University of Southampton

Faculty of Environmental and Life Sciences

School of Health Sciences

Detecting early signs of skin damage using non-invasive biophysical parameters

by

Nkemjika Sopuru Abiakam

Thesis for the degree of Doctor of Philosophy

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Abstract

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Pressure ulcers (PUs) and incontinence-associated dermatitis (IAD) are types of skin damage that result from prolonged exposure to external insults including pressure, shear, friction, and moisture. The clinical symptoms, which often lead to chronic wounds, can involve erythema, skin swelling, oedema and skin breakdown. It is imperative to detect early signs of these conditions prior to chronic damage. However, subjective visual and tactile skin assessments typically used by clinicians lack predictive capability. Objective biophysical approaches have been suggested to provide the means of monitoring the early signs of damage, although further evidence is required to establish their performance in a range of clinically relevant situations. The goal of the doctoral research is to utilise an array of non-invasive biophysical sensors to understand the pathophysiological changes in the skin following different insults associated with PUs and IAD.

This thesis presents the results from a series of complimentary retrospective and prospective studies. These involved the analysis of established data from the host lab, a survey of skin damage resulting from the application of personal protective equipment (PPE), and two prospective lab-based studies. The former involved the recruitment of able-bodied volunteers and healthcare workers who were exposed to moisture and mechanical loads through incontinence pads and lying postures, and respirator protective equipment (RPE), respectively. These findings were used to inform a cohort study of hospitalised patients with stage I pressure ulcers.

Results revealed that prolonged exposure of the skin to mechanical loads and moisture can disrupt its barrier function, as evidenced by enhanced values of trans-epidermal water loss and stratum corneum hydration. Furthermore, among the biophysical skin parameters evaluated, TEWL and hydration were able to distinguish between compromised and healthy adjacent skin sites, but not between different insults. During a COVID-19 study of adverse reactions to PPE, cohorts of healthcare workers revealed that they had indentation marks, pressure damage, itchiness, rashes, and spots. The subsequent study of biophysical changes in skin health identified distinct changes in skin barrier function and hydration over vulnerable bony landmarks on the face (bridge of the nose) associated with RPE application. Findings from this study showed that the outputs of these parameters were influenced by the subject extrinsic and intrinsic factors, namely body mass index and daily working hours. The final clinical study revealed highly localised changes (<5mm) in skin barrier function over the pressure ulcer site. In contrast to the lab-based studies, skin hydration did not differentiate between healthy and damaged skin. Complementary analyses also revealed the important role of inflammatory cytokines in the early detection of skin

compromise, as well as the potential of epidermal corneocytes as novel biomarkers reflective of changes in skin health.

The use of biophysical tools to monitor local changes in skin health could represent an important adjunct to clinical practice, which currently relies on subjective skin assessment. However, to support its wider translation in different care settings, future research is required to analyse time-dependent changes in these parameters, in both acute and long-term care facilities, to assess the prognostic value when determining skin status (healing or progressing to wounds). In addition, engagement with healthcare workers to analyse the barriers and facilitators to adoption along with the cost-effectiveness of introducing these tools is needed prior to wider adoption in practice.

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Research Thesis: Declaration of Authorship

Print name: Nkemjika Sopuru Abiakam

Title of thesis: Detecting early signs of skin damage using non-invasive biophysical parameters

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

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3. Where I have consulted the published work of others, this is always clearly attributed;
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5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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Dissemination

Publications

Abiakam, N., Worsley, P., Jayabal, H., Mitchell, K., Jones, M., Fletcher, J., Spratt, F., & Bader, D. (2021). Personal protective equipment related skin reactions in healthcare professionals during COVID-19. International Wound Journal, 18(3), 312-322.

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Currently under review

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Conference presentations

Abiakam N. (2021) Detecting the signs of skin damage using biophysical and biomarker approaches. European Pressure Ulcers Advisory Panel (EPUAP), 18 - 19 October, Virtual meeting.

Abiakam N. (2021) Biophysical and biomarker approaches for the early detection of skin damage. European Pressure Ulcers Advisory Panel (EPUAP), 18 - 19 October, Virtual meeting.

Abiakam N. (2022) Can non-invasive skin parameters reflect changes at grade 1 pressure ulcer skin sites? European Pressure Ulcers Advisory Panel (EPUAP), 14 - 16 September, Prague, Czech Republic

Conference Posters

Abiakam N. (2020) PPE related skin reactions in healthcare professionals during covid19. European Pressure Ulcers Advisory Panel (EPUAP), 24 September, Virtual meeting.

Abiakam N. (2021) Evaluating the implications of pH substrate and moisture-insulted skin using two different incontinence products. European Pressure Ulcers Advisory Panel (EPUAP), 18 - 19 October, Virtual meeting.

Abiakam N. (2021) Can two incontinence products with pH buffering capacity attenuate the impact of prolonged moisture exposure to the skin? Institution of Mechanical Engineers (IMechE). 16 - 17 November, Virtual meeting.

Personal motivation

“Never in the history of mankind, a pestilence of such extent and mortality was nowhere remembered…Neither medicine nor quackery helped” stated Thucydides concerning the lethal mysterious epidemic that devastated Athens in 430–429 B.C.E.

Numerous advancements have indeed been made in the medical field to avoid the repetition of the epidemic described by Thucydides. Nevertheless, healthcare systems worldwide are slowly, but steadily, consumed by a new “epidemic” namely, chronic wounds, which are reported daily by healthcare professionals, and yet very little has been done to reduce the unacceptable escalation of their prevalence. In the UK National Health Service, the diagnosis, treatment and management of these chronic wounds have been reported to cost over £8 Billion p.a. (Guest et al. 2020), corresponding to 4% of the total NHS budget. It is disappointing to note that most of this money is used for treatment and management instead of the detection and prevention of these chronic conditions.

Indeed, like many other clinical professionals working in acute and long-term care settings, I was of a strong belief that the primary strategy to eradicate chronic conditions was to develop novel smart technologies to monitor risk factors and develop personalised management interventions. This motivated and influenced my academic career both at the University of Parma (Italy) and the University College London (UK), where I spent a considerable amount of time working on the development of smart wound dressings able to release chemotactic factors necessary to induce the migration, proliferation and differentiation of stem cells at wound sites.

My motivation changed when I met Dr. Peter Worsley and Professor Dan Bader in June 2019. They both believed that a thorough understanding of the aetiology leading to the development of these chronic conditions needs to be mastered prior to interventions being put in place. In addition, they advocated for a shift of finance from treatment and management to early detection performed using novel bioengineering devices and biochemical markers able to detect minimal variation in skin physiology. To cut long story short, I was thrilled by their approach to the problem, which lead me to accept a role within their research group (Skin Health Research Group) at the University of Southampton. Under their mentorship and leadership, we have designed, conducted and managed research studies with the aim to shine a light on the fundamental understanding of how these tools can be used to monitor skin health, and what challenges there may be regarding their future translation to clinical practice. Furthermore, the future ambition was also to develop a point-of-care device, derived from the combination of both biophysical and

biochemical skin parameters, which would facilitate clinical decision-making in relation to two prominent chronic conditions, namely pressure ulcers and incontinence-associated dermatitis.

Although we have not created a definitive tool to monitor skin health, I trust Pete and Dan would agree that remarkable progress was made in assessing biophysical tools' ability to detect skin changes in pre-clinical and clinical cohorts. Therefore, the current thesis highlights all the efforts which have been promulgated in the last three and half years to understand the diagnostic and prognostic values of bioengineering tools, with associated parameters, which are widely used in skin health research but have never been implemented in clinical practice.

Dedication

The present thesis is dedicated to the exit of a great man

Professor Dan L. Bader*, Pioneer, Visionaire, Leader and Mentor.*

A lot has been said about the unexpected passing of Dan and many prominent figures have narrated various tales about his life and accomplishment. I am for sure not in the best position to illustrate his academic or personal achievements but what I could definitely talk about is his impact on my scientific and personal growth during the years we worked together.

I had the privilege to make Dan's acquaintance in June 2019 and from the get-go I could feel he was someone on a different level. Having a conversation with Dan can extend with him challenging you on some aspects of the topic to indulging in humour jokes. Dan was not a typical English professor. Indeed, he will clearly inform if he was not happy about something, and even if he did not communicate it, his facial expressions spoke for him. I remember being present at virtual meetings (with the webcams on) where he would have both his hands attached to his face in a sign of "disbelief", forgetting that his webcam was on and that everyone could see him. Nonetheless, given his inability to hide these facial expressions, you can also tell when he was pleased. Dan was not a man of many compliments, nevertheless, it was visible when he was delighted with a job well done or a well-written manuscript. Dan was an incredible source of knowledge and wisdom, and was relentless in his pursuit of novel ways of presenting scientific data and preparing a manuscript for journal submission. Indeed, drafting manuscripts with Dan was the most difficult and rewarding moment of my doctoral fellowship. Dan had a charming character and his 360-degree approach to everything he was involved in, motivated everyone around him to constantly improve and be the best they could be. Having Dan as my supervisor made me feel like I could challenge the entire scientific community and come back victorious. Indeed, under his constant supervision, my writing and communication skills, as well as my presentation abilities, have improved significantly in the last 3 years.

