents the first instance in which the measurement depth of the Corneometer® CM825, DermaStat®, MoistureMeter SC™, and MoistureMeter D® have been assessed in-vivo through the use of CRS.

8.1.2. Objective and Hypothesis

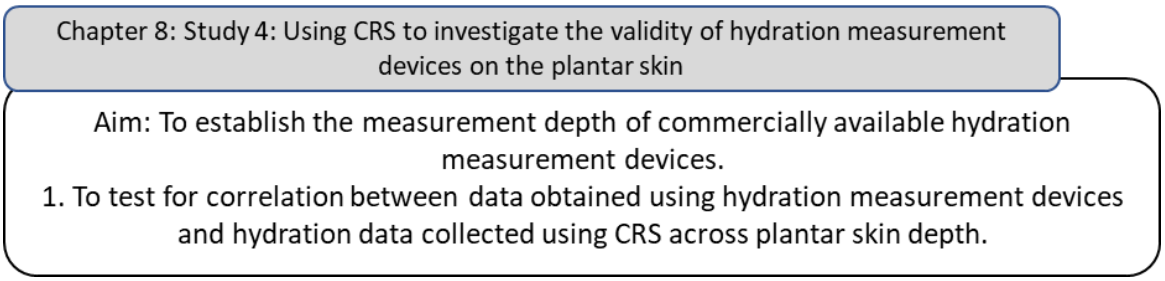


Figure 90. Objectives for Study 4: Using CRS to investigate the validity of commercially available hydration measurement devices on the plantar skin.

The following hypothesis is proposed for the outcome of this study:

Commercially available hydration measurement devices correlate strongly with CRS hydration data at the measurement depth described by the manufacturer, where these are given.

8.2. Method

The data analysed and presented within this chapter are the output of the study described in Chapter 7. The study protocol was reviewed and approved by The University of Salford Ethics Panel (application 6751),

8.2.1. Data processing

To explore the measurement depth of the hydration measurement devices, the data collected using these can be correlated with the water content of the skin across its depth measured using CRS. This analysis is described below:

8.2.1.1. CRS data set-up

For the purposes of this analysis, all CRS water content data collected are combined, irrespective of the age or diabetes status of the individual. Data from treated skin in the young healthy cohort has been excluded as the impact of emollient use on commercially available hydration measurement devices is not known, additionally, inclusion would have meant that each young healthy participants would have been represented twice within the dataset.

This produces a data set for each measurement depth for each measurement site:

Ventral forearm: 17 data sets representing measurement depths 0-50 µm

Medial arch: 20 data sets representing measurement depths 0-380 µm

Heel: 18 data sets representing measurement depths 0-400 µm

8.2.1.2. Data from commercially available hydration measurement devices set-up

Each of the four hydration measurement instruments (the Corneometer® CM825, the DermaStat®, the MoistureMeter SC™ and MoistureMeter D®) has been used to collect data from the ventral forearm, the heel, and the arch. This generates 12 data sets in total (four devices for each of the three skin-sites).

8.2.2. Statistical analysis

To examine how the hydration measures collected by each commercially available hydration measurement device correlated with the water content of the skin across its depth, a series of tests were completed in turn. i.e., Corneometer® CM825 data from the ventral forearm were examined for correlation with CRS water data collected at 0 µm depth, then 2 µm depth, then 4 µm depth (all on the forearm), until all measurement depths were exhausted. This process was then repeated for Corneometer® CM825 data from the medial arch and heel, and each other instrument. Due to the multiple comparisons made for each of these assessments, the Bonferroni correction was applied to reduce the likelihood of Type 1 errors arising (Curtin & Schulz, 1998).

Where all datasets were normally distributed (according to the Shapiro-Wilk test) Pearsons Correlation Coefficient was used, otherwise Spearman's Rank Order Correlation Coefficient was applied.

8.2.2.1. Presentation of results

For the Corneometer® CM825 a measurement depth is specified by the manufacturer. For this instrument, correlation with CRS data is of most relevance around this measurement depth; i.e., The Corneometer® CM825 is described by manufacturers as having a measurement depth of 10-20 µm (Courage & Khazaka electronics GmbH, 2010) and in vitro tests have found the measurement depth to be <15 µm (Fluhr et al., 1999a). Results are initially displayed for comparisons with CRS data for 10-20 µm depth (Table 65).

Due to the non-specific measurement depth of the MoistureMeter SC™ and MoistureMeter D®, and no measurement depth being given for the DermaStat®, there is no specific range of measurement depths that are directly suitable for their comparisons. Instead, all CRS measurement depths have been examined for correlation with measures for each instrument.

A demonstration of how the results of these analyses are displayed is given in Figure 91.

The correlation coefficient (r-value) for each device resulting from each correlation analysis is plotted on the secondary Y-axis, against the CRS measurement depth (X-axis). These data are overlaid on the individual hydration gradient data, which are plotted on the primary Y-axis. In Figure 90 the blue line represents hypothesised correlation coefficients for Corneometer® CM825 data and CRS data across tissue depth as an example. Hydration gradients are included to provide context to the correlation coefficients displayed across tissue depth (i.e., in some instances, a change in the strength of correlation coefficients aligns with changes in hydration gradients).

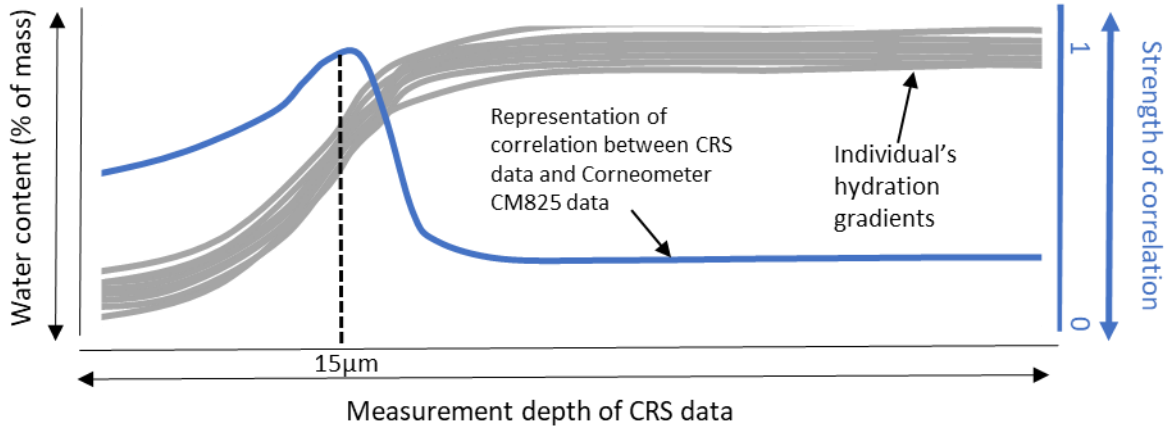


Figure 91. Schematic explaining the data displayed in Figures 93-95.

This arrangement provides a clear visual demonstration of how the strength of the correlations between the measures of skin hydration varies with skin site and measurement depth of CRS. For completeness, and additional insight beyond the measurement depths described by the manufacturers, these data are displayed for all four devices on each figure (represented by a different coloured line).

When interpreting the correlation data given in this format, it is important to consider the influence of the relationship between CRS data across different measurement depths on the correlation between CRS data and data collected using commercially available hydration measurement devices:

CRS data are not independent from one another at their different depths, these are part of a continuum. Superficial SC hydration is correlated with deeper SC hydration, and at some points (particularly on the plantar skin), CRS data are very consistent for an extended region in the skin. This influences the correlation between CRS data and data collected using commercially available hydration data. I.e., if data from a commercially available hydration measurement device correlates strongly with superficial SC hydration, it is likely that this correlation will remain beyond the anticipated measurement depth of the device due to the correlation between superficial and deep SC hydration.

When interpreting correlation data, therefore is it important to chiefly consider where the highest correlation exists and interpret correlation values lower than this with caution due to the likelihood that this is a consequence of correlation between CRS data and maybe not a

representation of the measurement depth of the commercially available hydration measurement device.

8.3. Results

A total of 34 individuals' data has been used for this exploration of correlation between CRS data across tissue depth and data obtained using commercially available hydration measurement devices. The demographics of the participants involved in this study are displayed in Table 64. CRS datasets were found to be primarily non-parametric in distribution by use of a Shapiro-Wilks test, therefore non-parametric statistical tests were employed.

Table 64. Participant demographics.

Participants	n=34
Age (Mean (SD))	53.53 (17.3) years
Sex (% female (n))	41% (14)
BMI	29.4 $kg.m^{-2}$
Ethnicity % (number of individuals)	85% (29) White British, 5.8% (2) Indian, 3% (1) White Irish, 3% (1) Pakistani, 3% (1) Black Caribbean

8.3.1. Corneometer® CM825

The measurement depth of the Corneometer® CM825 is reported between 10 and 20 µm (Courage & Khazaka electronics GmbH, 2010; Fluhr et al., 1999b). Below, the data collected using the Corneometer® CM825 is correlated with the CRS data collected at each measurement depth between 10 and 20 µm using the Spearmans correlation (See Table 65). Application of the Bonferroni correction for the multiple comparisons undertaken within this study generated a threshold for statistical significance of p-value = 0.0083 for the ventral forearm and arch, and p-value = 0.166 for the heel.

Table 65. Spearman's correlation coefficient between Corneometer® CM825 and water content at different skin depths.

Depth (µm)	Ventral Forearm		Arch		Depth (µm)	Heel	
	Correlation coefficient	Significance:	Correlation coefficient	Significance:		Correlation coefficient	Significance:
10	0.044	0.828	0.487	0.016	10	0.393	0.052
12	0.051	0.802	0.486	0.016	15	0.398	0.049
14	0.037	0.854	0.485	0.016	20	0.451	0.024
16	0.061	0.758	0.484	0.017			

18	0.096	0.634	0.457	0.025	
20	0.136	0.500	0.465	0.022	

At the ventral forearm site, no significant correlation was identified between the data obtained using the Corneometer® CM825 and water content of the skin between 10 and 20 µm (Dancey & Reidy, 2004). At the arch site, strong correlations were found between 10 and 20 µm depth and on the heel a medium strength correlation is evident at 10 and 15 µm depth, and a strong correlation at 20 µm. However, none of the p-values associated with these correlations reach the threshold of statistical significance.

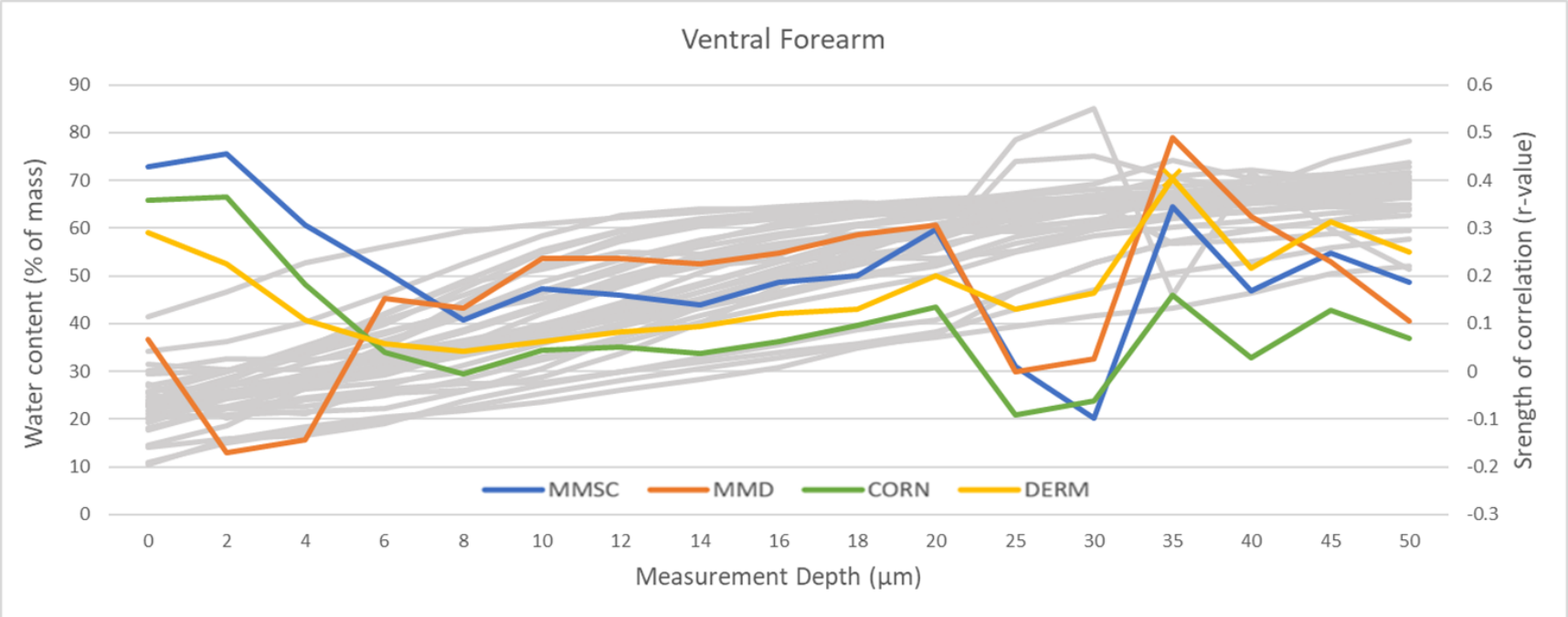
8.3.2. MoistureMeter SC™, MoistureMeter D®, and DermaStat®

Due to no specific measurement depth being provided for the MoistureMeter SC™, MoistureMeter D®, and DermaStat®, results of a Spearmans correlation between data collected using these devices and CRS data from each measurement depth are plotted against the depths at which these were collected (See Figure 91). For completeness, equivalent data are plotted for the Corneometer® CM825.

8.3.2.1. Ventral forearm

Below, the results of the Spearmans correlation conducted between CRS data at different depths and commercially available hydration measurement devices are plotted against CRS measurement depth. This graph reveals differences in the pattern of correlation demonstrated by each commercially available hydration measurement device across skin depth (See Figure 92).

Due to the multiple comparisons undertaken as part of this analysis, a p-value of 0.029 has been established as the threshold for statistical significance using the Bonferroni Correction.



		Raman Measurement Depth																
		0	2	4	6	8	10	12	14	16	18	20	25	30	35	40	45	50
MMSC (n=14)	Correla	0.429	0.455	0.305	0.209	0.108	0.174	0.160	0.138	0.187	0.200	0.297	0.011	-0.099	0.345	0.169	0.248	0.187
	Sig.	0.126	0.102	0.288	0.474	0.714	0.553	0.584	0.637	0.523	0.493	0.303	0.970	0.737	0.227	0.563	0.392	0.523
MMD (n=13)	Correla	0.066	-0.170	-0.143	0.154	0.132	0.236	0.236	0.225	0.247	0.286	0.308	0.000	0.027	0.489	0.324	0.231	0.104
	Sig.	0.831	0.578	0.642	0.616	0.668	0.437	0.437	0.459	0.415	0.344	0.306	1.000	0.929	0.090	0.280	0.448	0.734
CORN (n=27)	Correla	0.358	0.365	0.183	0.040	-0.006	0.044	0.051	0.037	0.062	0.096	0.136	-0.092	-0.062	0.159	0.029	0.127	0.069
	Sig.	0.067	0.061	0.362	0.842	0.976	0.828	0.802	0.854	0.758	0.634	0.500	0.647	0.758	0.427	0.887	0.528	0.732
DERM (n=32)	Correla	0.292	0.225	0.108	0.058	0.041	0.062	0.082	0.093	0.122	0.130	0.200	0.131	0.163	0.404*	0.216	0.312	0.251
	Sig.	0.105	0.216	0.557	0.751	0.822	0.735	0.655	0.611	0.507	0.479	0.272	0.475	0.372	0.022	0.236	0.082	0.166

Figure 92. Spearman's correlation coefficient for CRS data with the MoistureMeter SC™ (MMSC), MoistureMeter D® (MMD), Corneometer® CM825 (CORN) and DermaStat® (DERM) data from the ventral forearm. Overlaid on individual CRS hydration gradients (grey). Correlation coefficients with p-value <0.05 highlighted in grey.

MoistureMeter SC™

The MoistureMeter SC™ demonstrates a strong correlation with CRS data at 0 and 2 µm measurement depth, which reduces to a moderate and weak correlation at 4 and 6 µm (respectively). Across the deeper measurement depths the correlation strength varies but never reaches the strength of the superficial correlations. None of the correlations identified reach statistical significance ($p>0.0029$).

DermaStat®

At most tissue depths, the DermaStat® demonstrates no correlation or a weak correlation, with the CRS measures. At a measurement depth of 35 µm however, a strong correlation is evident between CRS data and DermaStat® data. Although the p-value for this result is <0.05, this does not reach the threshold for statistical signficance when the Bonferroni Correction is applied (p-value = 0.0029).