A book would not be sufficient to narrate all the tales about Dan however, I can firmly assert that I wouldn't be where I am now, in my career, if it was not for him. As such, I wish to express my powerless gratitude for all he has done for me throughout these years. Dan, I am sure that you are

up there in heaven smiling while you watch me write these paragraphs in an English language that you will probably define as “not convincing”. Nevertheless, I hope I was able to pay back the trust you confined in me over these years, but most importantly I hope this thesis will make you proud.

May your soul continue to rest in eternal peace. You will be forever in our hearts.

Ciao Dan!!

P.S. Don’t forget to teach the angels how to avoid incurring pressure ulcers!!

Acknowledgements

This thesis is the result of the constant support, cooperation and guidance of some prominent individuals to whom I wish to convey my appreciation.

Firstly, I would like to express my endless gratitude to you, Pete. You have been an incredible mentor, manager and most importantly a good friend. I wouldn't have been able to accomplish this doctoral fellowship without your outstanding support and guidance. Thank you for believing in the project even in those periods when I saw no hope and thank you for making the Skin Health Research Group a very warm place to work. Although studies have reported that managing me for 3+ years might have detrimental implications (e.g. growing grey hair earlier than predicted), I want you to know that I cherish everything you have taught me over these years and I will try to put them into practice.

A special thanks to Davide especially for supporting the reviewing of the current thesis. It was a breath of fresh air having you join the Research Group.

A huge thanks to my colleagues Hema and Silvia for supporting me with data analysis, drafting of manuscripts, and all the fun moments we have had both inside and outside the work environment.

My mother, brother, sisters, and my Zio & Zia for the spiritual support and guidance throughout these years of PhD.

A big thank you to my sweet Rozalia for holding everything down even during the craziest periods. Thank you for caring for me every day. I do not take it for granted.

To all the people that I met inside or outside of the work environment and that have supported in enabling me to achieve this thesis, I wish to express all my appreciation.

Last but not the least, I would like to thank the European Union Marie Curie International Training Network for this great opportunity and the funding of these 3 years. It is greatly appreciated.

To all the people that I met in Southampton over these years who have supported me in integrating into the city

THANK YOU ALL!!

Definitions and Abbreviations

APAM	Alternating Pressure Air Mattress
AU	Arbitrary Units
AUC	Area Under the (ROC) Curve
BAME	Black, Asian and Minority Ethnic
BMI	Body Mass Index
CE	Conformitè Europëenne (the European Union's (EU) mandatory conformity marking)
CI	Chemical Irritation
COVID-19	Coronavirus disease (2019)
CPAP	Continuous Positive Airway Pressure
CV	Coefficient of Variation
DEJ	Dermal-Epidermal Junction
DTI	Deep Tissue Injury
EPUAP	European Pressure Ulcer Advisory Panel
ERGO	Ethics and Research Governance Online
FFP	Filtering Face Pieces
FOHS	Faculty of Health Sciences
GDPR	General Data Protection Regulation
GICU	General Intensive Care Unit
GLOBIAD	(Ghent) Global incontinence-associated dermatitis (Monitoring Tool)
HAPU	Hospital Acquired Pressure Ulcer
HCA	Healthcare Assistant
HCW	Healthcare Worker

Definitions and Abbreviations

HRA	Health Research Authority
IAD	Incontinence Associated Dermatitis
IL	Interleukin
IL-1RA	Interleukin one receptor antagonist
IL-1(α)	Interleukin one (alpha)
IQR	Interquartile Range
IRAS	Integrated Research Application System
MDRPU	Medical Device Related Pressure Ulcer
MHC	Major Histocompatibility Complex
MIP	Macrophage Inflammatory Protein
mmHg	Millimetre of Mercury
MMPs	Matrix Metalloproteinases
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NICU	Neurological Intensive Care Unit
NIR	Near-Infrared
NIV	Non-Invasive Ventilation
NPIAP	National Pressure Injury Advisory Panel
pH	Potential of Hydrogen
PIS	Patient Information Sheet
PPE	Personal Protective Equipment
PPPIA	Pan Pacific Pressure Injury Alliance
PU	Pressure Ulcer
PUM	Polyethylene–Urethane Mattress

PURPOSE T	Pressure Ulcer Risk Primary or Secondary Evaluation Tool
RAS	Risk Assessment Scale
RCT	Randomised Clinical Trial
REC	Research Ethics Committee
ROC	Receiver Operating Characteristics (curves)
RPE	Respirator Protective Equipment
SAP	Superabsorbent Polymer
SC	Stratum Corneum
SEM	Subepidermal Moisture
SHDU	Surgical High Dependency Unit
SLDI	(Scanning) Laser Doppler Imaging
SLS	Sodium Lauryl Sulphate
SSWL	Skin Surface Water Loss
STINTS	Skin Tissue Integrity Under Shear
STROBE	Strengthening the Reporting of Observational studies in Epidemiology
SU	Synthetic urine
TEWL	Trans-Epidermal Water Loss
TNF-α	Tumour Necrosis Factor-alpha
TS	Tape Stripping
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VSA	Visual Skin Assessment

Chapter 1 GENERAL INTRODUCTION

1.1 SKIN MORPHOLOGY

The skin represents the largest organ in the human body with an average total surface area of approximately $2m^2$. It covers the whole body surface and connects with a number of systems of the body, such as the digestive system, through the mucose membrane, respiratory system (the nose), urogenital and conjunctiva of the eyelids, as well as coating the external surfaces of the eardrums and the meatus of the ears. It is composed of three different tissue layers, namely the epidermis, dermis, and hypodermis (Figure 1.1) which, together with the appendages, enable the skin to form a complex architecture that protects the body against harmful microbes and other factors in the external environment (Graham et al. 2019). Depending on the body region, there is a variation in the thickness of skin layers, with load-bearing sites (soles of feet and hands) having a thicker stratum corneum.

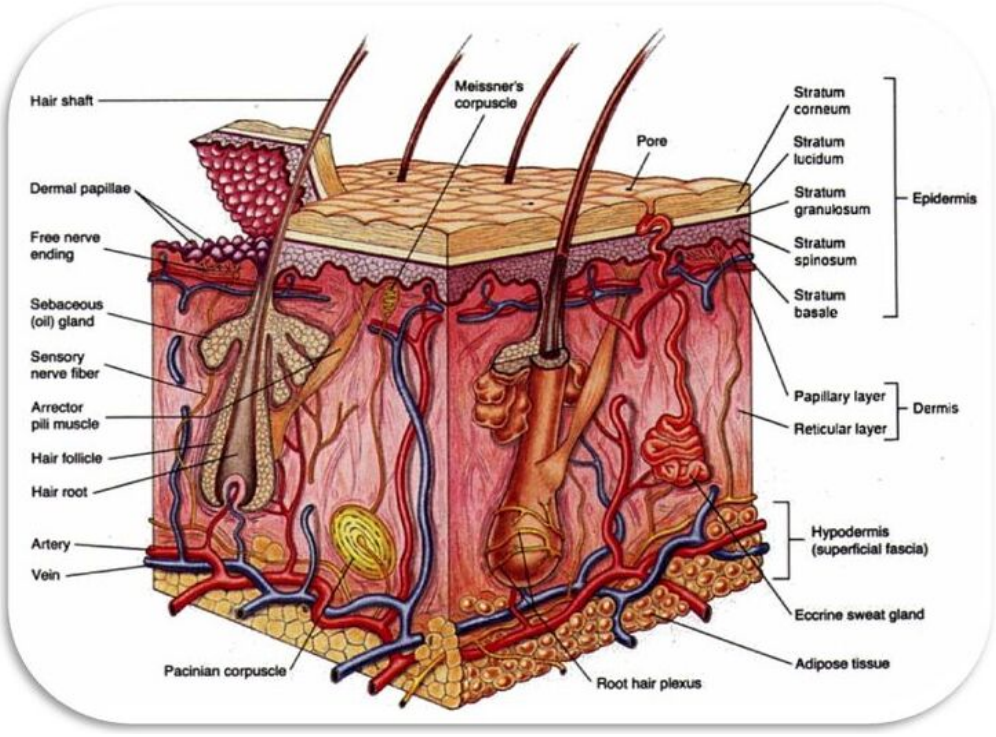


Figure 1.1. Illustration of the human integumentary system composed of the skin and its layers in addition to its appendages (Khavkin et al. 2011).

The skin also consists of integumentary components, such as sebaceous glands, hair, nail and sweat glands. The layers of the skin will be separately presented in the following sections.

1.1.1 EPIDERMIS

The epidermis is the outer layer of the skin. It is a highly cellularized but not vascularized layer, which is primarily responsible for the protection of the body against environmental insults such as pollution, dehydration, mechanical loading, radiation exposure and pathogens. It is mainly composed of keratinocytes both derived from the epidermis basal layer stem cells. More precisely, the latter transdifferentiate into keratinocytes and after a period of approximately 4 weeks, the keratinocytes progress upwards through the stratum corneum losing their nuclei and differentiating into corneocytes (Elsholz et al. 2014). The keratinocytes express proteins in the epidermis, such as nestin, vimetin, desmin and α-internexin (Graham et al. 2019). The epidermal layer is divided into four micro-layers namely *stratum corneum, stratum granulosum, stratum spinosum and stratum basale* (Figure 1.2). In addition to these, another micro-layer, called *stratum lucidum*, which lies between the stratum corneum and stratum granulosum, is found only on the palms of the hands and on the soles of the feet, where there is a thicker layer of epidermal tissues. The epidermis is viable in all its micro-layers except for the stratum corneum. For the purpose of the doctoral research project, only the *stratum corneum* will be detailed.

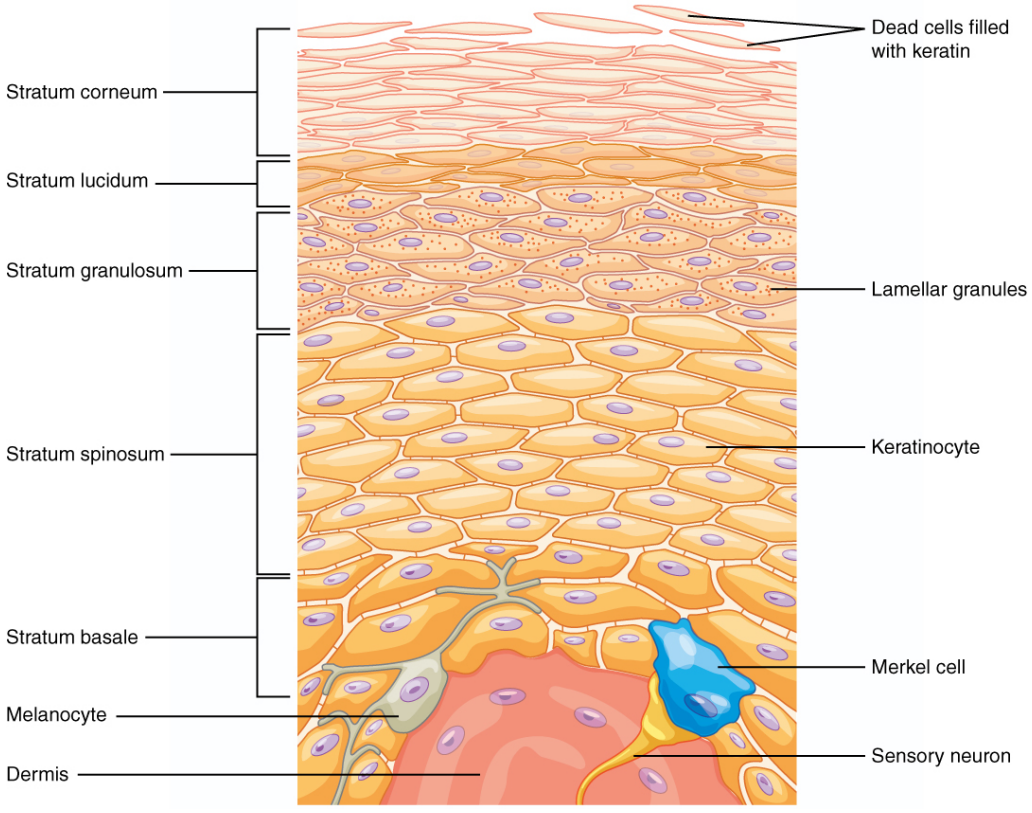


Figure 1.2. Micro-layers of the epidermis of thick skin in association with its relative cell population. Image from (Biga et al. 2020)

1.1.1.1 STRATUM CORNEUM

The *Stratum Corneum* (SC) is composed of a layer of dead and flattened keratinocytes, termed corneocytes, which are responsible for the protection of the body against external assaults, in addition to the maintenance of fluid exchange with the surrounding environment. The cells of the SC are subjected to a desquamation process (Figure 1.2), which ensures that the outermost layer of the skin is constantly replaced by newly formed corneocytes. The SC barrier function is provided by both the corneocytes and the presence of a surrounding lipid matrix, mainly composed of fatty acids, ceramides and cholesterol, which ensures adequate maintenance of the skin properties (Elias et al. 2010). Indeed, disruption of the SC has been associated with different kinds of skin diseases such as atopic dermatitis (Egawa et al. 2016), psoriasis, acne (Jordan et al. 2016), all of which lead to an increase in transepidermal water loss (TEWL) (Gao et al. 2012).

1.1.1.2 CELLS OF THE EPIDERMIS

In addition to being the main component of the SC, keratinocytes represent almost 85% of the cells found in the epidermis (Yousef et al. 2017). These cells are primarily responsible for the production of keratins, proteins that induce keratinization, which is a process necessary for the cornification of the skin. Keratinocytes are involved in the formation of skin barrier against water loss, UV radiation, heat, bacteria, viruses, and other external agents, and, when stimulated, produce a wide array of pro and anti-inflammatory cytokines such as IL-1α, IL-1RA, IL-1β, TNF-α, IL-8 IL-10 (Ito et al. 2005).

Other cells which are present at the epidermal layer are termed melanocytes, Langerhans' cells, and Merkel's cells (Figure 1.2).

- The main function of melanocytes is to produce melanin, necessary for the absorption and scattering of UV radiation, scavenging of free radicals, storage of ions, and the protection of DNA damage in keratinocytes (Hirobe 2014). They interact with keratinocytes via dendritic processes forming a complex termed the epidermal melanin unit system. There are about 1200 melanocytes per mm^2 of human skin, independent of ethnic background (Miot et al. 2009).
- Langerhans cells, known as antigenic-presenting cells, represent the outermost immunological defence barrier in the skin and constitute 3-5% of the cells in the *stratum spinosum* (Deckers et al. 2018). These cells are considered immature dendritic cells as they express lower levels of MHC class II and stimulate resting T cells compared to mature dendritic cells. When stimulated, they are able to produce inflammatory factors, such as interleukin 12 (IL-12), interleukin 15 (IL-15), IL-6, IL-1β, macrophage inflammatory protein alpha (MIP-1α), and MIP-1β. Interestingly, their function and phenotype are influenced by the cytokines produced by the surrounding keratinocytes (Moll 2013).

- Merkel cells, also known as tactile epithelial cells, account for 5% of the cell population in the epidermis (Maksimovic et al. 2014). They are found associated with cutaneous nerve terminal filaments, where they play an important role in touch sensation. In addition, Merkel cells are also involved in the development of hair follicles and eccrine sweat glands (Abraham et al. 2019), and inflammatory processes via the secretion of protein CD200 (Xiao et al. 2014).

1.1.2 DERMAL-EPIDERMAL JUNCTION

The dermal-epidermal junction (DEJ), also known as the epidermal basement membrane zone, is the undulating or wavy area that connects the dermis to the epidermis. The DEJ is mostly composed of keratinocytes and a small fibroblast population from the dermis. Keratinocytes are responsible for the production of type IV collagen and laminin. DEJ has a multitude of functions, including the critical roles of acting as the anchoring site between the epidermis and dermis and as a filter, allowing the exchange of nutrients and fluids between the two skin layers (James et al. 2011). The complex network of the DEJ together with the projections of rete ridges provide the skin with both structural integrity and mechanical stability (Langton et al. 2016). Other major functions of the DEJ include communication via biomolecules produced in the two layers, regulation of adhesion, polarity, growth, and movement of fibroblast and keratinocytes, mechanical support for the epidermis, and wound healing. With age, the DEJ tends to flatten, thereby affecting its function.

1.1.3 DERMIS

The dermis is a connective tissue layer made of elastic fibres, collagen fibres, and hydrated polymeric gel with extracellular features, including nerves, blood vessels, hair follicles, and both sebaceous and sweat glands. Its main functions are to support and protect the skin and the underlying soft tissue, assist in thermoregulation and participate in sensation. The dermis possesses various cell types including fibroblasts, histiocytes, mast cells, and adipocytes, which all together enable optimal performance of its functions (Sharma et al. 2017). The dermis is divided into two layers namely the papillary dermis and reticular dermis. The papillary dermis is the outermost layer of the dermis, composed of loosely arranged collagen fibres and interconnects with the rete ridges of the epidermis. Type I and III collagen are the most abundant in this layer, although it also contains elastic fibres. The subjacent layer, the reticular dermis, is made of both irregular connective tissues, as well as densely packed collagen fibres. Elastic fibres are also prominent in this layer in conjunction with reticular fibres, which confer strength, elasticity, and stiffness to the dermis (Marks et al. 2017).

1.1.4 APPENDAGES OF THE SKIN

Skin together with its appendages forms the so-called integumentary system, which has various roles including protection of the body against external insults, excretion of waste products, regulation of temperature, and sensation. Given the scope of this thesis, discuss will focus on the sebaceous glands.

Sebaceous glands are present in almost every area of human skin, with the exception of the palms and the soles. The distribution of sebaceous glands in the body has been reported to vary across anatomical locations, ranging from a low density at the limbs to a higher density at the scalp and foreheads, where it reaches 400-900 glands/cm^2 (Ludovici et al. 2018). Sebaceous glands are defined as holocrine-secreting tissue that develops adjacent to hair follicles and, as a result of such development, they empty their content into the follicular canal. The number of sebaceous glands is maintained constant throughout the life of an individual, although their size varies with ageing (Pappas et al. 2013). Sebaceous glands are responsible for the production of almost 90% of the lipids that coat the skin surface. Their main product, sebum, an oily to waxy mixture, is a combination of 30–60% triglycerides, 20–30% wax esters, 10–30% free fatty acid, and 10–20% squalene, as weight/weight percent (w/w %) (Picardo et al. 2009). In addition to lipids, sebum contains antimicrobial substances and matrix metalloproteinases (Zaballos et al. 2018). The main function of sebum is to keep the skin moist and avoid dehydration. Other functions include regulation of cutaneous steroid production, interaction with neuropeptides, and participation in pro and anti-inflammatory processes via the expression of inflammatory cytokines (Lovászi et al. 2017).