MoistureMeter D

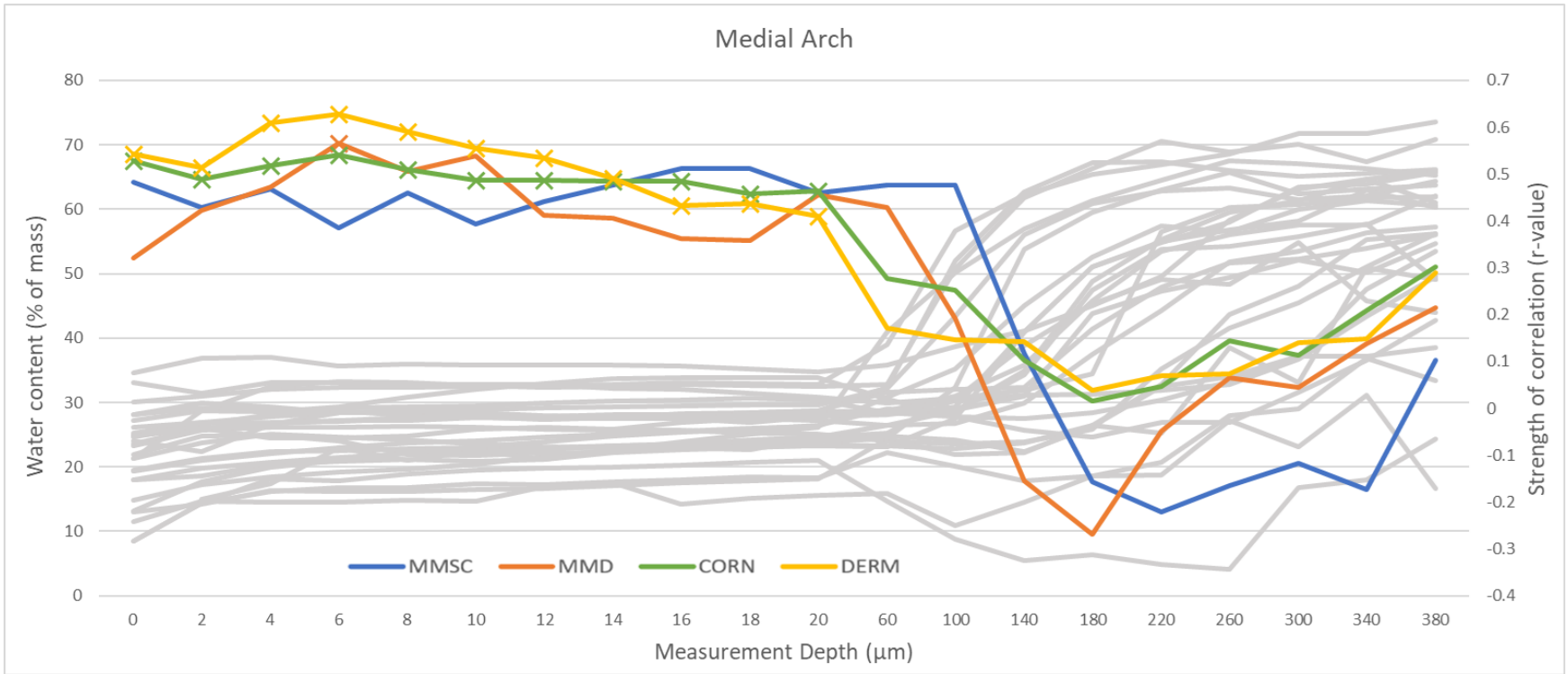
On the forearm, the strongest correlation between data collected using the MoistureMeter SC™ and CRS data is evident at 35 µm depth – a moderate positive correlation (0.489), however this is not statistically significant (p-value >0.0016). Elsewhere within the tissue, the correlation between these data is negligible-weak and highly variable.

Corneometer® CM825

At the ventral forearm, Corneometer® CM825 data demonstrates their strongest correlation (weak) with CRS data collected at a depth of 0 and 2 µm depth (r-value: 0.358 and 0.365 respectively) however neither of these reach statistical significance ($p>0.0016$). For the remainder of the tissue depth measured, the correlation between these data is negligible.

8.3.2.2. Medial arch

At the medial arch skin site, the Spearmans correlation coefficient between CRS data and data collected using commercially available hydration measurement devices generates similar profiles of correlation for each device used (See Figure 93). Due to the multiple comparisons undertaken as part of this analysis, a p-value of 0.0025 has been established as the threshold for statistical significance using the Bonferroni correction.



		Raman Measurement Depth																			
		0	2	4	6	8	10	12	14	16	18	20	60	100	140	180	220	260	300	340	380
MMSC	Correla	0.484	0.429	0.468	0.385	0.459	0.393	0.442	0.477	0.512	0.512	0.459	0.477	0.477	0.116	-0.156	-0.222	-0.165	-0.116	-0.174	0.103
(n=14)	Sig.	0.094	0.126	0.091	0.175	0.098	0.164	0.114	0.085	0.061	0.061	0.098	0.085	0.085	0.692	0.594	0.446	0.573	0.692	0.553	0.725
MMD	Correla	0.322	0.423	0.473	0.566*	0.505	0.538	0.412	0.407	0.363	0.357	0.456	0.429	0.192	-0.154	-0.269	-0.049	0.066	0.044	0.137	0.214
(n=13)	Sig.	0.308	0.150	0.103	0.044	0.078	0.058	0.162	0.168	0.223	0.231	0.117	0.144	0.529	0.616	0.374	0.873	0.831	0.887	0.655	0.482
CORN	Correla	0.529**	0.488*	0.519**	0.541**	0.510*	0.487*	0.486*	0.485*	0.484*	0.457*	0.465*	0.277	0.251	0.102	0.016	0.046	0.144	0.112	0.209	0.301
(n=27)	Sig.	0.009	0.016	0.009	0.006	0.011	0.016	0.016	0.016	0.017	0.025	0.022	0.191	0.236	0.635	0.941	0.832	0.503	0.602	0.327	0.152
DERM	Correla	0.542**	0.513**	0.609**	0.628**	0.590**	0.555**	0.534**	0.490*	0.432*	0.436*	0.409*	0.172	0.147	0.142	0.039	0.070	0.073	0.140	0.148	0.290
(n=32)	Sig.	0.006	0.009	0.001	0.001	0.002	0.004	0.006	0.013	0.031	0.029	0.042	0.412	0.483	0.498	0.852	0.738	0.730	0.504	0.479	0.159

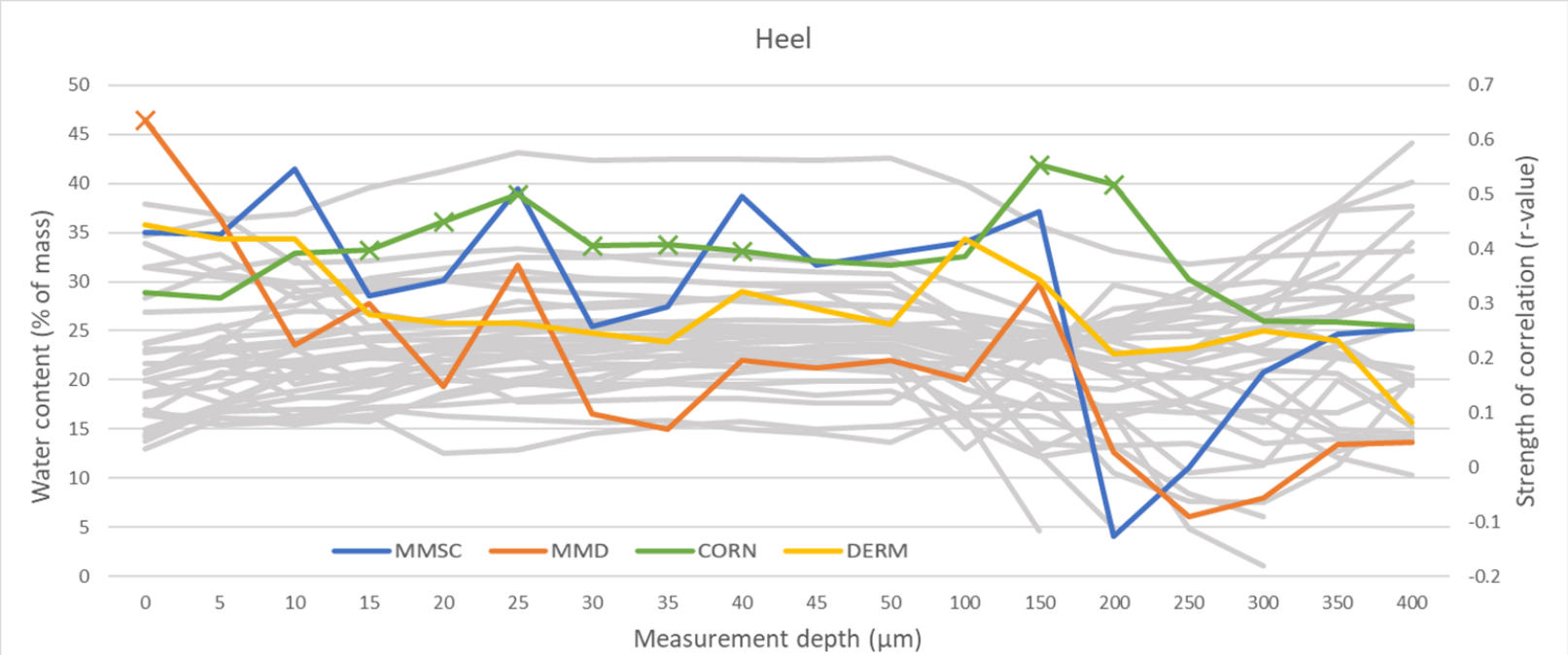
Figure 93. Spearman's correlation coefficient for CRS data with the MoistureMeter SC™ (MMSC), MoistureMeter D® (MMD), Corneometer® CM825 (CORN) and DermaStat® (DERM) data from the medial arch. Overlaid on individual CRS hydration gradients (grey). Correlation coefficients with p-value <0.05 highlighted in grey.

The DermaStat® and Corneometer® CM825 show a moderate-strong positive correlation with CRS data from the skin surface down to 20 µm depth. Each of these has a p-value <0.05, however, when the Bonferroni correction is applied, generating a p-value of 0.025, these are not considered statistically significant. Beyond 20 µm, the correlation between CRS data and the DermaStat® and Corneometer® CM825 reduces to a weak-negligible relationship for the remainder of the data.

The MoistureMeter SC™ and MoistureMeter D® demonstrate a similar pattern. These maintain a weak-moderate correlation with CRS data from the skin surface up to a depth of 60 and 100 µm (respectively). Beyond this depth, the strength of the correlations reduces markedly to a weak-negligible level for the remainder of the deeper CRS measures.

8.3.2.3. Heel

On the heel, Spearmans correlation has been applied to the CRS data collected at various tissue depths and data collected using commercially available hydration measurement devices (See Figure 94). Due to the multiple comparisons undertaken as part of this analysis, a p-value of 0.0028 has been established as the threshold for statistical significance using the Bonferroni Correction.



		Raman Measurement Depth																	
		0	5	10	15	20	25	30	35	40	45	50	100	150	200	250	300	350	400
MMSC (n=14)	Correla	0.430	0.427	0.545	0.315	0.343	0.510	0.259	0.294	0.497	0.371	0.392	0.413	0.469	-0.126	0.000	0.175	0.245	0.255
	Sig.	0.214	0.167	0.067	0.319	0.276	0.090	0.417	0.354	0.101	0.236	0.208	0.183	0.124	0.697	1.000	0.587	0.443	0.450
MMD (n=13)	Correla	0.636*	0.455	0.224	0.301	0.147	0.371	0.098	0.070	0.196	0.182	0.196	0.161	0.336	0.028	-0.091	-0.056	0.042	0.045
	Sig.	0.048	0.138	0.484	0.342	0.649	0.236	0.762	0.829	0.542	0.572	0.542	0.618	0.286	0.931	0.779	0.863	0.897	0.894
CORN (n=24)	Correla	0.321	0.309	0.393	0.398*	0.451*	0.501*	0.406*	0.408*	0.396*	0.379	0.371	0.386	0.554**	0.518**	0.345	0.269	0.266	0.257
	Sig.	0.136	0.132	0.052	0.049	0.024	0.011	0.044	0.043	0.050	0.062	0.068	0.057	0.004	0.008	0.107	0.215	0.220	0.248
DERM (n=25)	Correla	0.444	0.418	0.418	0.280	0.263	0.263	0.247	0.230	0.323	0.290	0.261	0.418	0.344	0.207	0.218	0.250	0.233	0.082
	Sig.	0.085	0.084	0.084	0.261	0.291	0.291	0.324	0.358	0.191	0.243	0.295	0.084	0.163	0.409	0.400	0.333	0.368	0.762

Figure 94. Spearman's correlation coefficient for CRS data with the MoistureMeter SC™ (MMSC), MoistureMeter D® (MMD), Corneometer® CM825 (CORN) and DermaStat® (DERM) data from the heel. Overlaid on individual CRS hydration gradients (grey). Correlation coefficients with p-value <0.05 highlighted in grey.

MoistureMeter SC™

At the heel the strength of the correlation between CRS data and MoistureMeter SC™ varies between weak, moderate, and strong between 0 µm and 150 µm, beyond 150 µm it is only weak or negligible. None of these correlations are statistically signficant. Due to the high variability in the CRS data collected at the heel, the limited data collected using the MoistureMeter SC™ (n=14) has resulted in increased variability being demonstrated between correlation coefficients at different depths.

DermaStat®

At the heel, a reduced volume of data is available for the DermaStat® due to a high incidence of data-collection failure. Out of 32 available participants, the DermaStat® was only able to collect three repeated successful measures of skin hydration from the heel from 25 people. Data-collection failure was found to occur when skin hydration was low, meaning that the correlation data presented here is only representative of the individuals with higher heel skin hydration. This issue is discussed in further detail in section 8.4.2.

Nonetheless, in the data that has been collected, a strong correlation is evident between the DermaStat® and CRS measures taken at 0-10 µm and 100 µm, otherwise, correlations are primarily weak or moderate. No results reach statistical significance.

MoistureMeter D®

At the heel, the MoistureMeter D® demonstrates a strong positive correlation (r-value = 0.636) with the CRS data at the skin surface (0 µm), albeit this does not reach statistical significance when the Bonferroni corrected p-value is considered. Beyond this depth, the strength of correlation reduces markedly and varies considerable across the remainder of the measurement depths, only achieving weak-negligible strength. This may be, in part, due to the limited participant numbers for the MoistureMeter D® (n=13) and the increased variability on CRS data on the heel.

Corneometer® CM825

On the heel, data collected using the Corneometer® CM825 correlates positively with CRS data with weak-moderate strength at measurement sites 15-40 µm and 150-200 µm, the strongest of which being at 150 µm (r-value = 0.554) albeit none of these reach the level of statistical significance required following application of the Bonferroni correction (p-value = 0.0025).

8.4. Discussion

The overarching aim of this investigation was to explore the measurement depth of commercially available hydration measurement devices on the plantar and non-plantar skin. It was hypothesised that the commercially available hydration measurement devices would correlate most closely with the CRS data at the measurement depth described by their manufacturers. i.e., The Corneometer® CM825 would demonstrate its strongest linear relationship with CRS data at 10-20µm depth, however this has been shown not to be the case for either the plantar or non-plantar skin.

No hypothesis was generated for the MoistureMeter SC™, MoistureMeter D®, or the DermaStat® as no specific measurement depth is described by the manufacturers for these devices, however, similarly, these were found to be representative of water content at different tissue depths within the plantar and non-plantar skin.

8.4.1. Corneometer® CM825

Through this work, it has been demonstrated that the measurement depth purported by the manufacturer (10-20 µm) is not reflected by correlation with CRS data. Instead, the Corneometer® CM825 appears to generate hydration data that correlates most closely with the superficial low-hydration area of the SC across all skin sites. This finding aligns with the work of Fluhr et al (1999) who established that the measurement depth of the Corneometer® CM825 was less than 15 µm. This is not the result anticipated for the Corneometer® CM825. This suggests that the measurement depth of the Corneometer® CM825 is influenced by the

structure of the skin, rather than consistently representing the water content of the uppermost 10-20 µm.

8.4.2. Consideration of all capacitance-based devices

Across all measurement sites, all three capacative-based devices broadly demonstrate the same pattern – high correlation with CRS data at the superficial, low hydration portion of the SC.

This is anticipated in the case of the MoistureMeter SC™, as the measurement mechanism of the MoistureMeter SC™ is described as being reflective of the dryness and thickness of the superficial dry layer of the skin, rather than representing tissue water at a specified depth (Alanen et al., 2004). Although, it is not possible to establish whether the 'thickness' of the SC impacts upon the data, as no SC thickness were collected within this study.

The manufacturers of the DermaStat® provide no indication of measurement depth or mechanism beyond 'capacitance' (see Chapter 4 for further information). Irrespective of how the manufacturer describes the output of these devices, however, these three devices appear to correlate in a similar manner with CRS data across the skin. This is major of importance, as one of these instruments costs a fraction of the price of the others: The DermaStat® costs £35, whereas the Corneometer® CM825 and MoistureMeter SC™ cost £2770 and £2750 respectively.

The DermaStat®, however, has a significant limitation: at low levels of skin hydration it has a high failure rate. This is particularly evident on the heel where 7 out of the 32 participants involved in this study data were not collected on the heel due to the low skin hydration.

The driving force behind foot-skin hydration research is the prevalence of xerosis on the plantar skin. If the DermaStat® is unable to consistently collect data from the heel skin of a population with no foot-health issues, it's successful application within a plantar xerosis trial is highly unlikely.

On non-plantar skin areas, however, the use of the DermaStat® is worthy of further consideration. If this device generates data equivalent to the Corneometer® CM825, this

could represent a much cheaper option for use within a clinical or commercial setting for assessment of xerosis on other bodily areas, including the dorsum of the foot.