1.2 SKIN DAMAGE

Although specifically organised with a very complex architecture, the human skin undergoes constant remodelling, which can impact both its function and appearance. The main causes of these changes are injuries, diseases, aging, and physical and chemical insults to which the skin is subjected (Boyle et al. 2019).

Skin injuries are events that can occur in normal day-to-day life, from abrasions or cuts, forming wounds. While a mild to moderate wound can resolve itself in a relatively short time, deep injuries may require clinical interventions. When a wound occurs within the epidermal layer of the skin, a scar-free healing process takes place, leading to a full restoration of skin function. By contrast, if the wound invades the dermal and sub-dermal layers, the reparative process is associated with a scar formation (Gawronska-Kozak et al. 2017). Skin healing is characterized by different consecutive phases, namely, inflammation, proliferation, and remodeling. A compromised inflammatory phase

precludes the initiation of the healing process, thereby resulting in the formation of chronic wounds (Frykberg et al. 2015). Various studies have reported the upregulation of different types of cytokines during the inflammation phase (Chaparro-Huerta et al. 2017). Although there are many types of skin injuries, only a few lead to a reduced quality of life for the individual as well as a major financial burden to the healthcare system. The four most prominent chronic skin conditions are Pressure Ulcers (PUs) and Incontinence Associated Dermatitis (IAD), which will be discussed separately, and Diabetic foot ulcers and leg ulcers.

1.2.1 PRESSURE ULCER

Pressure ulcers (PUs), also termed pressure injuries, pressure sores, or bedsores, represent a common form of a chronic wound. A PU has been defined as "a localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure, or pressure in combination with shear" (EPUAP/NPIAP/PPPIA 2019). PUs routinely occur adjacent to bony prominences, such as the sacrum, heel, occiput, malleoli, and trochanters. Based on the loss of the underlying tissue and the severity of skin damage, PUs can be classified into five categories/stages, namely I, II, III, IV and deep tissue injury, as illustrated in Figure 1.3 and detailed in *Table 1.1*.

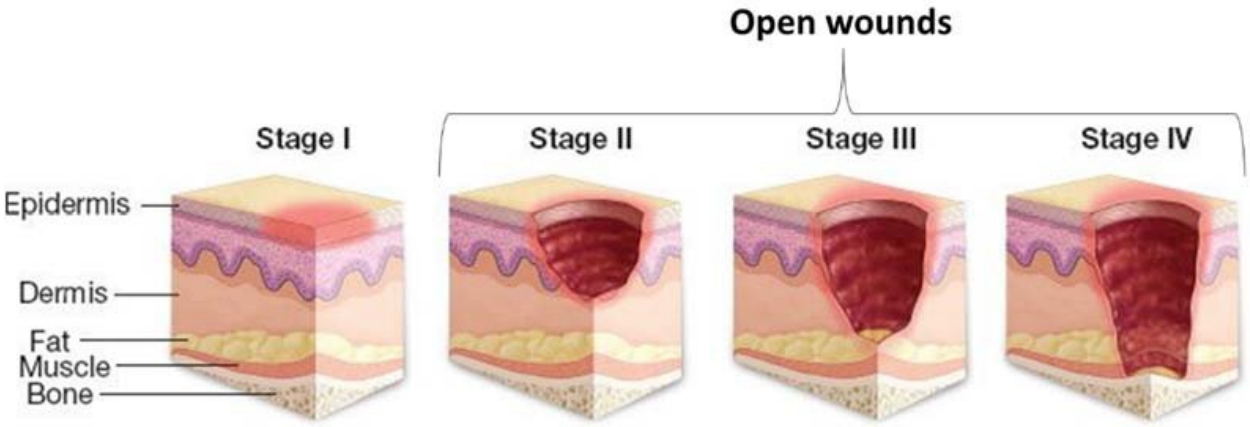


Figure1.3. Schematic illustration of the appearance of pressure ulcers across the stages. Image from NPUAP (2019).

Table 1.1. Pressure Ulcer stages. Table adapted from NPUAP (2019)-pressure ulcer treatment

Stage I	**Non-blanchable erythema** of intact skin under pressure. Preceding symptoms are redness, pain, softness of skin, increase in temperature
Stage II	**Partial-thickness** skin loss of the epidermis and/or dermis with visible symptoms such as red wound bed or visible serum blood-filled blister
Stage III	**Full-thickness skin loss** with the exposure of the subcutaneous fat without the exposure of the underneath bone
Stage IV	**Full-thickness tissue loss** with the exposure of bone, tendon, or muscle due to the extensive destruction induced by necrosis
Deep Tissue Injury	**Tissue damage beneath intact skin surface** characterized by a purple or dark area and/or the presence of blood-filled blister

Pressure ulcers represent a major economic burden to populations worldwide (*Table 1.2*) and, when compared to other dermatological conditions, have been attributed with the highest disability index (Hay et al. 2014). At an individual level, PUs have a detrimental effect on the quality of life (QoL) impacting emotional, physical, mental, and social well-being. In the UK, PUs represent a significant healthcare problem, with over 1,300 new cases reported each month (Source NHS Digital 2018) and an estimated 200,000 individuals developing a new pressure ulcer in 2017/18. Treating PUs costs the NHS more than £1.4 million every day (Guest et al. 2020). PU-associated costs have been reported to be influenced considerably by the length of stay in the hospital (Triantafyllou et al. 2021), as well as by the department in which the patient is admitted (McEvoy et al. 2021). Hence, strategies aimed at PU prevention, early diagnosis, and improvement of wound healing, would inevitably benefit both NHS, as well as individuals and their carers.

Table 1.2. PU-related annual costs incurred by various healthcare systems worldwide

COUNTRY	PU ANNUAL COST	REFERENCES
USA	$26.8 billion (€25.2 billion)	(Padula et al. 2019)
United Kingdom	$1.5 billion to $2.6 billion (£2.4 billion)	(Wood et al. 2019)
Spain	€435 million	(Soldevilla Agreda et al. 2007)
Azores (Portugal)	€10 million	(Andrade et al. 2016)
Australia	$13.61 million (€11.3 million)	(Graves et al. 2014)
Germany	€1 billion to €2.3 billion	(Wunden 1998)

1.2.1.1 INDIVIDUALS AT RISK OF A PRESSURE ULCER

Although no individual is exempt from developing a PU, there are specific sub-groups who are particularly at risk, as illustrated in the framework in Figure 1.4. These include the elderly, particularly if they present with impaired mobility and/or sensation, neuropathy, or issues with skin integrity. Many of these sub-groups may be largely bedridden with an inability to move or to reposition themselves, suffer from faecal and urinary incontinence with continuous moist skin in specific skin areas and present with other co-morbidities such as diabetes mellitus (Coleman et al. 2013). Indeed, various studies have suggested that PUs represent one of the most common and disabling conditions in people older than 70 years (Jaul et al. 2018) and in individuals presenting with spinal cord injury, who have been deemed vulnerable due to loss of their motor, sensory and autonomic mechanisms (Brienza et al. 2018). With regards to the elderly population, their skin exhibits reduced resistance to shear and frictional forces. In addition, biological ageing is associated with a decreased epidermal turnover, loss of dermal papillae, which results in the flattening of the Dermal-Epidermal Junction (DEJ) (Farage et al. 2013). With regards to spinal cord injury, it has been reported that up to 95% of individuals will experience at least one PU in their lifetime (Cowan et al. 2019), which can result in frequent re-hospitalization and could prove life-threatening (DeJong et al. 2013; Goodman et al. 2014).

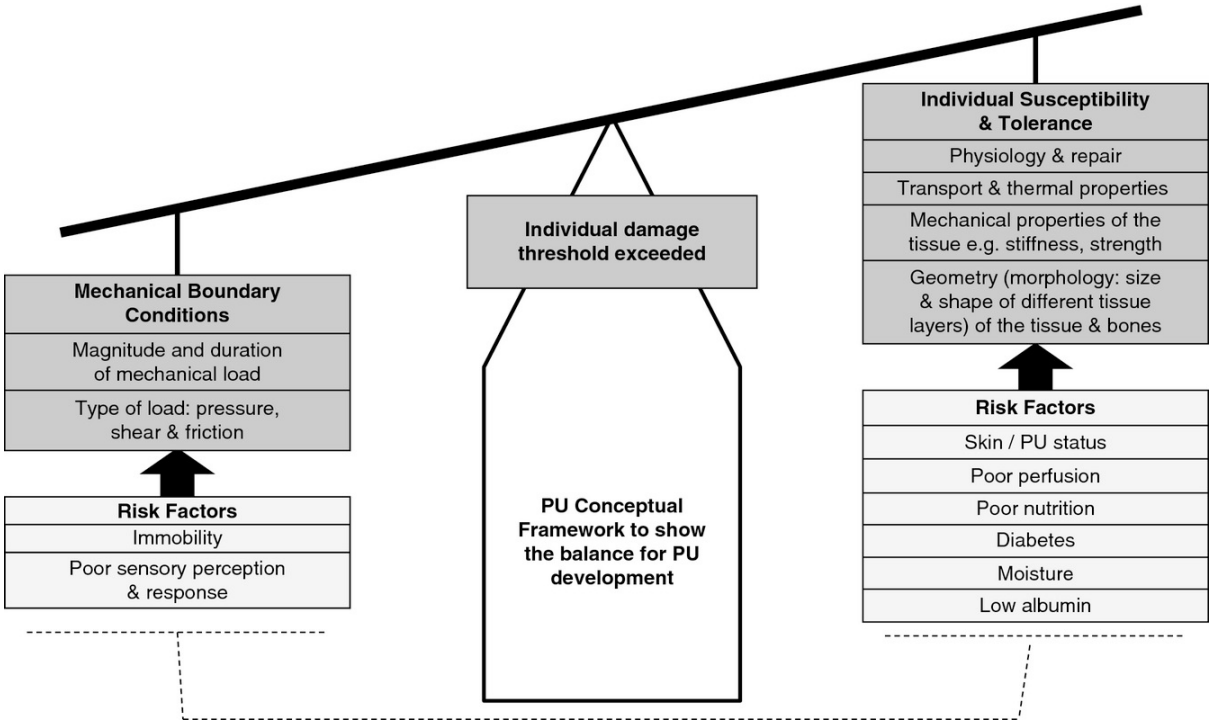


Figure 1.4. Conceptual framework detailing a clear linkage between the physiological and biomechanical determinants of pressure ulcer development and patient risk factors. Image from (Coleman et al. 2014)

1.2.1.2 HOSPITAL-ACQUIRED PRESSURE ULCER

Patients admitted to hospitals for a range of medical conditions are at potential risk of developing a PU involving mostly the superficial tissues of the skin (e.g. stage I PUs) (Figure 1.5). Despite the obvious care and attention afforded to these patients, it has been reported in the last few decades that there is an escalating number of what are termed hospital-acquired pressure ulcers (HAPUs) (Worsley et al. 2016). Indeed, several studies have reported that the prevalence of newly admitted patients that develop PU, most of which are categorised as stage I and II, during their stay in the hospital varies from 7% to 38% (VanGilder et al. 2017). In westernized countries, the prevalence of stage I to stage IV PUs in acute settings ranges between 6% to 18.5% (Tubaishat et al. 2018). A study suggested that the prevalence of HAPUs among inpatients varies between 13-14% and it can be as high as 70% in older patients treated with orthopaedic problems (Jaeblon 2010). The prevalence for hospitalised patients in a short-stay intensive care unit has been reported to be 13%, whilst it increased to 42% for a long stay (Cooper 2013). There are also factors associated with the nature of the hospital stay, for example, patients undergoing surgical procedures are at particularly high risk of developing PUs, due to prolonged periods of immobility under anaesthesia (Tschannen et al. 2012).

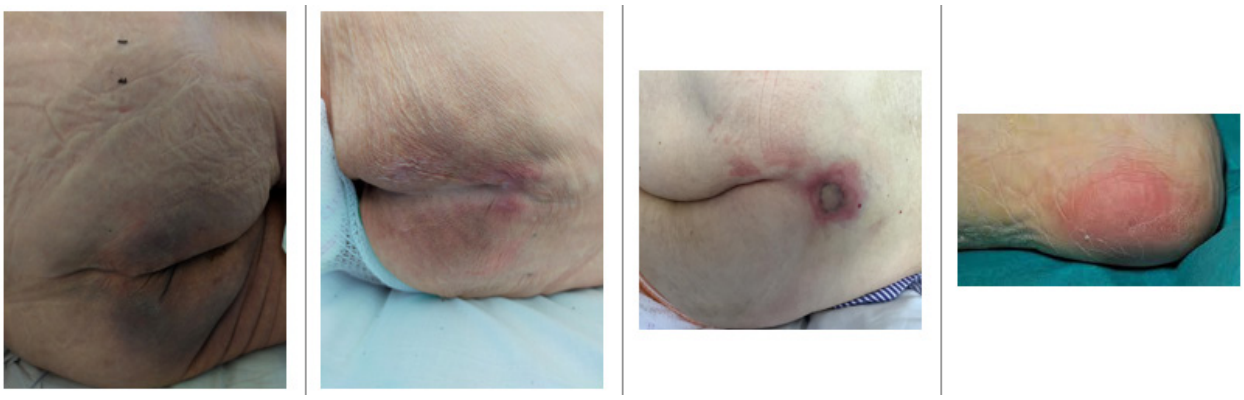


Figure 1.5. Hospital-acquired pressure ulcers at the sacrum, buttocks and heel of patients. Image courtesy of NHS patients (with permission).

Different strategies have been proposed to prevent pressure ulcers, typically recommended through local, national and international guidelines and policies. Indeed, some studies have reported that the education of nursing staff on the prevention of PUs has resulted in changes in nursing practice, improving the standard of care (Jankowski et al. 2011). Another study suggested that using experienced nurses in critical departments could reduce the incidence of HAPUs (Dunton et al. 2007). A more intransigent strategy to raise awareness of HAPUs was adopted by the US Medicare in 2008 when it was announced the intention of the national health insurance to no longer reimburse costs associated with hospital-acquired stage III and stage IV PUs. As a result of this implementation, it was reported a significant decrease of HAPU incidence from 11.8 cases per 1,000 inpatients in 2008 to 0.8 cases per 1,000 in 2012 (Padula et al. 2015). However, it is unclear whether this was a significant change in practice and improvement in prevention or a change in reporting practice due to the financial penalties.

1.2.1.3 MEDICAL DEVICE-RELATED PRESSURE ULCER

Prolonged contact with a medical device attached to the skin can also lead to damage, commonly termed a medical device-related pressure ulcer (MDRPU) (Figure 1.6). The incidence and prevalence of MDRPU have been reported to be 12% and 10%, respectively (Jackson et al. 2019). Of the relatively few studies describing MDRPU, there has been a focus on how both patient-device interactions can induce a change in skin microclimate and the tightness of attachment of these devices can cause skin damage in areas remote from bony prominences. For example, studies involving patients with cervical collars reported the development of decubitus ulcers in 44% of cases, of which 52% with full-thickness PUs and 28% with stage 1 PUs (Lacey et al. 2019). In a further study involving more than 100000 patients, it was reported that medical devices induced ulcer development at different anatomical locations including 19.9% on the ears, 14.3% on the sacrum/coccyx, 10.2% on the heel and 8.8% on the buttock (Van Gilder et al. 2012). Non-invasive ventilation (NIV) masks represent a common cause of MDRPUs. To achieve the inspiratory and

expiratory therapeutic air pressures and oxygen delivery, the devices are tightened, by clinicians and/or patients, causing an increase in applied pressures particularly at the bridge of the nose site (Worsley et al. 2016; Bader et al. 2019). Studies have highlighted how patient repeated usage of non-invasive ventilatory masks, could lead to broken skin or open wounds (Carron et al. 2013; Brill et al. 2018). Various case studies have reported the development of stage II (Rathore et al. 2016) and stage IV (Maruccia et al. 2015) PUs over the nasal bridge following continuous and intermittent usage of NIV masks. Usage of other medical devices, such as prosthetics and orthotics, splints, and indwelling urethral and epidural catheters, have been implicated in the formation of pressure ulcers (Ham et al. 2017; Pellegrino et al. 2017; Kayser et al. 2018).

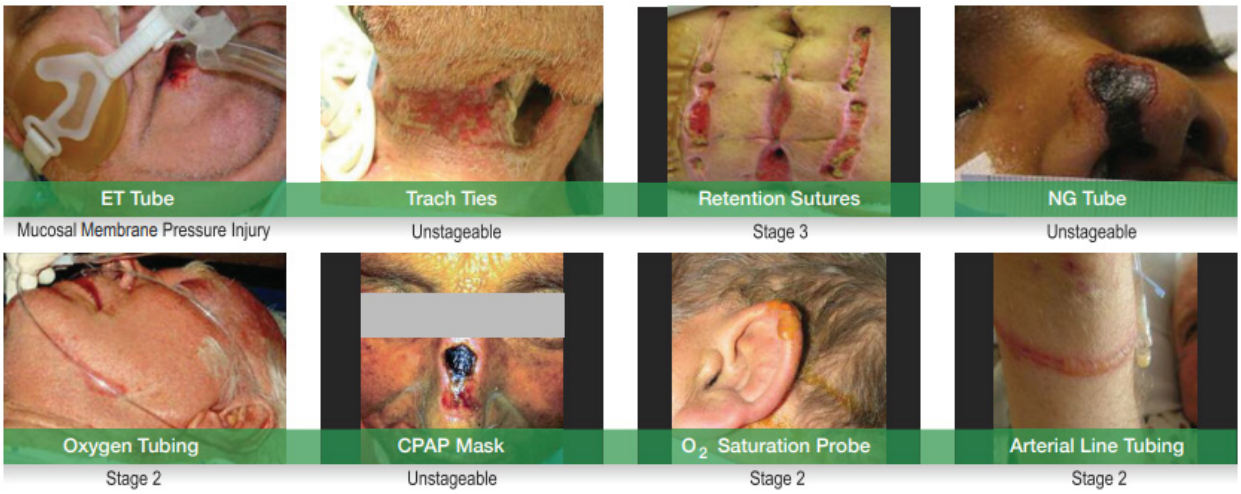


Figure 1.6. Medical device-related pressure ulcers at different anatomical locations of the body. Image adapted from NPIAP 2019

Given the potentially detrimental implication of medical devices, it is necessary to implement effective strategies aiming at minimising the escalating incidence of these MDRPUs. This could include higher vigilance by nurses, more efficient ways for reporting and recognising devices that are more likely to induce damage, as well as informing manufacturers (Pramod 2021).