8.4.3. MoistureMeter D®

The 'effective' measurement depth of this instrument, as described by the manufacturers, is 500 µm. As discussed in Chapter 4, this does not mean than the value reported by this device is representative of the volume of water within the tissue at 500 µm depth, but rather of the dielectric properties of the tissues up to this depth. The influence of material dielectric properties on data is also heavily weighted towards superficial tissues (Meaney et al., 2016).

The depth at which correlation strength is highest for CRS data and MoistureMeter D® data within the current study varies depending upon the site of the skin surface measured: At the medial arch the strongest is found at the mid-portion of the initial low-hydration plateau within the SC, and at the heel the strongest correlation is found at the skin surface. As the thickness of the superficial, low-water content portion of the SC increases, the measurement depth at which the MoistureMeter D® correlates most strongly with CRS data reduces. It is possible that is a reflection of the reduced penetration depth of the MoistureMeter D® due the thickness and dryness of the plantar SC.

There is some evidence that this might be the case from previous work. In 2013, Mayrovitz et al (2013) found that data collected using MoistureMeter SC™ correlated most strongly with data collected using the MoistureMeter D® 0.5, 1.5 and 2.5 mm probes at the plantar and palmar skin. As discussed, the data collected using the MoistureMeter D® is heavily influenced by the dielectric constant of the superficial material within its measurement range (Meaney et al., 2016). On the plantar and palmar skin, the SC is thick and dry – therefore having a large influence on the data collected using MoistureMeter D® probes. The MoistureMeter SC™ also collects data from the area within the SC where hydration is low (as demonstrated within the current study). This means that on the plantar skin, these two instruments are collecting data from equivalent tissues within the skin, therefore generating similar data.

Mayrovitz et al (2013) also identified a difference in the direction of change in the water content of tissues when measured using the three different probes between plantar and non-

plantar tissues. Values collected using probes of increasing 'effective' measurement depth decrease at the non-plantar skin - this is presumed to be due to the transition from the superficial measures (0.5 mm probe) where the thin SC, epidermis and uppermost dermis are measured (high hydration), to deeper measures where the subcutaneous fat is within measurement range (low hydration) (Mayrovitz, Bernal, et al., 2013). On the plantar and palmar skin this is reversed (i.e., the deeper measures generate higher values) the authors propose that this is due to an increased volume of deep-lying eccrine sweat glands at these locations.

However, within the currently study the MoistureMeter D® has been shown to collect data at a superficial level on the heel and arch. It is proposed that the marked and sustained low hydration of the plantar SC impedes the penetration depth of the 0.5 mm probe, generating this result. If this is also the case with the 1.5 mm and 2.5 mm probe, this would mean these devices would be collecting data from an area superficial to their proposed 'effective' measurement depth, i.e., these would be collecting data from slightly deeper within the tissue than the 0.5 mm probe, but not to their full 'effective' measurement depth. This would generate increasing values as the probe measurement depth increased to include higher hydration evident at the viable epidermis and superficial dermis.

To test this theory, a repeat study could be undertaken using CRS simultaneously with MoistureMeter D® probes of different 'effective' measurement depths. Alternately, in-vitro testing could be undertaken using an inhomogeneous testing material with dielectric properties similar to that of the plantar epidermis.

8.5. Limitations of study design and future recommendations

Although some clear patterns have been identified in the relationships between CRS data and data collected using commercially available hydration measurement devices, it would be advantageous to be able to support these findings with some correlation coefficients that were of statistical significance. Unfortunately, due to the number of comparisons undertaken within this study, the Bonferroni correction generated a very high threshold of statistical significance. Additionally, the participant numbers within this study were relatively low (particularly for the MoistureMeter D® and MoistureMeter SC™), further reducing the power

of this study. In future, it would be beneficial to repeat this study with a larger number of participants, and more consistent access to the commercially available hydration measurement devices (reducing the inconsistency in n-numbers between devices). With a larger dataset it may also be possible to use a regression analysis to calculate a correction factor, allowing data collected using commercially available hydration measurement devices to be equated to a percentage value of SC water content (Bansal et al., 2016). This could be particularly useful in a clinical setting where CRS data collection may be impractical, but an understanding of the volume of material within the tissue would be advantageous.

Irrespective of the intricacies of statistical design used within these works, consideration of the use of CRS data as a 'standard' from which other measures of skin hydration can be judged must be considered carefully.

Despite standardised procedures for CRS data analysis being available in SkinTools 3, these are unsuitable for application to plantar foot skin data due to its inconsistency and low-quality. Instead, as demonstrated in Section 7.3.9.2., a protocol to assure the quality of CRS data outside of these mechanisms has been developed and applied to the CRS data collected within this study. Some aspects of this protocol are subjective in nature, primarily stage 3 in which the reviewer is required to assess the data by eye and identify sets of hydration gradient data that are not consistent with other equivalent data.

The subjectivity of this data assurance protocol has a negative impact on the validity of the data, and therefore on its use as a 'standard' comparator for alternative methods of skin hydration. In future, demonstration of adequate inter and intra-assessor reliability of the data assurance technique would support the use of CRS data in this manner.

Following on from the work described in this chapter, it would be beneficial to further explore the penetration depth of the MoistureMeter D® probes in relation to the structure of the plantar SC, to establish whether this thickened, dry layer attenuates the signal generated by the probe. This could be achieved through the use of MoistureMeter D® probes of different depth alongside CRS, or through in-vitro testing of penetration depth using a material with similar dielectric properties as the plantar soft tissues.

8.6. Conclusion

Through the conduction of this study, novel insight has been generated relating to the relationship between the hydration gradient demonstrated within the plantar and non-plantar skin, and measures obtained using commercially available hydration measurement devices.

It has been established that capacitance-based commercially available hydration measurement devices collect data from equivalent tissue structures on the plantar and non-plantar skin, representing the water content of the superficial, low portion of the SC hydration gradient.

Additionally, it has been found that the MoistureMeter D® 0.5 mm collects data from inequivalent tissues within the plantar and non-plantar skin, with its measurement depth appearing to be reduced on plantar skin due to the dry, thickened SC at this location.

Chapter 9. Discussion

This PhD project aimed to generate knowledge of the biochemical composition of the foot skin in health, age, and diabetes that can be applied in a clinical or commercial setting and inform the formulation and test the efficacy of emollients, and monitor foot skin health. In the preceding four chapters, experimental work has been described which fulfils of the aims and objectives described in Chapter 3.

In Chapter 5, methodological uncertainties surrounding the use of commercially available hydration measurement devices on the foot were addressed (i.e., repeatability, feasibility of use, impact of consecutive use), which were used to inform the study design of later studies. In Chapter 6, the correlation between skin hydration (as measured using commercially available devices) and physical characteristics of the foot skin were examined, providing insight into their use for assessing emollient effectiveness in the future. In Chapters 7 and 8, CRS was used to collect data on the composition of skin on the foot in participants with varied foot-risk and with and without emollient, generating an understanding of how skin composition changes with age, diabetes status and emollient use. The findings of these studies have been discussed in detail, including their contribution to accepting or rejecting hypotheses and in relation to data published in the literature.

In this chapter, the results of these studies are brought together, not to further examine the composition of the foot skin but to generate a more nuanced, informed understanding of how this data can be applied.

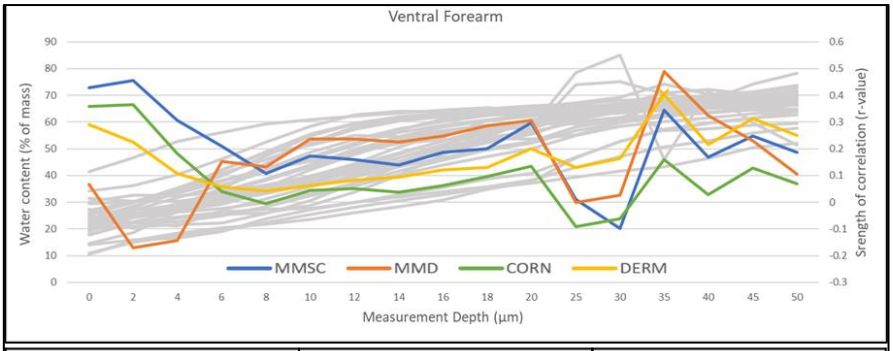
Although CRS has been successfully used to quantify the composition of the plantar skin, the conduction of the study detailed in Chapter 7 has given some insight into the limitations of this device. Data-collection from the plantar foot is challenging (due to the inhomogeneous skin surface and complex foot morphology) and time-consuming, particularly for individuals with poor motor control. Due to this, and the high cost of device rental, this method is not necessarily suitable for use in a clinical or commercial environment. However, the commercially available hydration measurement devices used across this PhD are generally very fast to use, easy to apply to all foot-skin surfaces, portable, relatively affordable, and therefore represent an opportunity to help address some of the contemporary issues facing foot health care delivery.

Within this chapter, the understanding gained of commercially available hydration measurement devices on the plantar foot is considered collectively with the author's experience of using these devices, resulting in a set of recommendations being made for their application across a range of environments. Additionally, recommendations are made for future research to advance foot skin healthcare.

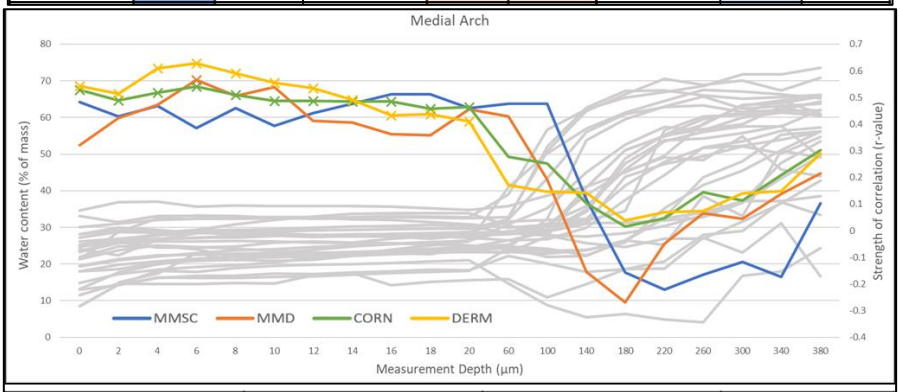
9.1. Measurement depth of commercially available hydration measurement devices and correlation with physical characteristics

Within this section, the measurement depths of the commercially available hydration measurement devices (extracted from Chapter 8) are considered alongside the correlation these display with the physical characteristics of the skin (extracted from Chapter 6) Figure 95. This is except for the DermaStat, as this device was not used within the study described in Chapter 6.

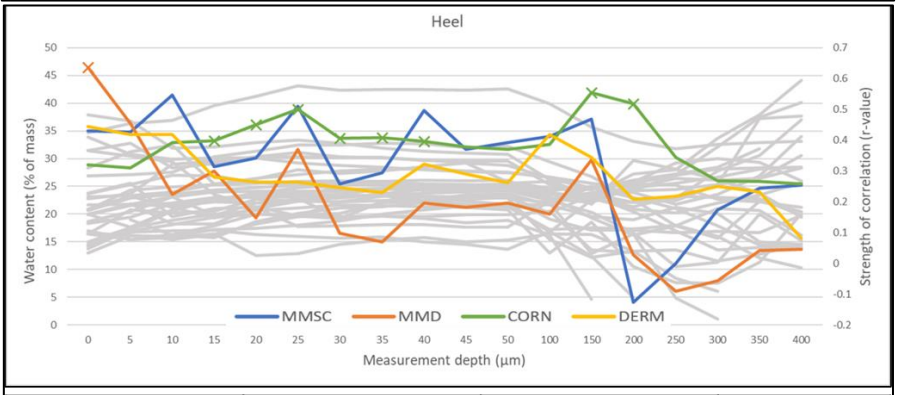
This discussion is intended to elucidate whether there are any clear connections between the measurement depths of the devices used and their correlation with the skin characteristics on the plantar and non-plantar skin. i.e., if a device collects data from deep within the SC of the non-plantar skin and at the superficial SC of plantar skin, does this correspond with the correlation the devices display with the physical characteristics of these tissues? If so, data collected from the plantar or non-plantar skin using this device must be interpreted differently.



Durometer (r-value)			Retraction speed (r-value)			Roughness (r-value)		
	Left	Right		Left	Right		Left	Right
Corneometer	0.185	0.341	Corneometer	-0.488	-0.488	Corneometer	0.170	0.291
MMSC	0.387	-0.052	MMSC	-0.253	-0.376	MMSC	0.058	0.114
MMD 0.5	0.483	0.084	MMD 0.5	-0.234	-0.269	MMD 0.5	0.205	0.024



Durometer (r-value)			Retraction speed (r-value)			Roughness (r-value)			FSkHQ (r-value)	
MA	Left	Right	MA	Left	Right	MA	Left	Right	Medial arch	
Corneometer	-0.468	-0.234	Corneometer	-0.176	-0.175	Corneometer	-0.552	-0.550	Corneometer	-0.261
MMSC	-0.277	-0.259	MMSC	-0.354	-0.395	MMSC	**-0.596**	**-0.646**	MMSC	-0.230
MMD 0.5	-0.080	-0.021	MMD 0.5	-0.231	-0.252	MMD 0.5	**-0.537**	**-0.590**	MMD 0.5	-0.336



Durometer (r-value)			Retraction (r-value)			Roughness (r-value)			FSkHQ (r-value)	
H	Left	Right	H	Left	Right	H	Left	Right	Heel	
Corneometer	**-0.633**	-0.491	Corneometer	0.214	0.200	Corneometer	-0.430	-0.383	Corneometer	-0.443
MMSC	**-0.552**	-0.378	MMSC	-0.065	0.307	MMSC	-0.472	-0.509	MMSC	-0.089
MMD 0.5	**-0.692**	-0.494	MMD 0.5	-0.063	0.368	MMD 0.5	**-0.615**	**-0.609**	MMD 0.5	-0.383

*Figure 95. Demonstration of the measurement depth of commercially available hydration measurement devices for each skin site overlaid on hydration gradient data, presented alongside the correlation of hydration data with the physical characteristics of the skin.****

*** Correlations with a p-value <0.05 indicated by a cross on the graph and with bold and underlined text in table.

In both their measurement depth and correlation with the characteristics of the skin, the Corneometer® CM825 and MoistureMeter SC™ behave similarly. Hydration data collected using these devices correlates consistently with the water content of the superficial SC, both on the plantar and non-plantar skin surfaces. On the plantar skin, they demonstrate similar negative correlation strengths (weak-strong) with skin hardness, roughness, and patient perception of foot skin features (FSkHQ) (only at the medial arch). However, they have no or weak correlation with skin elasticity. On the non-plantar skin, however, their direction of correlation with skin characteristics is markedly different: Positive correlations (albeit weak, inconsistent, and non-significant) are demonstrated with skin hardness and roughness and a consistent weak-moderate (non-significant) negative correlation is demonstrated with skin elasticity.

This difference in the direction of correlations at the plantar and palmar skin shows that although these capacitance-based devices collect data from the same depth at these locations, this does not necessarily mean hydration data from these different skin sites correlate in the same manner with the skin behaviour.

A similar pattern is demonstrated in the direction of correlations between plantar and non-plantar skin for the hydration data collected using the MoistureMeter D®: On the non-plantar skin, the MoistureMeter D® has a weak-moderate positive correlation with skin hardness and roughness, and a weak negative correlation with elasticity (non-significant results). This is reversed on the plantar skin, most evidently at the heel. Conversely, the MoistureMeter D® does appear to collect data from a different measurement depth on the plantar skin (superficial SC) and the non-plantar skin (beyond the SC).

Several conclusions can be drawn from this unusual phenomenon:

1. The measurement depth of the commercially available hydration measurement devices tested in this study does not appear to impact their ability to collect hydration data correlated with skin characteristics.
2. The direction of correlation between skin hydration and skin characteristics is reversed on the plantar skin, mostly due to the measures of skin characteristics chosen within this study and the structure of the SC and underlying tissues at the locations tested (as discussed in Section 6.4.3.).

These findings have an impact on how commercially available hydration measures are used on the foot. The measurement depth of these devices is now perceived to not be of consequence for their application on plantar and non-plantar skin. What is of importance, however, is their ability to detect skin hydration that is indicative of the effectiveness of the skin barrier.

The skin hydration of individuals with at-risk foot skin is monitored as xerosis is associated with tissue damage, i.e., hard skin resulting in the breakdown or splitting of tissues. In a clinical setting, therefore, the hydration measurement device that most closely correlates with the physical behaviour of the skin is most appropriate for assessing skin hydration. In a commercial environment, however, the nature of testing may vary. Some emollients are indeed marketed to consumers wanting to reduce 'hard skin'; however, many are also sold to consumers claiming that they will reduce skin roughness or promote 'healthy-looking skin'. Under these circumstances, the devices most suitable for use are those which correlate most closely with skin roughness, or patient perception of foot skin health.

Additionally, these findings have indicated that the correlations these devices demonstrate with skin characteristics are not consistent between the skin across the full surface of the foot. For this reason, the location of skin measurements used within studies must inform the choice of device used. For example, if the scope of a study is limited to a single location (for example, if the express focus of the research team was to reduce the hardness of plantar heel callus), then only the correlation strength between physical characteristics and hydration measures at this site are of relevance to device choice. However, if a study entails the assessment of skin sites across the foot, including plantar and non-plantar sites (as in the case of an all-purpose foot skin emollient), then the device which correlates most consistently with skin characteristics across these locations is most suitable for use.