1.2.2 INCONTINENCE ASSOCIATED DERMATITIS

Incontinence-associated dermatitis (IAD) represents a common problem and a major challenge both for the affected individual and for the healthcare professionals, who are trying to maintain a healthy skin status. IAD is moisture-associated skin damage caused by the continuous interaction of the skin with body waste products, namely urine, and faeces (McNichol et al. 2018). The WHO ICD-11 EK02.22 describes IAD as an irritant contact dermatitis from prolonged contact with urine or faeces as a result of incontinence. Urine and or feacal incontinence, are defined by the ICD-11 as

the loss of voluntary control of the bladder and/or the anal sphincters with involuntary passage of urine and leakage of faeces and flatus (WHO, 2018). The typical clinical signs of IAD include erythema, erosions, excoriations, and pain (Figure 1.7), while the most affected areas for IAD are the perineum, perianal region, inner thighs and the convex areas of the buttocks (Koudounas et al. 2020). The type of exposure and the time period necessary for skin inflammation and damage to occur remains unknown. Urine is considered to be the main cause of IAD formation due to its ability to overhydrate the skin, release harmful enzymes or MMPs when interacting with the stool, and decrease the tolerance of skin tissue to friction and pressure (Voegeli 2016). In addition, combined urinary and faecal incontinence, defined as double incontinence, has been suggested as the most debilitating and severe manifestation of skin disease associated with pelvic floor disease (Rodríguez-Palma et al. 2021). Exposure of the skin to waste products induces changes in the microclimate, overhydration of stratum corneum, an increase in pH and permeability, and a decrease in the skin protection function (Gray et al. 2012). In addition, the presence of faecal enzymes has been reported to play an important role in the degradation of skin status (Mugita et al. 2015).

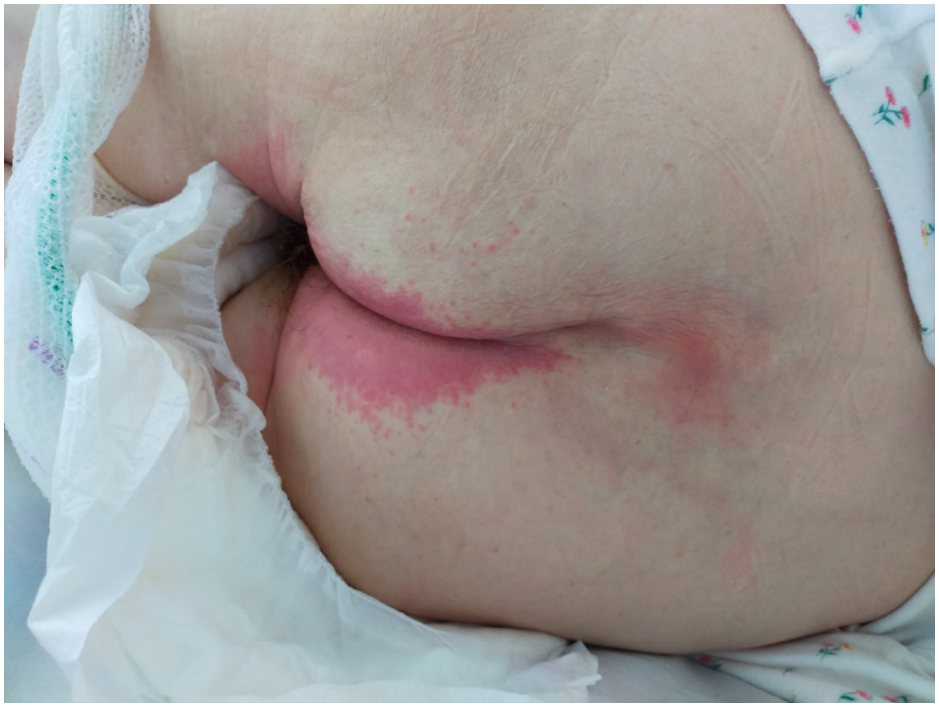


Figure 1.7. Illustration of a typical site of the body with erythema as a result of the impact of IAD. Images from NHS patient (with permission)

Based on the severity of skin damage, IAD can be classified into 3 different categories termed 0, 1, and 2 (Table 1.3*)*. Estimation of worldwide costs associated with IAD has been reported, although due to the difficulties in differentiating between direct, indirect, and intangible costs they are complicated to interpret. One recent systematic review estimated that 17 million people were affected by urinary incontinence and that the daily cost for the treatment and prevention for a

patient ranges between $0.23 and $20.17 (Raepsaet et al. 2021). From a psychological perspective, IAD has been reported to have a detrimental impact on individuals diminishing their quality of life. Indeed, IAD is commonly associated with great discomfort, loss of confidence, and embarrassment. Individuals affected have reported this condition as being troublesome, bothering, and interfering with daily activities (Van Damme et al. 2015).

Table 1.3. Incontinence-associated dermatitis categories. Table adapted from (Beeckman et al. 2015)

Category 0	**At risk of IAD.** Presence of intact skin without skin breakdown in individuals suffering from incontinence
Category 1	**Mild IAD.** Patient with recurrent erythema and oedema but without the presence of skin breakdown
Category 2	**Severe IAD.** Presence of persistent erythema with skin breakdown

1.1.1.3 INDIVIDUALS AT RISK OF INCONTINENCE-ASSOCIATED DERMATITIS

IAD can affect individuals of all ages, ranging from neonates and infants to the elderly. Initially associated with diaper dermatitis, the impact of IAD has been extensively studied in paediatrics (Miyauchi et al. 2016). The skin of the infant has been shown to be very fragile and thin with only a few cell layers constituting the SC, with an associated limited barrier function (Lagier et al. 2015). Prolonged exposure to moisture, derived from incontinence events, can lead to changes in the skin status of the infant. These are revealed in changes in skin properties, such as an increase in transepidermal water loss (TEWL), following the exposure of the skin of neonates to moisture (Stamatas et al. 2014). In addition, due to the defective skin status, infants are more likely to develop IAD as a result of inadequate fluid intake, ineffective thermoregulation, increased percutaneous absorption of toxins, increased susceptibility for infection, and delayed wound healing (Atherton 2016).

A further sub-group of individuals affected by IAD is the elderly. This cohort is characterized by diminished skin barrier function with decreased skin elastic properties, hence elderly skin is most vulnerable to abrasions when exposed to frictional forces. Elderly skin is also characterized by low water content in the SC, decreased thickness and epidermal turnover, and a reduction in sebum production by as much as 60% (Farage et al. 2009). In addition, baseline TEWL decreases with age, as does the recovery process following exposure to moisture (Kottner et al. 2013). Exposure to moisture can also reduce the strength and stiffness of the SC, thereby reducing its tolerance to mechanical loading (Kottner et al. 2018). Although the presence of incontinence is a prerequisite

for the development of IAD, not every individual suffering from incontinence presents with IAD. This indicates that there are many other factors involved which can increase or reduce the probability of acquiring this skin condition. Different measures have been put in place through international guidelines to assist individuals presenting with IAD. Furthermore, studies have suggested that IAD patients are more likely to receive higher-quality skin care compared to incontinent individuals without the condition (Jacobson et al., 2015; Lichterfeld et al., 2015). Nevertheless, there is no clear recommendation and superiority of a care procedure or topical product, although there is evidence that mild skin cleansing and the application of a skin protecting leave-on product might possess beneficial effects (Beeckman et al., 2016; Lichterfeld-Kottner et al., 2020). Similar to many other irritant dermatitis, IAD is an inflammatory response to prolonged exposure of the skin to moisture, with lesions characterized as top-down injuries, which initially present as erythema of intact skin but may progress to vesicle formation and epidermal loss, especially when the area is also exposed to friction.

1.2.3 SKIN ASSESSMENT: INCONTINENCE-ASSOCIATED DERMATITIS OR PRESSURE ULCER?

Diagnosis of IAD has often been difficult due to certain similarities to PUs. Indeed, many clinicians have reported issues distinguishing IAD from PUs, and other moisture-related skin conditions (Barakat-Johnson et al. 2022). Misdiagnosis of IAD as PU can have serious consequences in terms of reimbursement, litigation, and legal proceedings, as the altered condition is associated with the quality of care delivered by the health care institution. However, it is also important to recognise that in some patients these conditions can co-exist.

Both IAD and PU represent conditions that affect the skin and the underlying soft tissue. While IAD initiates at the skin surface and spread into the underlying tissues (top-down form of damage), PUs can either start from the skin surface (top-down) or can be initiated in deep tissues adjacent to bony prominences (bottom-up) (Beeckman 2017). There is a paucity of research on the pathophysiological and histopathological differences between IAD and PU. Indeed a meta-analysis review suggested that IAD could play a crucial role in the development of PU (Beeckman et al. 2014). These findings were confirmed by a separate study, which highlighted that prolonged exposure of the skin to moisture and irritants induces changes to the mechanical properties of skin tissues e.g. tissue stiffness as well as increases in the coefficient of friction (Luboz et al. 2015). Although there are clear clinical similarities, it is important to distinguish between the aetiology of both conditions, to provide a diagnosis that will lead to the adoption of the appropriate treatment, for reporting and as evidence of adequate quality of care. This concept was emphasised by the international guidelines for PU prevention in 2019 (Kottner et al. 2019) and the IAD best practice document in 2015 (Beeckman et al. 2015).