Below, a set of recommendations are made for commercially available hydration measurement devices that consider the above factors, and also the author's reflections on the suitability for use in different environments and their cost.

9.2. Recommendations for the future use of devices for the measurement of foot skin composition

Through the conduction of the experimental work described in this PhD thesis, insight has been gained into the suitability of devices for measuring skin composition on the foot. These data may be used clinically to assess and monitor foot-skin risk and in a commercial setting to assist the assessment of emollient efficacy. Below, these findings are distilled into recommendations for using devices on the foot skin in these environments, followed by a discussion of where future research work would benefit.

9.2.1. Use of commercially available hydration measurement devices for the assessment of emollient efficacy on the foot skin.

Prior to selecting a commercially available hydration measurement device for the assessment of emollient effectiveness, three questions should be considered:

1. Which foot skin sites will be measured within this study?
2. What aspect of foot skin behaviour is intended to be modified by using the product?
3. In what environment will data-collection take place?

If skin hydration data is collected from the plantar and dorsal skin, the Corneometer® CM825 is the most suitable device due to its consistent correlation with the physical characteristics of the foot skin across the surface. It also most closely reflects consumer perception of their skin dryness. The MoistureMeter SC™ has been shown to demonstrate similar correlations with foot skin characteristics associated with hydration, however, it does not reflect consumer perception of their skin dryness as closely.

Aspects related to the practicalities of testing are also worthy of consideration. The Corneometer® CM825 collects data in approximately 1/3 of the time the MoistureMeter SC™ does; However, this device must be wired to a base unit and PC during data-collection, restricting its movability. Therefore, this device is unsuitable for use outside of a laboratory environment. The MoistureMeter SC™ is a wireless handheld unit. Data can be transmitted to a PC nearby via Bluetooth or recorded manually by the user (i.e., using pen or paper). This device is suitable for use in a clinical or home environment.

If skin hydration data is collected only from the plantar skin, the MoistureMeter D® is the commercially available hydration measurement device used within this study that most closely correlated with the physical behaviour or the plantar skin. This is a wired unit that can be used with software to automatically record data, or data can be manually recorded. In a commercial setting, hydration measures may be collected exclusively from the plantar skin when this is the only skin site relevant to the intervention being used, i.e., where therapy is intended to reduce hyperkeratosis on the plantar metatarsal heads or heel (such as callus removal plasters).

Irrespective of whether only plantar measures are collected, if the primary interest of the research team is consumer perception of foot skin health, the Corneometer® CM825 should be used for data-collection.

Similarly, if data is to be collected in a home or clinic environment, the MoistureMeter SC™ is the most appropriate device for use as this is easily transportable and still correlates well with plantar skin characteristics (albeit to a lesser level than the MoistureMeter D®).

9.2.1.1. Product claims

Often, emollients are marketed to customers using product claims that relate to changes in skin-hydration, the physical behaviour of skin, or consumer perception of skin features: i.e., ‘doubles skin hydration in 24 hours’, ‘decreases skin hardness in 7 days’, ‘softer-feeling skin in 48 hours’. The first two can be supported using hydration measurement devices or physical skin testing equipment, such as the SATRA STD 226 Digital Durometer, for example.

Such investigations of product on consumers requires repeatable data to be captured to explore the influence of emollient over periods of time (24, 48 hours or 7 days). The pilot study described in Chapter 5 explored within- and between- day repeatability of the Corneometer® CM825, MoistureMeter SC™ and MoistureMeter D®. Further participant numbers are required to increase the confidence in the findings of this study, but observations include that: Data collected using each of these devices is relatively repeatable between and within days, and between and within-day repeatability is not seemingly influenced by skin hydration levels (i.e., individuals with low skin hydration did not show more

or less variability between data-collection sessions than individuals with high skin hydration). These findings support the use of these three devices in longitudinal studies of emollient effectiveness.

Finally, following the completion of study 'An investigation into the hydration of the foot skin and associated skin characteristics', individual components of the FSkHQ have been shown to display internal (FSkHQ skin scores correlate with overall dryness score) and external validity (correlation with objective measures of skin characteristics). Therefore, this is recommended as a self-administered tool for assessing foot skin features and overall perception of foot skin health, offering opportunities for consumer perception-based product claims to be developed.

9.2.2. Use of commercially available hydration measurement devices for the monitoring of foot skin health in a clinical environment

The suitability of commercially available hydration measurement devices for use to assess foot skin health in a clinical environment depends on the risk-factors being addressed and the skin sites of interest.

With advanced age and diabetes, skin pathology commonly arises on the plantar foot where high pressures coincide with xerosis, resulting in hyperkeratosis forming. These lesions are characterised by their increased hardness.

The MoistureMeter D® is the commercially available hydration measurement device that collects data from the plantar foot skin that most consistently correlates with foot skin hardness and is therefore perceived to be the most suitable device for detecting changes to plantar skin hydration that are indicative of hardness. For example, this device would be most suitable for use when a patient has recurring heel fissures, when managing the hardness of the plantar heel skin is paramount to prevent fissures from developing.

The MoistureMeter D®, however, is considerably more expensive (£7650) than the Corneometer® CM825 (£2770) and MoistureMeter SC™ (£2750), which would also be suitable for use on the plantar foot, although these generate data that correlates with the physical behaviour of this tissue to a slightly lesser degree.

9.2.3. Opportunities for application of the DermaStat

The DermaStat® has so far been excluded from discussions around application within a clinical or commercial setting, in part due to the lack of data supporting its use outside of this PhD project, but also due to its inability to collect data from areas of skin with low hydration (see Section 8.4.2.).

However, this device has been shown to collect data that is comparable to the other capacitance-based devices used in this work (the Corneometer® CM825 and MoistureMeter SC), and its extremely low cost (£35) makes it appropriate for use in very different circumstances to other devices.

The primary limitation associated with the DermaStat® is its inability to record data consistently at skin sites with low water content. This inhibits using this device to assess existing xerosis in any circumstance (as baseline values could not be recorded). However, this does not preclude its use to monitor healthy foot skin for the development of xerosis. For example, the DermaStat® could be provided to patients to monitor their foot skin hydration prior to the development of xerosis. Whether the skin hydration data observed by the patient decreases or hydration measurements begin to fail, this is still an indicator of a change in foot skin hydration that a participant could report to their healthcare provider. This could be a helpful tool in identifying xerosis early and through providing emollient and monitoring its effectiveness, preventing tissue damage resulting from xerosis and hyperkeratosis. Additionally, providing patients with the tools to monitor their foot health aligns with the increasing focus on patient-centred care and encouraging self-management.

As described in Chapter 4, the manufacturer of this device (Arche Healthcare) is already developing a device for self-testing foot skin hydration for the American healthcare system. Although this device uses bioimpedance (a different mechanism to the capacitance based DermaStat) and is intended for use on the dorsal foot. Further research on the bioimpedance methods suitability for foot skin, and the correlation between dorsal and plantar foot skin would be required before this product was similarly endorsed.

9.3.4. Recommendations for future research

As alluded to in each experimental chapter of this thesis, there are significant opportunities for future work to build upon the project outcomes. In the below section, these are presented formally as defined areas for future work:

9.3.4.1. Application of Confocal Raman Spectroscopy to the at-risk foot.

In this study, the successful application of CRS to the foot skin has been demonstrated, however the participant numbers involved in this study and the demographics of the participant groups recruited have limited the statistical significance and the transferability of the data collected.

In future, larger participant numbers would increase the statistical confidence associated with comparisons between groups of people with varied foot risk. Post-hoc power calculations indicate that a sample number of 30 to 40 per group would be sufficient to detect statistically significant differences between the water content of the skin between young people and older people in the arch and heel, and people with and without diabetes in the arch (with 80% power) (Kane, 2018). To detect a statistically significant difference in the water content of the heel SC between age-matched individuals with and without diabetes, 240 participants would be required for each group. This is due to the small difference observed between groups at this location. These calculations have been made using data obtained at the SC depth where differences between water volume are most evident.

Table 66. Demonstration of post-hoc power calculations.

Comparison	Skin site	Measurement depth (µm)	Group 1 (% water content of SC) (mean ± SD)	Group 2 (% water content of SC) (mean ± SD)	N per group	Power
Young healthy Vs older healthy	arch	4	28.65 ± 5.31	24.78 ± 5.03	29	81.3%
	heel	0	27.4 ± 8.73	21.41 ± 7.34	29	80.7%
People without diabetes and people with diabetes	arch	0	22.91 ± 6.81	18.66 ± 6.31	38	80.6%
	heel	5	21.41 ± 7.34	19.88 ± 4.15	240	80.3%

These calculations do not consider the potential for the threshold for statistical significance to be modified if multiple comparisons are undertaken during statistical testing, i.e., if the

same statistical model of multiple T-tests is used, so could be an underestimation of the participants required to achieve significant results dependant on the study design.

However, as individuals with more advanced diabetic foot disease are anticipated to deviate further from the non-diabetic cohort than the current cohort with diabetes, fewer participants would likely be required to observe statistically significant changes between groups if more high-risk people with diabetes were recruited. As previously described, it would be useful to collect CRS data from the feet of people with different stages of diabetic foot disease to observe any changes in foot skin composition with the disease advancement. Emollients expressly designed to deliver compounds lost from the skin could then be developed, and their efficacy assessed using further CRS investigation.

Additionally, examining plantar areas of prior ulceration or high-risk tissue using CRS could offer insight into the composition of skin that is immediately vulnerable to tissue damage. This could be achieved by recruiting individuals who have a history of diabetic foot ulceration and collecting data from sites that have previously ulcerated or are identified as being at risk of ulceration (as indicated by peak plantar pressures) (Abbott et al., 2022).

9.3.4.2. Use of CRS to assess foot skin xerosis and efficacy of emollient.

In this study, only healthy foot skin was examined using CRS. Through collecting these same data on xerotic foot skin, it would be possible to identify where within the skin composition changes with xerosis, and which particular compounds are lost due to this process. This would generate an opportunity for emollients to be formulated specifically to supplement these lost compounds.

It would also be pertinent to test the effectiveness of emollients on xerotic skin, as this is a more authentic representation of their use in the real world. This would allow for exploration of how compounds lacking in xerotic skin may, or may not, be restored using a topical applicant and could also provide insight into the use of penetration-enhancing materials on the plantar skin.

9.3.4.3. Use of commercially available hydration measurement devices within participant groups with varied foot-health risk.

In the study described in Chapter 7, four commercially available devices were used to measure the hydration of healthy foot skin in young people, older people, and people with diabetes. Unfortunately, due to the limited data due to instrument breakages and restricted participant numbers, it was not possible to make meaningful comparisons between the hydration data from each participant group for each device.

In future, it would be useful to have a dataset from each device for individuals of varied foot risk, both from a range of ages and of different diabetes status (with a range of levels of diabetic foot disease). Access to a large volume of data such as this would allow researchers, clinicians, and industry professionals to understand what a 'typical' skin hydration is for people according to their demographic, and even offer the opportunity for a clinically important difference to be established for each device. This benchmark would have implications on the testing of emollients, and the monitoring of foot skin health over time.

Finally, to support further research using the commercially available hydration measurement devices used within this project, it would be pertinent to further explore the methodological uncertainties remaining following the completion of this project: Primarily the identification of a suitable plantar skin acclimatisation period. This could be resolved through the repetition of the acclimatisation-period aspect of the pilot work described in Chapter 5, with a larger number of participants and a broader range of acclimatisation periods.

Chapter 10. Conclusion

Worldwide, the population is ageing, and diabetes is becoming more prevalent (National Cardiovascular Intelligence Network, 2022). Age and diabetes are associated with an increase in the incidence of foot-skin health problems, which can limit an individual's ability to live independently and without pain and represent a significant cost to health services (Armstrong et al., 2020; Farndon et al., 2009; Kerr et al., 2019).

Foot skin xerosis is common and often precedes the development of more serious foot skin pathology, such as diabetic foot ulcers (Pavicic & Korting, 2006). Xerosis can be treated using emollients readily accepted and used by the general public (Voegeli, 2007). However, a paucity of data on the composition of the healthy foot skin, and the composition of skin in a population with increased foot-health risk, has limited the development and assessment of effective treatments for foot xerosis.

Within this PhD project, novel data have been collected on the composition of the foot skin using CRS. These data have revealed a different hydration gradient on the plantar skin than non-plantar skin and identified differences between the composition of the ventral forearm skin in people of different ages and diabetes status.

As part of an emollient study, urea was found to penetrate the plantar SC; however, this was not necessarily found to increase SC hydration. This has generated insight into the structure of the plantar SC and provided evidence to support the use of CRS to assess emollient effectiveness on the plantar foot.

Across these works, a variety of commercially available hydration measurement devices were also applied to the foot skin. This has resulted in the generation of a large normative dataset for each of these devices.

Data collected using these devices has been correlated with the characteristics of the foot skin associated with skin hydration (hardness, elasticity, roughness, and perception of skin dryness) and CRS hydration data collected across skin depth. These comparisons have generated novel data on the measurement depths of commercially available hydration measurement devices when applied to different skin surfaces, and their varied implications of the data they collect on plantar and non-plantar skin.

By combining these novel data with their experiences of using these devices, the author has generated a series of recommendations for using commercially available hydration measurement devices on the foot skin in future.

Finally, a series of suggestions have been made for areas for further research relating to this area of study, including the wider application of CRS, and opportunities that could be taken to further understand the suitability of the use of commercially available hydration measurement devices on the at-risk foot.

10.1. References

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Chapter 11. Appendices

Appendix 1. Dissemination

During the course of the PhD Studentship, the author has presented their work, or reflections of their experiences as a student of the EPSRC Centre for Doctoral Training in Prosthetics and Orthotics or as a clinical-academic working in partnership with industry, at several academic conferences. Additionally, the author has presented the findings of their work to their industry sponsor for implementation into Scholl research and development processes, and in one instance ran an education session for the wider Scholl staff. These activities are in Table 67.

Table 67. Record of presentations.

Academic Conferences	
Venue and date	Title of presentation
London Skin Club March 2023	An evaluation of the biochemical components of the foot skin using in-vivo Confocal Raman Spectroscopy
ISPO 'Building Research into Prosthetic Clinical Practice' 2023	Centre for Doctoral Training in Prosthetics and Orthotics: Experiences of a PhD Student
Royal College of Podiatry Annual Conference 2022	Contemporary research in private practice, the NHS and industry
OT World Leipzig 2022	EPSRC Centre for Doctoral Training in Prosthetics and Orthotics – Experiences of a PhD Student and the organisation of CDT in P&O conference
Industry Communication	
Setting	Title
Internal presentation to Research and Development team and supervisory team 2023	Non-invasive methods of quantifying the composition of the plantar epidermis: The interpretation of data in health, ageing and disease.
Internal presentation to staff across the business 'Breakfast club' 2022	Skin Hydration and Emollient Therapy

During the course of their PhD, the author has also contributed to, or independently generated, several published works (See Table 68).

Table 68. Record of publications.

Authors (Date) Title *Publisher or Conference*	Format
Parker D, Andrews J, Price C (2023) Validity and reliability of the XSENSOR in-shoe pressure measurement system *PLOS ONE 18(1): e0277971*. Accessible at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0277971	Paper
Andrews, J (2021) Foot skin hydration: Quantification, interpretation, and opportunities for modification *The Royal College of Podiatry Annual Conference 2021*	Digital Poster
Andrews, J (2019) Skin Surface pH of the Healthy Adult Foot *The College of Podiatry Annual Conference and Exhibition 2019*	Poster
Andrews, J (2019) An Investigation into the pH of the Foot Skin in Relation to Prevalence and Presentation of Tinea Pedis: A Project Proposal *The College of Podiatry Annual Conference and Exhibition 2019* - 'Highly Commended' Student Poster	Poster

Appendix 2. Foot Skin Health Questionnaire - Pre-pilot

Consumer perception of foot skin features questionnaire

There are two sections within this questionnaire:

Section A is about your general foot skin health and comprises a mix of Visual Analogue Scale questions and Yes/No tick boxes.

Section B will require you to look at and feel the skin on different parts of your feet and provide information on how scaly, rough, hard or cracked you feel these skin areas are. If you need any assistance with this process, the researcher will be in place to support you.

Guidance for completion of this questionnaire:

Using Visual Analogue Scales

Within this document, you will be asked to answer several questions that require you to use a Visual Analogue Scale. Within these questions, there will be a line drawn on the page with a statement at either end, and you will be asked to answer the question by making a mark along the line between the two statements.

To demonstrate, please see the example below:

How tired do you feel now?

Not tired at all Extremely tired

The individual answering this question would make a small mark on this line at a location they think represents their current level of tiredness.

I.E. if they were feeling very tired, they may make a mark here:

Not tired at all Extremely tired

But if they were not feeling very tired at all they may place a mark here:

Not tired at all Extremely tired

Section A

1. How healthy do you think the skin on your feet is? (mark on the horizontal line with a small vertical line)

Very healthy Very unhealthy

2. Would you describe the skin on your feet as ‘dry’?

Yes ☐

No ☐

I don’t know ☐

If you have answered ‘yes’ to question 2., move on to question 3. If you have answered ‘no’ or ‘don’t know’, move on to question 4.