1.2.4 SKIN DAMAGE VIEWED THROUGH THE PRISM OF COVID-19 PANDEMIC

Since 2020, the COVID-19 pandemic has placed healthcare systems worldwide under considerable strain. An immediate consequence of this unprecedented event was the rapid increase in the number of patients requiring intensive care treatment, in particular mechanical ventilation (Sorbello et al. 2020). To provide optimal ventilation, patients are kept in prone posture for prolonged periods. This has resulted in the development of PUs mostly occurring on areas of the face, in addition to MDRPUs resulting from the prolonged attachment of medical devices to the skin of patients for therapeutic and monitoring purposes (Worsley et al. 2020).

In addition, managing patients with COVID-19 has resulted in skin damage in many healthcare workers (HCWs) from the prolonged application of personal protective equipment (PPE) (Akl et al. 2021). Although the prolonged use of PPE is critically important both in acute settings and long-term care facilities to keep HCWs and patients safe, it has resulted in secondary complications, particularly when considering the maintenance of skin health. Among the conditions, PUs, erythema, contact dermatitis, and moisture-associated dermatitis have been the most commonly reported (Wang et al. 2019). Typical areas of skin damage include the nasal bridge, cheeks, forehead, ears, and hands. Evidence of this has been recently reported in a qualitative study, where 97% of clinical staff were reported to present with skin symptoms due to the continuous usage of PPE (Darlenski et al. 2020). Among the protective equipment in use, respiratory protective equipment (RPE) has been most associated with skin damage, particularly over the nasal bridge (Caggiari et al. 2022). One recent study showed that scarring over the nasal bridge and facial itching was reported in 68.9% and 27.9% of healthcare workers, respectively, due to prolonged use of N95 masks (Hu et al. 2020).

Although clinical staff engaged in all types of COVID-19 care settings are consistently reporting facial skin erythema, indentation marks, and lesions caused by prolonged use of PPE, PPE-related skin damage is often considered an inevitable consequence which must be accepted in managing the patients. However, mild irritation can lead to gross changes in skin integrity, which represents a portal for the penetration of pathogens and other hospital-acquired bacterial and viral infections (Gefen et al. 2020). Therefore, there is an urgent need for research to examine the underlying biochemical and biophysical mechanisms that precede the physiological changes in skin status in response to prolonged loading associated with PPE usage.

1.3 AETIOLOGY AND PATHOPHYSIOLOGY OF PRESSURE ULCERS AND INCONTINENCE-ASSOCIATED DERMATITIS

1.3.1 AETIOLOGY OF PRESSURE ULCER

PUs can develop as a result of extrinsic and intrinsic factors (Figure 1.8), each of which will be briefly discussed.

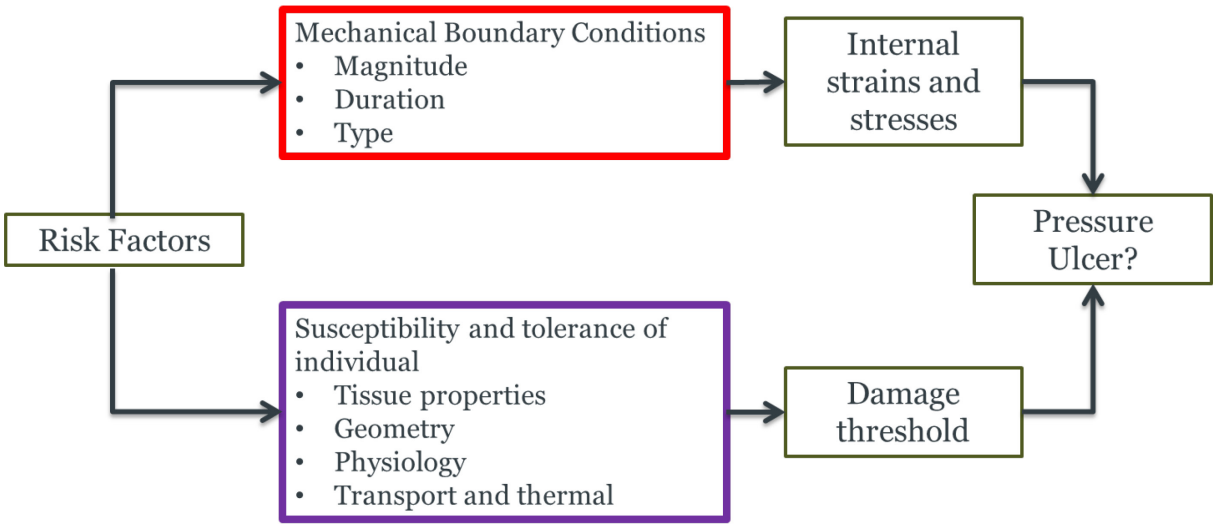


Figure 1.8. A conceptual framework consisting of the risk factors for pressure ulcer development. Image adapted from (Coleman et al. 2014).

1.3.1.1 EXTRINSIC FACTORS

Extrinsic or primary factors involve external conditions to which the body can be exposed and include pressure, shear and frictional forces, temperature and/or moisture. Pressure is defined as the ratio between the force acting perpendicular to the body surface and the skin contact area. Shear is a force that acts parallel to the skin surface, which generally results from the motion of the body relative to its support surface, for example, when a reclined body slides down a bed (Figure 1.9) or a subject is transferred between support surfaces. External shear forces affect both the skin and the underlying soft tissues and will cause deformation and shearing of blood vessels, thereby reducing blood flow and compromising tissue viability. Indeed, shear forces are often considered as important as pressure in the initiation of PUs (Tabloski 2013). Frictional forces are exerted between the skin and the materials of the support surfaces. They need to be overcome for relative motion between the two surfaces. Repeated friction due to periodic movements can lead to abrasive loss of the stratum corneum. External mechanical loading will result in tissue deformation and associated strain, which are determined by the stiffness, morphology and composition of the underlying skin, fat, muscle and bone (Agrawal et al. 2012).

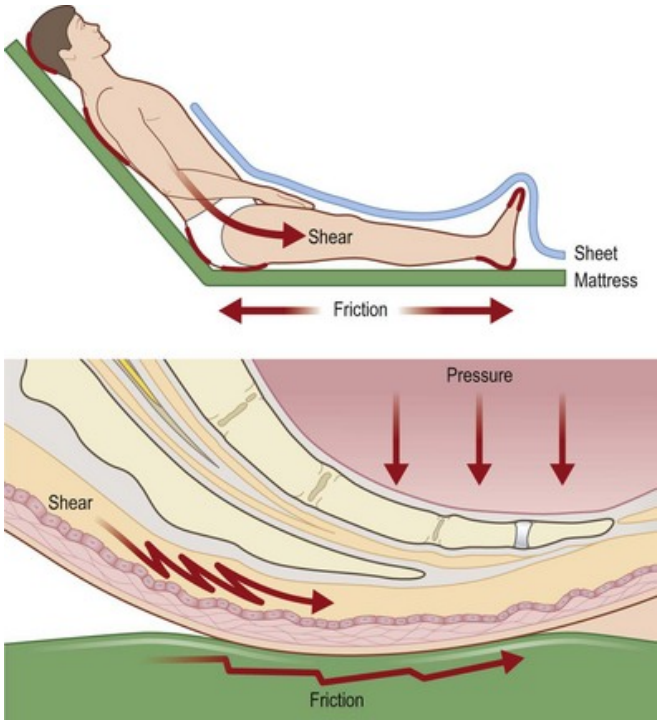


Figure 1.9. Forces applied to the skin surface. Image from Kwon, Janis et al. 2013

1.3.1.2 INTRINSIC FACTORS

Intrinsic factors are causes of PU development which are inherent in the individual or sub-group. Impaired mobility, nutritional compromise, underlying health conditions, ageing of skin tissues and incontinence represent the most prominent intrinsic factors involved in the development of PUs. Several studies have elucidated the role played by health conditions in the development of PUs. Comorbidities including, vascular diseases, diabetes, chronic pulmonary disease (COPD), neurodegenerative disorders, and musculoskeletal disorders have all been implicated in the aetiology of PU (Jaul et al. 2018). For example, there was a reported association between peripheral arterial disease and the development of heel ulcers in hospitalized patients (Delmore et al. 2015; Corniello et al. 2014). Age has been associated with the risk of PUs. Indeed, ageing induces a loss in tissue elasticity, thinning of the epidermis, and reduction of functional capacity of the skin, making it more susceptible to shear and frictional damage (Farage et al. 2013). In addition, the elderly often present with impaired mobility, which prolongs their time period in sitting and/or lying positions, subjecting the skin to sustained unrelieved pressure. The vulnerability of the elderly to PUs has been extensively addressed in numerous studies (Mervis et al. 2019; Jaul et al. 2018).

In addition to extrinsic and intrinsic factors, other factors can lead to PU development and progression. For example, an excess length of hospital stay has been cited as a cause of enhanced risk of new hospital-acquired PUs (Lima Serrano et al. 2017). Recurrent PUs and prolonged surgical procedures can also result in PU development (Coleman et al. 2014).