3. How ‘dry’ is the skin on your feet? (mark with a vertical line)

Skin is well-hydrated Extremely dry, worst ever

4. Would you describe the skin on your feet as ‘hard’?

Yes ☐

No ☐

I don’t know ☐

If you have answered ‘yes’ to question 4., move on to question 5. If you have answered ‘no’ or ‘don’t know’, move on to question 6.

5. How ‘hard’ is the skin on your feet? (mark with a vertical line)

Skin is not hard Extremely hard, worst ever

6. Would you describe the skin on your feet as ‘rough’?

Yes ☐

No ☐

I don’t know ☐

If you have answered ‘yes’ to question 6, move on to question 7. If you have answered ‘no’ or ‘don’t know’, move on to question 8.

7. How ‘rough’ is the skin on your feet? (mark with a vertical line)

Skin is not rough Extremely rough, worst ever

8. Do you have foot pain as a result of problems with your foot skin?

Yes ☐

No ☐

I don’t know ☐

If you have answered ‘yes’ to question 8, move on to question 9. If you have answered ‘no’ or ‘don’t know’, move on to question 11.

9. How would you rate the level of pain resulting from the skin on your feet? (mark with a vertical line)

No pain Extremely painful, worst ever

10. If you answered 'Yes' to the above, please describe what you think the cause of this pain may be:

_____

_____

11. Do you use any products to treat your foot skin at home? (Please tick those applicable)

Emollients:		Tools to remove hard skin:	
Body cream/lotion	☐	Foot file	☐
Foot-specific cream/lotion	☐	Pumice stone	☐
Heel balm	☐	Foot mask to remove skin	☐
Hydrating foot mask	☐	Other skin treatments:	
Oil	☐	Foot bath or soak	☐

If you use products that are not listed above, please tell us about them in the space below.

_____

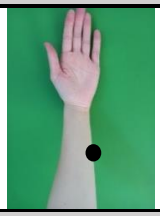
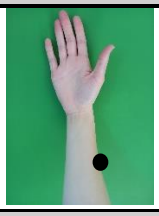
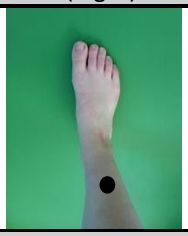
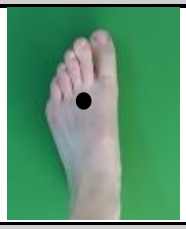
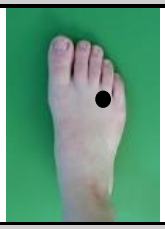
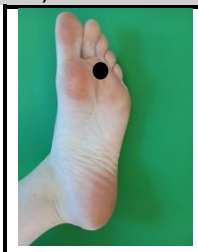
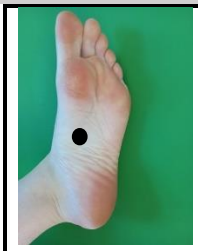
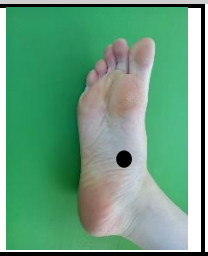
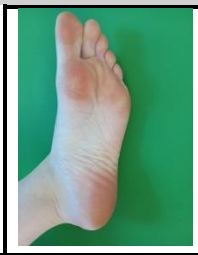
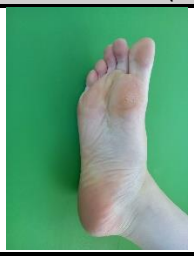
Section B

For this section, you will need to examine 6 different areas on each of your feet (12 in total) and provide information on how scaly, rough, hard, or cracked the skin is. If some of this section is not applicable to you, please write N/A in the relevant section.

To complete this, you will need to identify each area of skin, using the diagrams embedded in Table 1 and score these according to the Modified Specified Symptom Sum Score Reference Table 2. The Researcher will talk through this scale with you beforehand.

The Modified Specified Symptom Sum Score Table provides a brief description of skin features that align with scores of 0-4 for four categories: scaliness, roughness, hardness, and severity of cracking of the skin. For each area of skin, please provide a value for each of these categories.

Table 1. Skin Scoring Matrix

Forearm (Left)			Forearm (Right)		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	
Shin (Left)			**Shin (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	
Top of 3rd toe base joint (Left)			**Top of 3rd toe base joint (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	
Underside of 3rd toe base joint (Left)			**Underside of 3rd toe base joint (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	
Arch under foot (Left)			**Arch under foot (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	
Centre of heel (Left)			**Centre of heel (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Roughness				Roughness	
Hardness				Hardness	
Cracking				Cracking	

Modified Specified Symptom Sum Score – Reference Table

Please use the table below to score each skin location described in Section B for its scaliness, roughness, skin thickening and hardness and the severity of cracks/fissures. We would recommend that you use the side of the fingertip if you would like to feel the area of skin, rather than the pulp.

Table 2. Modified Specified Symptom Sum Score

Original Score	Descriptor	
	Scaling (visual evaluation)	
0	Absent	
1	Slight	Small scales only, surface lightly dull in colour
2	Moderate	Small scales in combination with larger scales (>0.05 mm), surface opaque or whitish
3	Severe	Larger and large scales (flakes >1 mm) are prominent, surface whitish
4	Extreme	Large flakes (flakes >1 mm) covering almost all of the skin surface
	Cracks fissures (visual evaluation)	
0	Absent	
1	Slight	One crack, or a few superficial cracks present
2	Moderate	Single or grouped superficial and more deep cracks
3	Severe	As 2 but with deep cracks
4	Extreme	Numerous deep cracks
	Roughness (tactile evaluation)	
0	Absent	Perfectly smooth
1	Slight	Slightly rough skin surface
2	Moderate	Rough skin surface
3	Severe	Very rough skin surface
4	Extreme	Skin surface is extremely rough and scratchy. Skin markings are disturbed.
	Thickening and hardening (tactile evaluation)	
0	Absent	Skin is pliable
1	Slight	Skin is pliable but feels slightly thickened
2	Moderate	Skin is of increased thickness and hardness.
3	Severe	Skin feels very thick and hard.
4	Extreme	Skin is extremely thick and hard. Appears callused.

Appendix 3. Foot Skin Health Questionnaire – Post pilot

Foot Skin Health – Participant Questionnaire

There are two sections within this questionnaire. Please complete both sections.

Section A is about your general foot skin health and comprises a mix of Visual Analogue Scale questions and Yes/No tick boxes.

Section B will require you to look at and feel the skin on different parts of your feet and provide information on how scaly, rough, hard or cracked you feel these skin areas are. If you need any assistance with this process, the researcher will be in place to support you.

Guidance for completion of this questionnaire:

Using Visual Analogue Scales

Within this document, you will be asked to answer several questions that require you to use a Visual Analogue Scale. Within these questions, there will be a line drawn on the page with a statement at either end, you will be asked to answer the question by making a mark along the line between the two statements.

To demonstrate, please see the example below:

Q1. How tired do you feel now?

Not tired at all Extremely tired

The individual answering this question would make a small mark on this line at a location they think represents their current level of tiredness.

I.E. if they were feeling very tired, they may make a mark here:

Not tired at all Extremely tired

But if they were not feeling very tired at all they may place a mark here:

Not tired at all Extremely tired

If this is unclear, please contact your podiatrist.

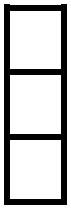
Section A

12. How healthy do you think the skin on your feet is? (mark on the horizontal line with a small vertical line)

Very healthy Very unhealthy

13. Would you describe the skin on your feet as ‘dry’?

Yes
No
I don’t know



If you have answered ‘yes’ to question 2., move on to question 3. If you have answered ‘no’ or ‘don’t know’, move on to question 4.

14. How ‘dry’ is the skin on your feet? (mark with a vertical line)

Skin is well-hydrated, best ever Extremely dry, worst ever

15. Would you describe the skin on your feet as ‘hard’?

Yes ☐
No ☐
I don’t know ☐

If you have answered ‘yes’ to question 4., move on to question 5. If you have answered ‘no’ or ‘don’t know’, move on to question 6.

16. How ‘hard’ is the skin on your feet? (mark with a vertical line)

Skin is not hard, best ever Extremely hard, worst ever

17. Would you describe the skin on your feet as ‘rough’?

Yes ☐
No ☐
I don’t know ☐

If you have answered ‘yes’ to question 6, move on to question 7. If you have answered ‘no’ or ‘don’t know’, move on to question 8.

18. How ‘rough’ is the skin on your feet? (mark with a vertical line)

Skin is not rough, best ever Extremely rough, worst ever

19. Do you have foot pain as a result of problems with your foot skin?

Yes ☐

No ☐

I don't know ☐

If you have answered 'yes' to question 8, move on to question 9. If you have answered 'no' or 'don't know', move on to question 11.

20. How would you rate the level of pain resulting from the skin on your feet? (mark with a vertical line)

No pain Extremely painful, worst ever

21. If you answered 'Yes' to the above, please describe what you think the cause of this pain may be:

_____

_____

22. Do you use any products to treat your foot skin at home?

In this section, please tick all boxes that apply.

Emollients:	
Body cream/lotion	☐
Foot-specific cream/lotion	☐
Heel balm	☐
Hydrating foot mask	☐
Oil	☐
Tools to remove hard skin:	
Foot file	☐
Pumice stone	☐
Foot mask to remove skin	☐
Other skin treatments:	
Foot bath or soak	☐

23. If you use products that are not listed above, please tell us about them in the space below. Feel free to include brand names of products.

_____

_____

_____

Guidance for completion of Section B:

For this section, you will need to examine 6 different areas on each of your feet and one location on each of your forearms (14 in total). You will be asked to provide information on how scaly, rough, hard, or cracked the skin is. If some of this section is not applicable to you, please write N/A in the relevant section.

To complete this, simply identify each area of skin using the photographs embedded in Table 2 and score these according to the Modified Specified Symptom Sum Score Reference Table on Page 6. The Researcher will talk through this scale with you beforehand.

The Modified Specified Symptom Sum Score Table provides a brief description of skin features that align with scores of 0-4 for four categories: scaliness, roughness, hardness, and severity of cracking of the skin. For each area of skin, please provide a value for each of these categories.

Modified Specified Symptom Sum Score – Reference Table

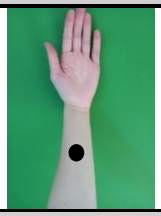
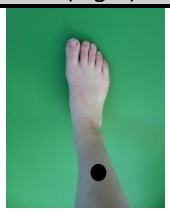
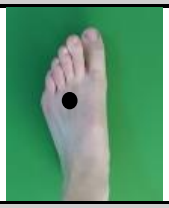
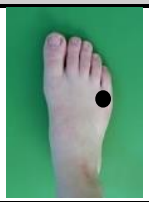
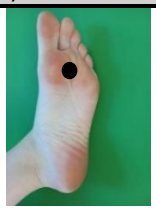
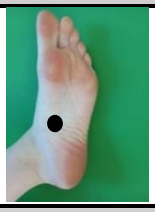
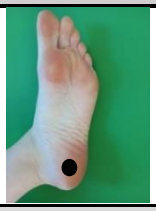
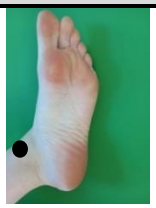
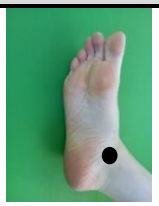
Please use the table below to score each skin location described in Section B for its scaliness, roughness, skin thickening and hardness and the severity of cracks/fissures. We would recommend that you use the side of the fingertip if you would like to feel the area of skin.

Table 1. Modified Specified Symptom Sum Score

Original Score	Descriptor	
	Scaling (visual evaluation)	
0	Absent	
1	Slight	Small scales only, surface lightly dull in colour
2	Moderate	Small scales in combination with larger scales (>0.05 mm), surface opaque or whitish
3	Severe	Larger and large scales (flakes >1 mm) are prominent, surface whitish
4	Extreme	Large flakes (flakes >1 mm) covering almost all of the skin surface
	Cracks fissures (visual evaluation)	
0	Absent	
1	Slight	One crack, or a few superficial cracks present
2	Moderate	Single or grouped superficial and more deep cracks
3	Severe	As 2 but with deep cracks
4	Extreme	Numerous deep cracks
	Roughness (tactile evaluation)	
0	Absent	Perfectly smooth
1	Slight	Slightly rough skin surface
2	Moderate	Rough skin surface
3	Severe	Very rough skin surface
4	Extreme	Skin surface is extremely rough and scratchy. Skin markings are disturbed.
	Thickening and hardening (tactile evaluation)	
0	Absent	Skin is pliable
1	Slight	Skin is pliable but feels slightly thickened
2	Moderate	Skin is of increased thickness and hardness.
3	Severe	Skin feels very thick and hard.
4	Extreme	Skin is extremely thick and hard. Appears callused.

Table 2. Skin Scoring Matrix

Please refer to Table 1 for scoring guidance.

Forearm (Left)			Forearm (Right)		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Shin (Left)			**Shin (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Top of 3rd toe base joint (Left)			**Top of 3rd toe base joint (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Underside of 3rd toe base joint (Left)			**Underside of 3rd toe base joint (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Arch under foot (Left)			**Arch under foot (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Centre of heel (Left)			**Centre of heel (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	
Inside Ankle (Left)			**Inside Ankle (Right)**		
Category	Score (0-4)			Category	Score (0-4)
Scaliness				Scaliness	
Cracking				Cracking	
Roughness				Roughness	
Hardness				Hardness	

Appendix 4. Study 1 recruitment material

Recruitment Letter

Hello

I am a PhD student at the University of Salford. I would like to collect some data from a small group of volunteers. I have sent this email to you, as I believe that you are currently working on the University campus. Please read the information below and contact me, using the details at the end of this form, if you would like to be involved.

Thank you for your consideration.

Jennifer Andrews

Post-graduate Research Student

Title of study:

Quantification of foot skin hydration: A pilot study

What is the purpose of the study?

The foot skin is a common location for dry skin to develop. To monitor the effectiveness of treatments for dry foot skin, knowledge is required of 'healthy' foot skin hydration and how this varies over time. The data obtained within this study will inform how several hydration measurement devices are used for this purpose in future.

Who are the participants?

People aged 20-40 who have no skin disease on their feet or a systemic illness that may influence their skin (I.E. Diabetes or Scleroderma).

What will happen if I choose to take part?

You will be asked to attend six data-collection sessions within the Skin Lab at the University of Salford. These will be booked across three days, and you will be required to attend the lab twice per day (10.00AM and 14.00PM). During these appointments, you will have a series of measurements taken from the skin on your feet, legs, and arms. You should not experience any discomfort as a result of the measurements. Each appointment will take approximately 40 minutes (AM and PM)

If you have any questions about this study or are interested in taking part, please contact the researcher using the contact details below. A more detailed information pack will be distributed to you.

Thank you for taking the time to consider your involvement in this study.

Jennifer Andrews

Post-graduate research student

j.r.andrews@edu.salford.ac.uk

07546 984 420

Appendix 5. Study 1 participant information sheet

Participant information sheet

Title of study:

Quantification of foot skin hydration: A pilot study

Study Details:

When the skin on the foot becomes dehydrated its surface can become hardened and cracked. This can lead to pain, disability, and an increased likelihood of soft tissue infections. There are lots of treatments available that claim to 'hydrate' the foot skin, such as lotions and creams. To assess the effectiveness of these products, the water content of the skin must be accurately measured.

To interpret hydration measures from foot skin correctly, it is important to understand how these data are influenced by the circumstances surrounding their collection (such as environmental conditions, time of day and repeat measurements). The purpose of this study is to gain an understanding of how the data collected by three hydration measurement devices varies according to these. The outcomes of this study will contribute to a larger study where the relationship between foot skin hydration and mechanical properties are explored.

Participants demographics:

Participants must be between the ages of 20 and 40, have no skin disease on the foot and have no systemic disease that may influence the skin (for example Diabetes or Scleroderma). Participants must not have any current symptoms of COVID 19 or have experienced these within 14 days before attending a data-collection appointment. Participants must be able to abstain from using any topical applicants (other than cleansing products) on the skin testing sites in the 7 days before data-collection. Finally, participants must not wash their feet for 5 hours before the data-collection session begins.

Do I have to take part in this study?

No, you have been given this information today as you expressed an interest in being involved in this study following an invitation email. If you do decide to be a participant, you will have an opportunity to ask questions and express any concerns before data collection taking place and you are free to withdraw from the study at any time. If you choose to withdraw your involvement from the study (before the publication of any data collected within this study) your data will be removed and the researcher will not contact you further regarding this project.

What will happen if I choose to take part?

You will be asked to attend six data-collection sessions within the Skin Lab at the University of Salford. These will be booked across three days and you will be required to attend the lab twice per day (10.00AM and 14.00PM). As you arrive at the clinic each morning you will call the researcher using the mobile number listed at the bottom of this form and the researcher will ask you to confirm that you do not have any current or recent (14 days) symptoms of Coronavirus. If this is the case, you will be greeted by the researcher at the clinic doors and asked to don a mask and apply hand sanitiser before entering. You will then be led to the data-collection space to begin the data collection session.

Each appointment will take approximately 40 minutes (AM and PM)

You will initially be given the opportunity to ask any questions you may have about the study before being asked to complete a written consent form that confirms your willingness to undergo data collection. If you are happy to continue after this process you will complete a COVID 19 'Track and Trace form' and a brief demographic form (day 1 only). You will then be asked to remove your shoes, hosiery and roll up any clothing to expose the lower leg and the forearms. Following a short period of rest, the researcher will take a series of measurements from the skin on your feet, lower leg and forearm using non-invasive devices.

Measurements will be taken from 14 skin sites, which will be marked using a skin-safe marker.. The mark will wash off after several days. You should not experience any discomfort as a result of the data collection procedure. All measurements will be taken using CE marked commercially available devices. These devices are not experimental and are regularly used in the cosmetics industry Once all data has been collected, you will re-dress and be escorted out of the clinic by the researcher.

Are there disadvantages of taking part?

We do not expect there to be any disadvantages to taking part in this study, the techniques used are non-invasive and should not cause any harm or discomfort.

What do I do if there is a problem?

If you have any concerns or complaints about the study, please contact the primary researcher using the details below. If this is not appropriate, then please contact Dr Hashmi, a supervisor on this project, using f.hashmi@salford.ac.uk.

Will my involvement in this project be kept private?

Yes, any personal data collected will be stored and accessed in line with the General Data Protection Regulations (May 2016). All documents, bar the consent forms and 'Track and Trace' forms, will be anonymised through the use of a participant identification number.

Written consent forms will be stored within a locked cabinet in the Brian Blatchford Building and destroyed following scanning and storage on a secure computer drive hosted by the University of Salford. 'Track and Trace' forms also will be retained within a locked cabinet but will be destroyed 21 days after data collection as per government guidelines. The secure drive and locked cabinet are only accessible by the researcher and their immediate supervisors. All other anonymised data will also be stored within the secure drive.

What will happen to the results of the study?

The data collected will be analysed by the researcher and findings will be published in a PhD thesis, as well as in scientific journals and conferences. The data may also be used by the industry sponsor who is funding this project, Scholl Wellness Co. Some of the data collected may also be shared via the University of Salford data repository. All data will be entirely anonymised and you will not be identifiable.

Data sharing as a result of the 'Track and Trace' system

The University will only share your personal data with public health authorities, where necessary, who may contact you as part of their contact tracing scheme. We will retain your data in line with government recommendations which is currently 21 days. Information on exercising your data protection rights can be found here: https://beta.salford.ac.uk/privacy

Who has reviewed and approved this study?

This study has been reviewed by an independent group called the Research Ethics Committee. Their role is to protect the safety and wellbeing of participants in research. If you have any concerns or complaints about this study, please contact the Research Ethics Committee using the following email: ethics@salford.ac.uk

Thank you for taking the time to consider your involvement in this study.

If you have any questions regarding this, please contact me using the details below:

Jennifer Andrews

Post-graduate Research Student

j.r.andrews@edu.salford.ac.uk

07546 984 420

Appendix 6. Study consent form

Participant Consent Form

Title of study: Quantification of foot skin hydration: A pilot study

Study overview: Participants will be required to expose the skin on their feet, lower leg and forearm. Non-invasive measurements of skin hydration will then be obtained from these skin areas following a short acclimatisation period. Full details of the protocol may be found within the Participant Information Sheet (see attached).

Please place an initial in the appropriate box and complete your name and signature at the base of this form.

	Yes	No
I have read and understood the patient information sheet and I understand what my contribution will be.		
I have had the opportunity to ask questions about the study and understand that I am free to ask questions throughout the appointment.		
I understand that during this study multiple measurements will be obtained from the skin on my feet, arm and lower leg.		
I understand that my participation in the study is voluntary and I may withdraw my data from use within this study up to two weeks following data collection.		
I agree for the measurements taken from my feet to be used for further research and publication within the University of Salford.		
I understand that my anonymised data may be shared with Scholl Wellness Co, and I consent to this.		
I agree to provide demographic information (see attached sheet).		
I agree to take part in the study discussed above.		

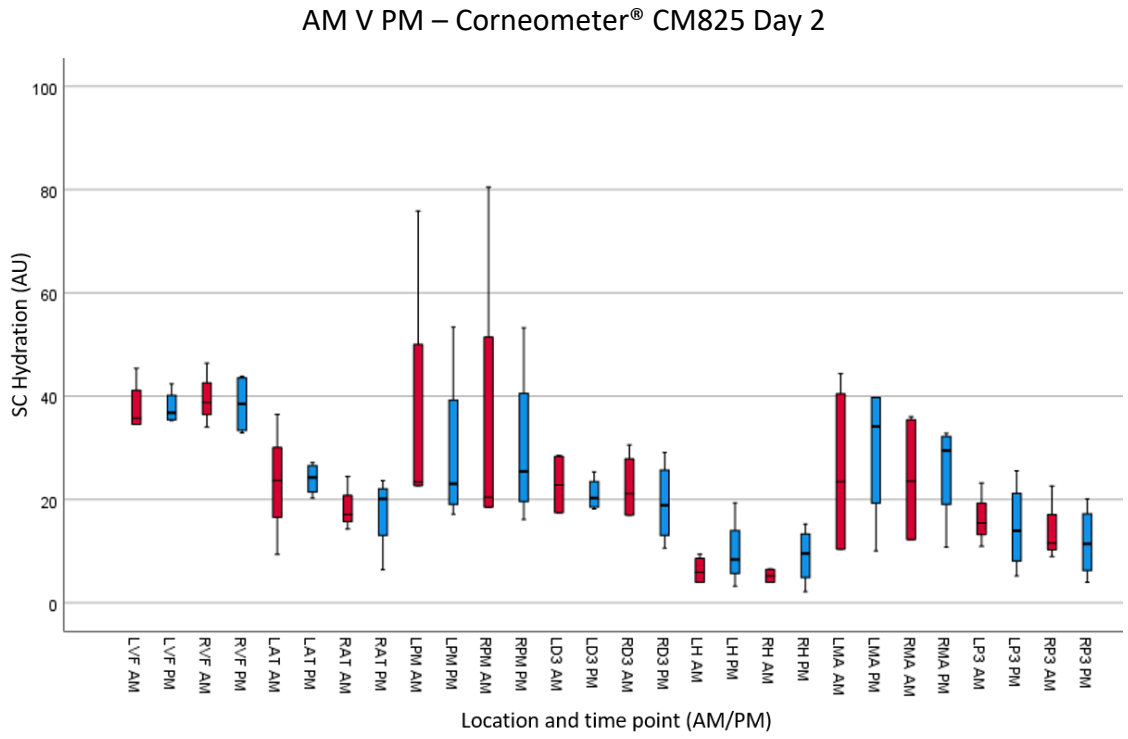
Participant Name……..

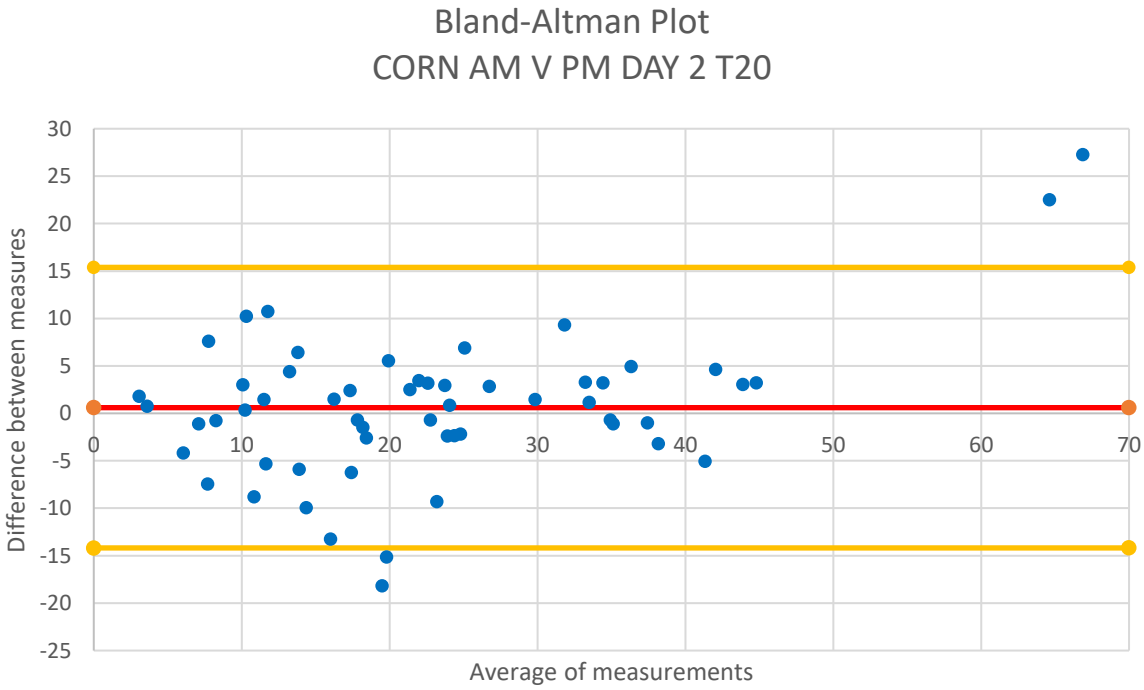
Participant Signature……

Researcher Signature……..

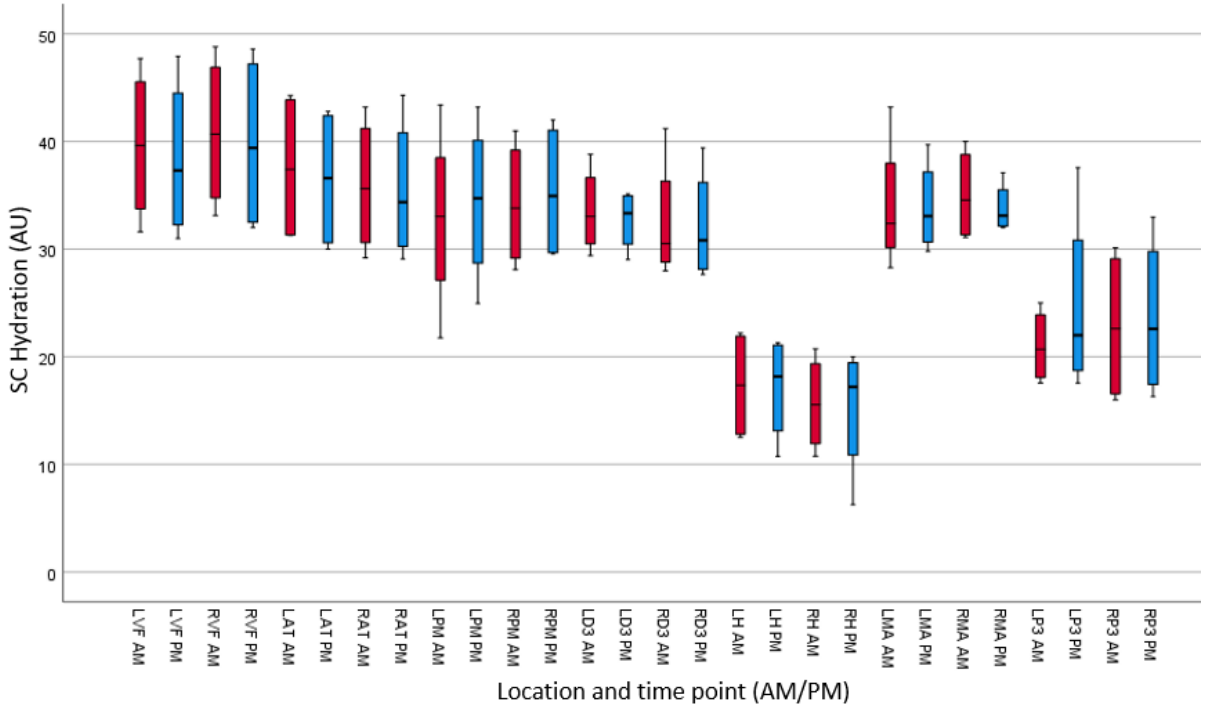
Date……
Participant number _____

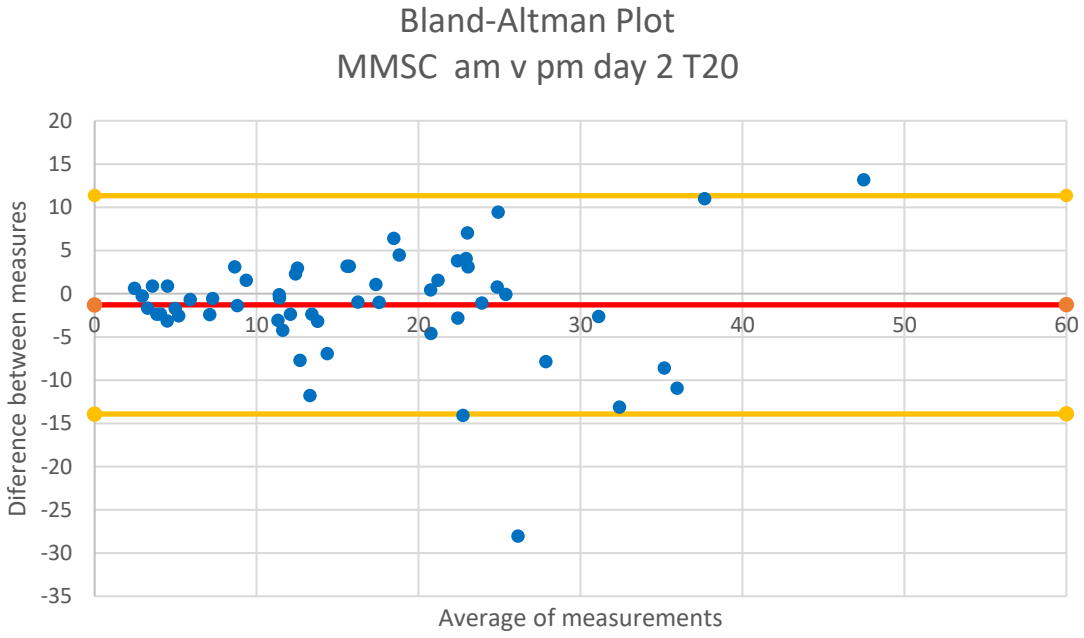
Appendix 7. Study 1 Repeatability Data



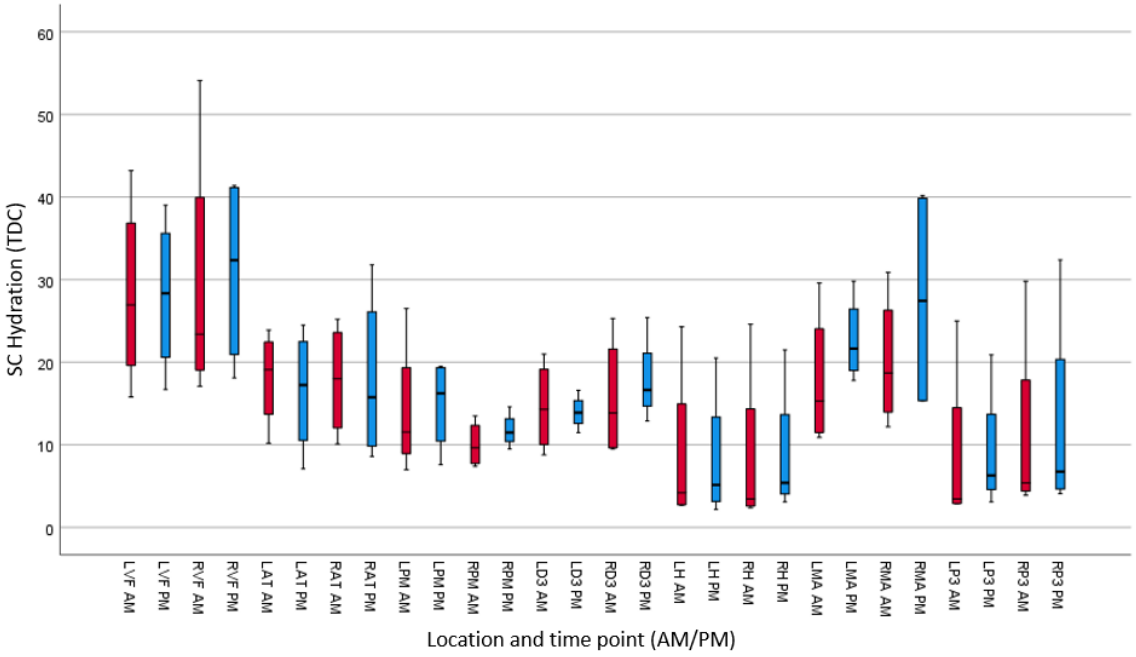


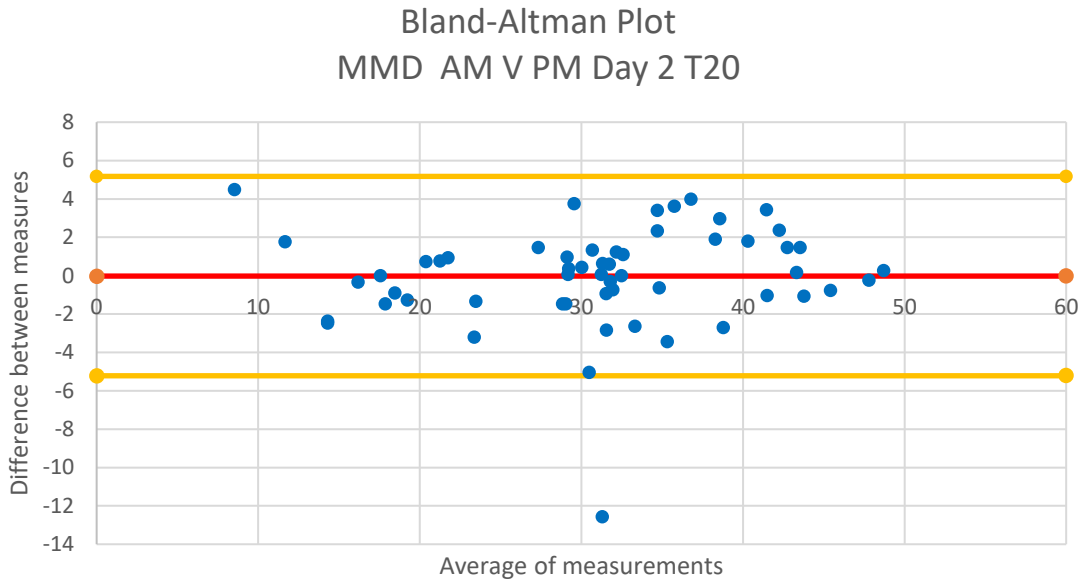
AM V PM - MoistureMeter SC™ Day 2



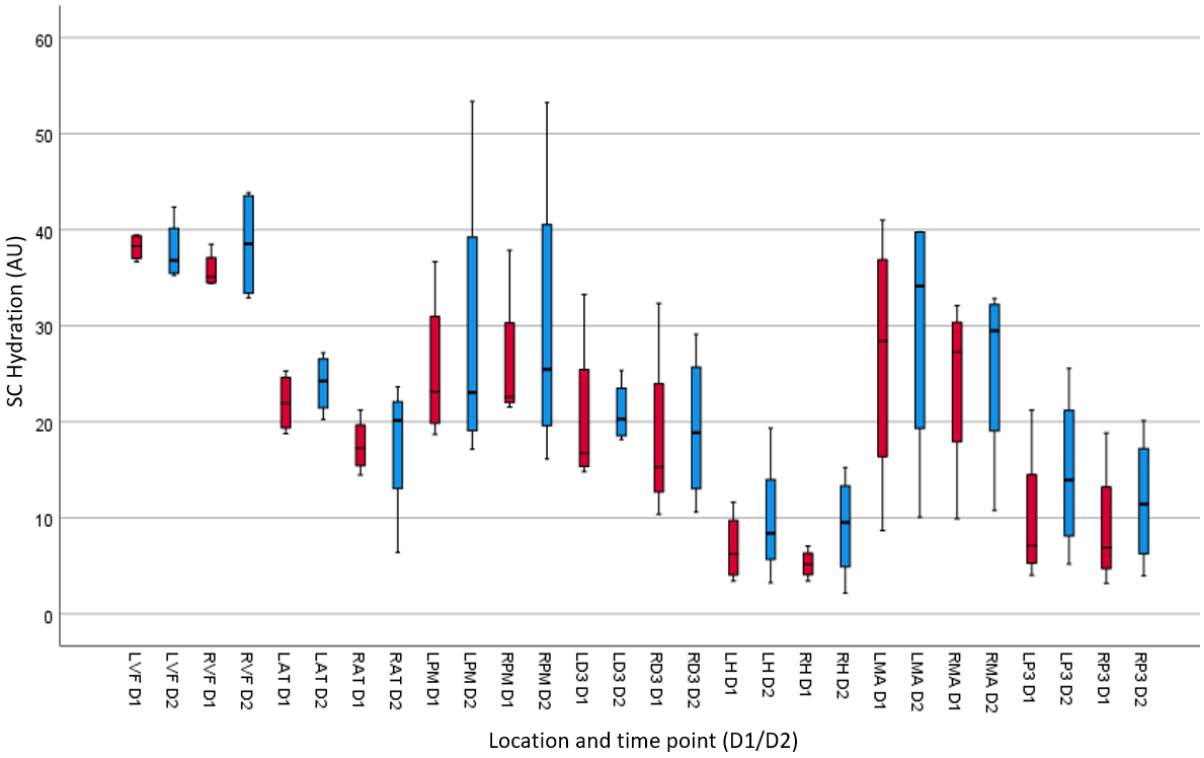


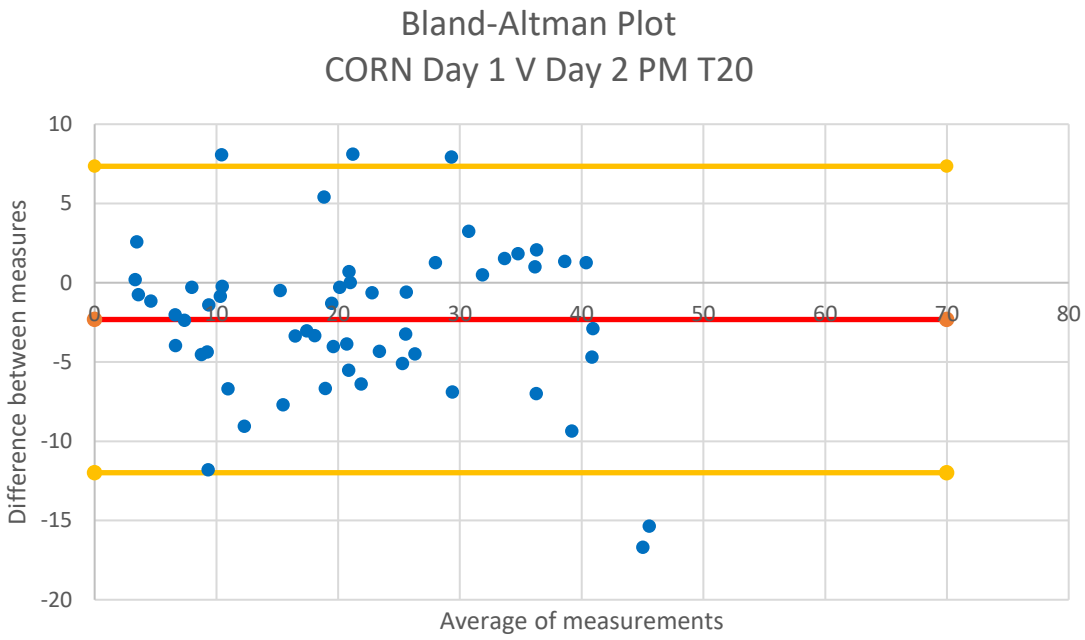
AM V PM - MoistureMeter D® Day 2



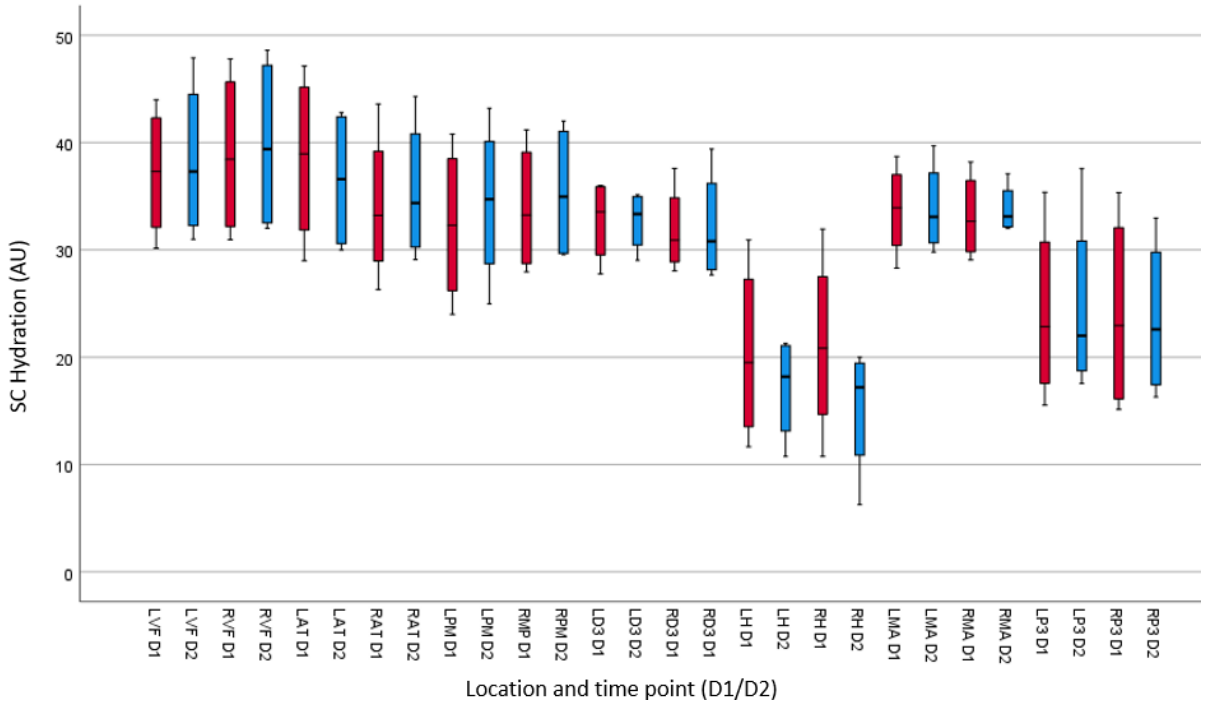


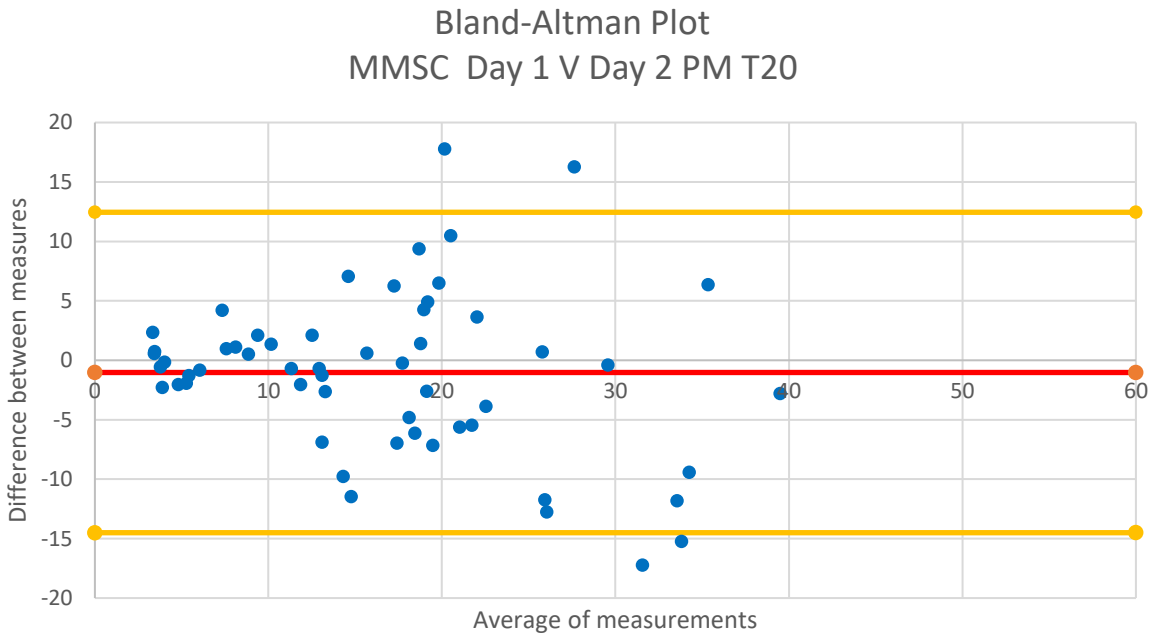
Day 2 V Day 2 – Corneometer® CM825 PM



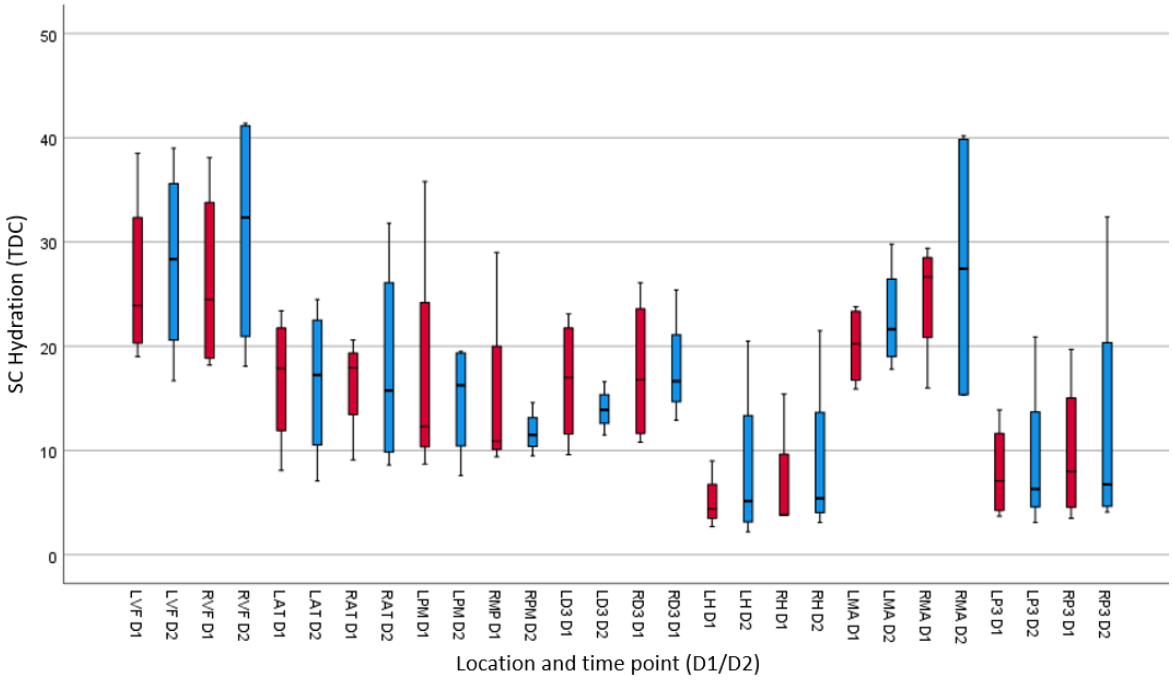


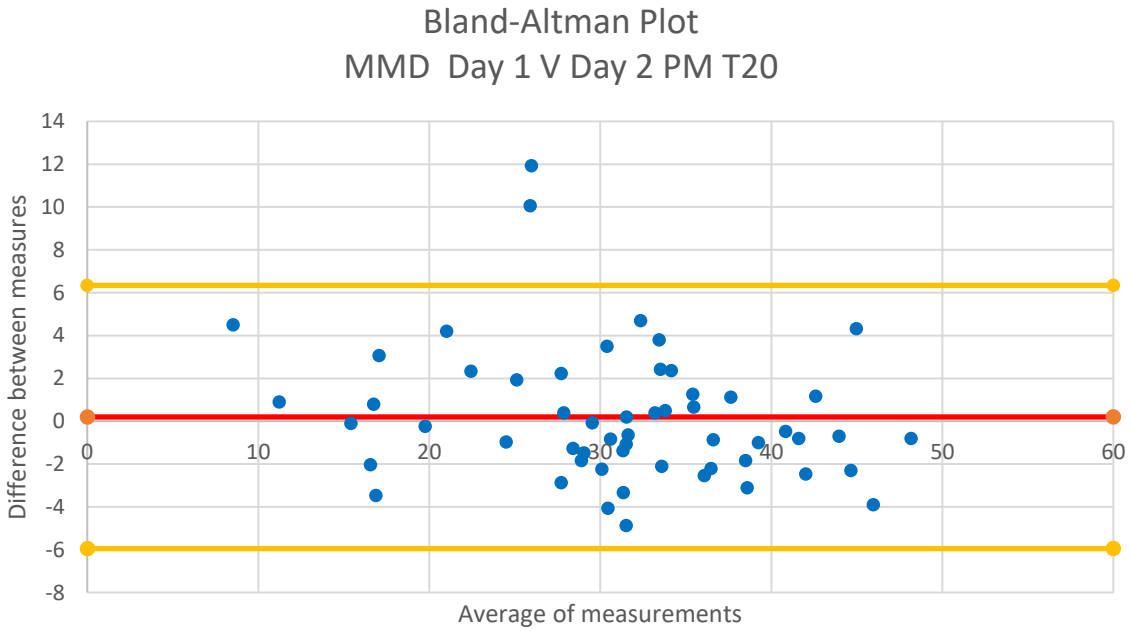
Day 1 V Day 2 - MoistureMeter SC™ PM





Day 1 V Day 2 - MoistureMeter D® PM





Appendix 8. Study 2 recruitment material

Recruitment Message

Hello

I am a PhD student at the University of Salford. I would like to collect some data from a small group of volunteers. I have sent this email to you, as I believe that you are currently working on the University campus. Please read the information below and contact me, using the details at the end of this form, if you would like to be involved.

Thank you for your consideration.
Jennifer Andrews
Post-graduate Research Student

Title of study:
An investigation into the hydration of the foot skin and associated skin characteristics.

What is the purpose of the study?
The foot skin is a common location for dry skin to develop. To monitor the effectiveness of treatments for dry foot skin, knowledge is required of 'healthy' foot skin hydration. Currently, little information is held on foot skin hydration. The available data is of uncertain value as it is collected using devices that haven't been evaluated for their usefulness on the foot skin.

This study aims to provide data that informs how hydration measurement devices are used in future and to provide more information on the 'healthy' hydration of the foot and how this relates to other skin characteristics.

Who are the participants?
We are looking for participants aged 20-40 who have no skin disease on their feet or a systemic illness that may influence their skin (I.E. Diabetes or Scleroderma).

What will happen if I choose to take part?
You will be asked to attend a data-collection session within the Skin Lab at the University of Salford. During these appointments, you will be asked to complete a short amount of paperwork, and a series of measurements will be taken from the skin on your arm, lower leg and foot. You should not experience any discomfort as a result of the measurements. An appointment takes approximately 60 minutes

If you have any questions about this study or are interested in taking part, please contact the researcher using the contact details below. A more detailed information pack will be distributed to you.

Thank you for taking the time to consider your involvement in this study.
Jennifer Andrews
Post-graduate Research Student
j.r.andrews@edu.salford.ac.uk
[redacted]

Appendix 9. Study 2 participant information sheet

Participant information sheet

Title of study:
An investigation into the hydration of the foot skin and associated skin characteristics.

Study Details:
The foot is a common location for dry skin conditions which can lead to the development of problems with the skin, such as heel cracks, soft tissue infections and ulcers. It is possible to manage dry skin conditions using topical applicants such as lotions and creams.

This purpose of this study is to help expand our understanding of the hydration of foot skin and how this influences its behaviour. To understand how effective lotions and creams are, we need to understand how much water is typically present in 'healthy' foot skin. Also, how this influences the behaviour of the skin (such as how the skin responds to being squashed) and what a 'significant' change in skin hydration looks like.

Currently, little data is available on the typical water content of foot skin, and the devices used to collect this data have not been tested for their suitability for use on the foot. Within this study we will be using three different devices designed to measure skin water content across the surfaces of the foot, arm, and leg. Other measurements will be taken of other skin characteristics known to vary with skin hydration (roughness, hardness, elasticity, frictional behaviour).

With this information we will be able to better understand foot skin water content and related tissue characteristics. This will inform how treatments for dry foot skin are developed and tested in future, helping those with problems with their foot skin.

Participants demographics:
Participants must be between the ages of 20 and 40, have no skin disease on the foot and no systemic disease that may influence the skin (for example Diabetes or Scleroderma). Participants must not have any current symptoms of COVID 19 or have experienced these within 14 days before attending a data-collection appointment. **Participants must be able to abstain from using any topical applicants (other than cleansing products) on the skin testing sites in the 7 days before data-collection.** Finally, participants must not wash their feet for 5 hours before the data-collection session begins.

Do I have to take part in the study?
No, you have been given this information today as you expressed an interest in being involved in this study. If you do decide to take part, you will have an opportunity to ask questions and express any concerns before undergoing data-collection. After your appointment, if you no longer want to be included in the study, your data can be removed as long as you let the researcher know within 2 weeks of attending the lab. After this time your information may have already been included in reports and papers.

What will happen if I choose to take part?

You will make an appointment for data collection within the Skin Lab at the University of Salford.
On the day of your appointment, you will attend the main entrance of the Salford University Podiatry Clinic and inform the researcher of your arrival via telephone. You will be greeted by the researcher at the clinic doors and asked to don a mask and apply hand sanitiser before entering. If you do not have a mask one will be provided to you. You will then be led to the data-collection space to begin the data collection session.
The appointment will take approximately 90 minutes.
You will be able to ask any questions you have about the study before being asked to complete a written consent form that confirms your willingness to undergo data collection. If you are happy to continue after this process you will complete a short questionnaire about your foot skin, and a brief demographic form. You will then be asked to sit comfortably and remove your shoes, hosiery and roll up any clothing to expose the lower leg and the forearms.

Measurements will be taken from 14 skin sites, which will be marked using a skin-safe marker. The mark will wash off after several days.

Following a short period of rest, the researcher will take measurements from the skin on your feet, lower leg and forearm using several non-invasive devices. These devices are all specifically designed to measure skin and you should not experience any discomfort. They involve a small probe being placed on the skin and held for a period of seconds. One device required a small adhesive disc to be applied to the skin which will be peeled off before the session is completed.

Once all data has been collected, you will re-dress and be escorted out of the clinic by the researcher.

Are there disadvantages of taking part?
We do not expect there to be any disadvantages to taking part in this study, the techniques used are non-invasive and should not cause any harm or discomfort.

What do I do if there is a problem?
If you have any concerns or complaints about the study, please contact the primary researcher using the details below. If this is not appropriate, please contact Dr Hashmi, a supervisor on this project, using f.hashmi@salford.ac.uk. If your queries remain unanswered, you can contact Prof Andrew Clark, Chair of the University's School of Health & Society Research Ethics Panel on a.clark@salford.ac.uk

Will my involvement in this project be kept private?
Yes, any personal data collected will be stored and accessed in line with the General Data Protection Regulations (May 2016). All documents, bar the consent forms and 'Track and Trace' forms, will be anonymised through the use of a participant identification number.

Written consent forms will be stored within a locked cabinet in the Brian Blatchford Building and destroyed following scanning and storage on a secure computer drive hosted by the University of Salford. The secure drive and locked cabinet are only accessible by the

researcher and their immediate supervisors. All other anonymised data will also be stored within the secure drive.

What will happen to the results of the study?
The data collected will be analysed by the researcher and findings will be published in a PhD thesis, as well as within scientific journals and abstract format for presentation at conferences. The data obtained within this study may also be used by the industry sponsor who is funding this project, Scholl Wellness Co. Some of the data collected may also be shared via the University of Salford data repository. All data will be entirely anonymised and you will not be identifiable.

Who has reviewed and approved this study?
This study has been reviewed by an independent group called the Research Ethics Committee. Their role is to protect the safety and wellbeing of participants in research. If you have any concerns or complaints about this study, please contact the Research Ethics Committee using the following email: ethics@salford.ac.uk

Thank you for taking the time to consider your involvement in this study.

If you have any questions regarding this, please contact me using the details below:

Jennifer Andrews
Post-graduate Research Student
j.r.andrews@edu.salford.ac.uk
[redacted]

Appendix 10. Study 2 consent form

Participant Consent Form

Title of study: An investigation into the hydration of the foot skin and associated skin characteristics.

Study overview: Participants will be required to expose the skin on their feet, lower leg and forearm. Non-invasive measurements of skin characteristics will then be obtained from these skin areas following a short acclimatisation period. Full details of the protocol may be found within the Participant Information Sheet (see attached).

Please place an initial in the appropriate box and complete your name and signature at the base of this form.

	Yes	No
I have read and understood the patient information sheet and I understand what my contribution will be.		
I have had the opportunity to ask questions about the study and understand that I am free to ask questions throughout the appointment.		
I understand that during this study multiple measurements will be obtained from the skin on my feet, arm and lower leg, some of which will involve limited soft tissue compression/shear.		
I understand that my participation in the study is voluntary and I may withdraw my data from use within this study up to two weeks following data collection.		
I agree for the measurements taken from my feet to be used for further research and publication within the University of Salford.		
I understand that my anonymised data may be shared with Scholl Wellness Co, and I consent to this.		
I agree to provide demographic information (see attached sheet).		
I agree to take part in the study discussed above.		

Participant Name……..

Participant Signature……

Researcher Signature……..

Date……

Participant number _____

Appendix 11. Study 2 demographic collection form

Demographic Collection Form

Age: _____

Sex:_____

When was (approximately) the last time you washed the skin on your lower legs and arms?

Date: ____/____/____ Time: ____:____ AM/PM (please circle)

What is the current date and time?

Date: ____/____/____ Time: ____:____ AM/PM (please circle)

In the last 14 days what has been your primary footwear choice? (Please tick one)

Closed-toed shoes without socks/hosiery ☐

Closed-toed shoes with socks/hosiery ☐

Open toes shoes (such as sandals or flip flops) ☐

Other (please explain below)

_____

_____

_____

For researcher use only:

Participant number: _____

Hours since cleansing: _____

Appendix 12: Scholl 'Intense Nourish' Ingredients

Aqua, Glycerin, Liquid Paraffin, Urea, Polyglyceryl-3 Methylglucose Distearate, Cyclopentasiloxane, Glyceryl Stearate, Myristyl Alcohol, Cyclohexasiloxane, Dimethicone, Paraffin, Phenoxyethanol, Panthenol, Parfum, Methylparaben, Allantoin, Bisabolol, Tocopheryl Acetate, Ethylparaben, Butylparaben, Propylparaben.

Appendix 13: Emollient application instructions

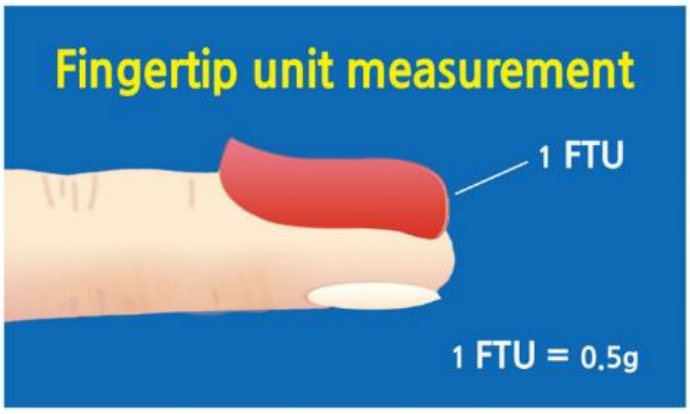


Image extracted from: Topical application of Jaungo in atopic dermatitis patients: study protocol for a randomized, controlled trial – Yun et al 2017 DOI 10.1186/s13063-017-1920-9

Please apply the cream included within this package to your _____ foot once-daily. As a rough guide, you should use one finger-tip unit (see the image on the left) to cover the whole of the foot, from the ankle downwards. Do not apply any cream to your other foot. Please inform the research team if you are unable to do this.

Thank you

Jennifer Andrews

Post-graduate Research Student and Podiatrist

j.r.andrews@edu.salford.ac.uk

07546 984 420

Appendix 14. Study 3 Recruitment Material

Recruitment Letter: An investigation into the composition of the foot skin and associated skin characteristics.

Dear _____

I am a Podiatrist and PhD student at the University of Salford. Between September and November this year I will be conducting a study investigating the characteristics of the foot skin of people with Diabetes and people without Diabetes.

I am recruiting participants from the patients of the University of Salford Podiatry Clinic. You have been contacted as you fit the description of participants I require for this study.

Within this letter is a detailed information sheet that describes the purpose of the study, what the data-collection entails, and how your data would be used. Please review this carefully and consider whether you would like to be involved.

If you choose to take part you would be asked to attend an appointment at the University of Salford Podiatry Clinic where a series of non-invasive measures would be taken from the skin on your forearm, lower leg, and foot. Your routine foot care (nail cutting, skincare, footwear advice etc.) would then be provided free-of-charge at the end of this session. This process would take approximately 3 hours in total.

Please feel free to contact me if you have any questions about this study, or you would like to discuss your involvement.

Thank you for taking the time to consider your involvement in this study.

Jennifer Andrews

Post-graduate Research Student and Podiatrist

j.r.andrews@edu.salford.ac.uk

[redacted]

Appendix 15. Study 3 Participant Information Sheet

Participant information sheet

Title of study:

An investigation into the composition of the foot skin and associated skin characteristics.

Study Details:

People often experience dry skin on their feet and this can lead to heel cracks, infection and in some case ulcers on the feet. It is possible to improve dry skin using lotions and creams.

This purpose of our study is to better understand how the skin stays hydrated with water or becomes dry and how moisture in the skin affects things like skin texture, hardness, elasticity, and how it responds to friction (rubbing). We are interested in how much water is in 'healthy' foot skin and then how conditions such as diabetes change the water content of skin. Once we know this we will be better able to design new lotions or creams to add water back into dry skin.

We would like to use several different ways to measure the skin because no one method measures everything we need to know. Also, it is useful to measure skin at different sites on the body, because we know some skin areas (like the soles of feet) are more specialised than other areas.

We will be collecting data from people with and without diabetes and comparing the results. This will allow us to identify how diabetes affect skin health and ways we might improve this with lotions and creams that target this difference in skin.

Participants demographics:

Participants must be 18+ and have no skin conditions or overall medical problem that may influence the skin (other than Diabetes). Importantly, **participants must**

1. not use any products on the skin where testing will take place (other than cleansing products or products provided to them by the researcher) for 7 days before data-collection.
2. not wash their feet for 5 hours before the data-collection session begins.

Do I have to take part in the study?

No, you have been given this information today as you are a patient at the University of Salford Podiatry Clinic, and we think you fit our criteria. If you do decide to take part, you will have an opportunity to ask questions and express any concerns before going ahead. After your appointment, if you no longer want to be included in the study then your data can be removed as long as you let the researcher know within 2 weeks of attending. After this time your information may have already been included in reports and papers.

What will happen if I choose to take part?

You will be asked to attend an appointment at the University of Salford Podiatry Clinic. On the day you will attend the Clinic just as you would for any other appointment, where you will be greeted by the researcher who will take you through to the area we are using for our study. You can choose to take a break whenever you please during your appointment and will have access to toilet facilities nearby.

The appointment will take no longer than three hours.

You will be able to ask any questions you have about the study before completing a written consent form and a short questionnaire. **Within this questionnaire you will be asked to share your age, weight, height, ethnicity and your most recent HbA1c result (if you have one) and medication**. You will then be asked to sit comfortably and remove your shoes, hosiery and roll up any clothing to expose the lower leg and the forearms.

Measurements will be taken from 11 skin sites, which will be marked using a skin-safe marker. The mark will wash off after several days.

Following a short period of rest, the researcher will take measurements from the skin on your feet, lower leg and forearm using a series of devices. These devices are all specifically designed to measure skin and you should not experience any discomfort. They involve a small probe being placed on the skin and held for a period of seconds. For a few of these measurements you may be asked to kneel or lie on a cushioned bench.

Once all data has been collected, you will undergo your usual Podiatry treatment (free of charge) and be escorted back to the waiting room by the researcher.

Are there disadvantages of taking part?

We do not expect there to be any disadvantages to taking part in this study, the techniques used are non-invasive and should not cause any harm or discomfort.

Are there advantages of taking part?

During your time at the clinic, you will undergo routine Podiatry treatment just as you would at your usual appointment, but to recognise your contribution this will be free of charge on this occasion. This includes routine nail care, skin care and foot care advice and will be carried out by a HCPC registered Podiatrist.

What do I do if there is a problem?

If you have any concerns or complaints about the study, please contact the primary researcher using the details below. If this is not appropriate, then please contact Dr Hashmi, a supervisor on this project, using f.hashmi@salford.ac.uk.

Will my involvement in this project be kept private?

Yes, any personal data collected will be stored and accessed in line with the General Data Protection Regulations (May 2016). All documents, bar the written consent forms, will be anonymised through the use of a participant identification number.

Written consent forms will be stored within a locked cabinet in the Brian Blatchford Building and destroyed following scanning and storage on a secure computer drive hosted by the University of Salford. The secure drive and locked cabinet are only accessible by the researcher and their immediate supervisors. All other anonymised data will also be stored within a secure drive.

What will happen to the results of the study?

The data collected will be analysed by the researcher and findings will be published in a PhD thesis, as well as within scientific journals and abstract format for presentation at conferences. The data obtained within this study may also be used by the industry sponsor who is funding this project, Scholl Wellness Co, and some may be used for secondary analysis at a later date by other researchers. Some of the data collected may also be shared via the University of Salford data repository. All data will be reported anonymously and stored securely. Personal identifiable data will be destroyed 5 years after data-collection is completed.

Who has reviewed and approved this study?

This study has been reviewed by an independent group called the Research Ethics Committee. Their role is to protect the safety and wellbeing of participants in research. If you have any concerns or complaints about this study, please contact the Research Ethics Committee using the following email: ethics@salford.ac.uk

How do I get involved?

If you are interested in taking part in this study, please call the researcher using the number below and you can discuss the study and book a data-collection appointment. Appointments will be available throughout September, October, and November.

Thank you for taking the time to consider your involvement in this study. If you have any questions about the study or you would like to discuss being involved, please contact me using the details below:

Jennifer Andrews

Post-graduate Research Student

j.r.andrews@edu.salford.ac.uk

[redacted]

Appendix 16. Study 3 consent form

Participant Consent Form
Title of study: An investigation into the composition of the foot skin and associated skin characteristics.

Study overview: Participants will be required to expose the skin on their feet, lower leg and forearm. Non-invasive measurements of skin composition will then be obtained from these skin areas following a short acclimatisation period. Full details of the protocol may be found within the Participant Information Sheet (see attached).
Please place an initial in the appropriate box and complete your name and signature at the base of this form.

	Yes	No
I have read and understood the patient information sheet and I understand what my contribution will be.		
I have had the opportunity to ask questions about the study and understand that I am free to ask questions throughout the appointment.		
I understand that during this study multiple measurements will be obtained from the skin on my arm, legs and feet.		
I understand that my participation in the study is voluntary and I may withdraw my data from use within this study up to two weeks following data collection.		
I agree for the measurements taken from my feet to be used for further research and publication within the University of Salford, The University of Southampton, and Imperial College London.		
I understand that my anonymised data may be shared with Scholl Wellness Co, and I consent to this.		
I understand that some of the data collected within this study may be used for secondary analysis in future.		
I agree to provide demographic information (see attached sheet).		
I agree to take part in the study discussed above.		

Participant Name……..
Participant Signature……
Researcher Signature……..
Date…….