1.3.1.3 MODELS OF PRESSURE ULCER PATHOPHYSIOLOGY

In the last decades, different mechanisms have been proposed to be important in the development of PU formation (Ceelen et al. 2008). Traditionally, compression-induced ischaemia has been considered to be the primary mechanism, however, depending on the intensity and duration of an external mechanical load, four main aetiological processes, have been identified to co-occur with skin damage. These processes include:-

- Ischemic-induced damage resulting from impaired delivery of oxygen and nutrients to the microenvironment due to occlusion of blood vessels which, if sustained, can result in the build-up of anaerobic metabolites and eventual cell death (Jan et al. 2012).
- Impaired lymphatic drainage from compressed vessels resulting in the accumulation of cellular waste products which can prove toxic to the cells (Worsley et al. 2020; Gray et al. 2016).
- Reperfusion damage, which is promoted by the up-regulation of harmful oxygen-free radicals released locally following the restoration of the blood supply. The severity of this mechanism is associated with the intensity of the external pressure to which the skin is subjected (Hoogendoorn et al. 2017).
- Direct tissue deformation damage, which could lead to disruption of the cell membrane, impaired cell viability and capacity to remodel (Oomens et al. 2015). This results from high tissue strains and can be exacerbated by the presence of frictional forces, such as sliding and rubbing (*Figure 1.9*) (Coleman et al. 2013).

1.3.2 AETIOLOGY OF INCONTINENCE-ASSOCIATED DERMATITIS

In contrast to PU literature, there is a limited understanding of the pathophysiology of IAD, considered to be complex and multifactorial in nature (Beele et al. 2018). Factors influencing the development of IAD can be divided into both external provoking and underlying predisposing factors. The pathophysiology that leads to the development of IAD is summarised in *Figure 1.10*.

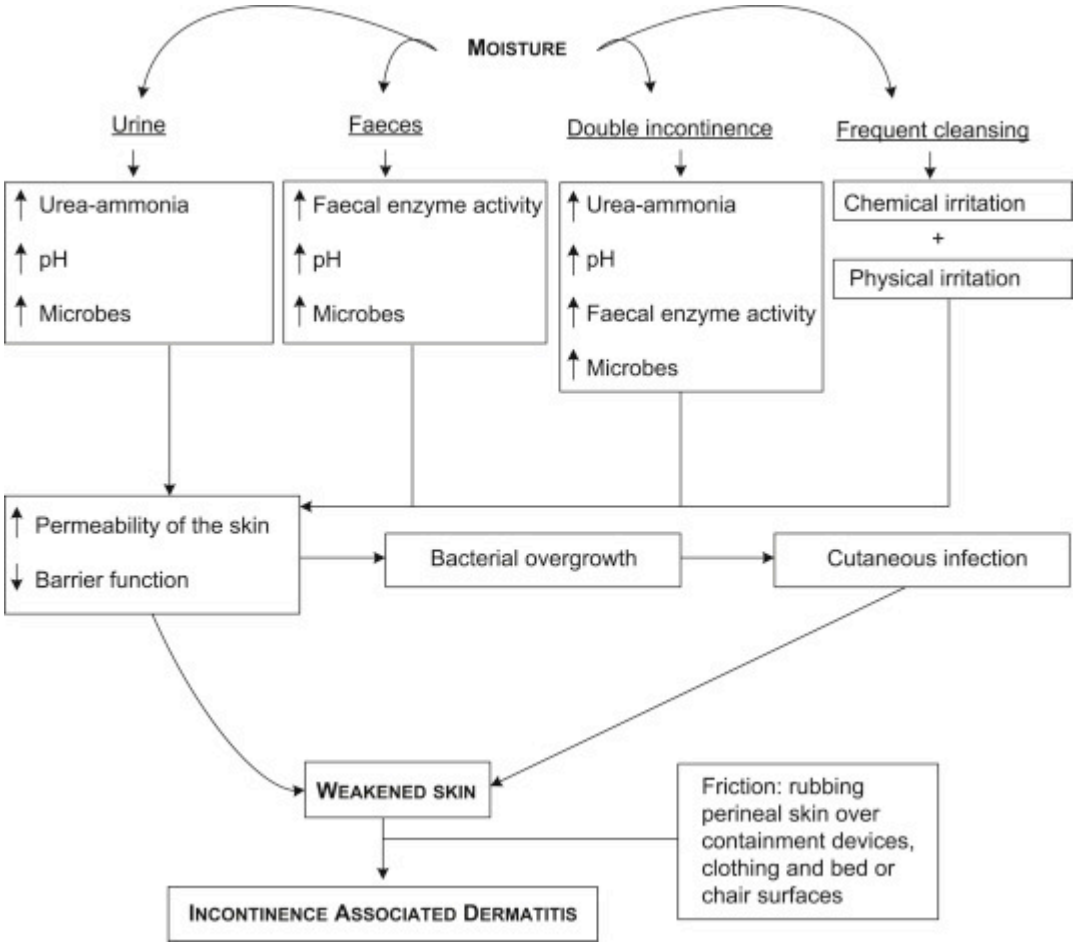


Figure 1.10. Combination of events that promote incontinence-associated dermatitis formation. Image from (Beeckman et al. 2009)

1.3.2.1 EXTERNAL PROVOKING FACTORS

Prolonged exposure to urine and/or stool alone or in combination with secondary infections is considered to play a critical role in the development of IAD. Indeed, the interaction of skin with chemical irritants within the urine has been suggested to induce changes in its status. In particular, ammonia in urine is capable of shifting the skin pH from acid to neutral or even alkaline levels. This causes a disruption of the acidic mantle of the skin, alteration of the skin flora, in terms of high bacterial colonization and overgrowth, diminished stratum corneum cohesion, and impaired skin barrier function (Odio et al. 2014). One study suggested that continuous exposure to alkaline urine, not only causes irritation but delays the recovery of the skin pH to its baseline levels (Larner et al. 2015). In addition, the presence of urine can activate proteolytic and lipolytic enzymes, present in the stool, which can degrade proteins and fat in the stratum corneum (Mugita et al. 2015). Furthermore, exposure of skin to moisture can result in an increase in the coefficient of friction at the skin-support interface and, as a consequence, aggravate the shear stresses that are generated during moving and handling the patient (Beele et al. 2018).

In the presence of moisture in the form of urine and physical friction caused by motion against clothing, bed linen or containment materials, the barrier function of the skin will also be reduced (Atasağun et al. 2022). In addition, vigorous or excessive cleaning of the skin in order to remove incontinent products may exacerbate skin damage (Black et al. 2011). Indeed, repetitive and energetic use of water and skin cleaning agents has been reported to induce both chemical irritation, as well as mechanical irritation (Voegeli 2008). Individuals with urinary incontinence are most likely to wear absorbent materials, and most of these devices can contribute to the promotion of IAD. Indeed it is well established that prolonged contact between the skin and incontinence pads induces its occlusion, leading to an increase in sweat production and changes in microclimate (Falloon et al. 2018)

1.3.2.2 UNDERLYING PREDISPOSING FACTORS

Among the predisposing factors, old age has been associated with increased vulnerability to IAD (Stalder et al. 2014). Elderly skin is characterised by diminished cell remodelling, delayed wound healing processes, and reduced barrier function (Al-Nuaimi et al. 2014). In addition, the elderly exhibit less natural skin moisturising content as a result of decreased production of sebum, sweat, and stratum corneum lipids. This will lead to dry skin which can easily be disrupted by, for example, mechanical irritation. Furthermore, other comorbidities which can promote the development of IAD in the elderly include chronic diseases i.e. diabetes mellitus, delayed immune response, and prolonged drug intake i.e. corticosteroids (Beele et al. 2018). Other factors that can increase the risk of acquiring IAD include reduced mobility and independence in toileting (Kottner et al. 2014).

1.3.2.3 MODELS OF INCONTINENCE-ASSOCIATED DERMATITIS PATHOPHYSIOLOGY

Various theories, both historical (Brown and Sears 1993, Jeter and Lutz 1996) and more recent (Beeckman et al. 2014; Beele et al. 2018), have been proposed to explain the pathophysiology of IAD. However, most of these were an adaptation of diaper dermatitis and considered only a single factor as the main causation of this skin condition or were merely based on clinical experience without robust empirical evidence. Indeed, with reference to diaper dermatitis, the latter is defined as a group of skin disorders in infants as a consequence of the skin being subjected to physical, chemical, enzymatic, and microbial factors challenge in the diaper environment. This compromises the integrity of healthy skin of the infant which is not designed to tolerate prolonged exposure to urine and faeces. In addition, the presence of moisture and occlusion, due to the use of diaper products, causes an increase in the coefficient of skin friction. Increase in skin hydration and pH result in impaired barrier function, and the presence of faecal enzymes leads to the skin being further degradated beyond its normal ability to cope with its environment. In this weakened state,

the skin is susceptible to a number of biological, physical and chemical insults that can aggravate diaper dermatitis.

Differently from diaper dermatitis, IAD occurs in elderly individuals or those with neurological impairment, especially if they present with limited toileting capacity and the ability to control bladder and bowel movements. In addition, another important difference between the two condition is given by the structure of the skin of the elderly, which differs considerably from that of infants as follows:

- There are significant differences in skin barrier function of the elderly compared to infants;
- The size of corneocytes is bigger in the elderly;
- The stratum corneum of the elderly present lower water content;
- The epidermal thickness of the elderly is higher compared to the infants
- The Elderly presents a decreased TEWL

There are clearly a number of similarities between the two conditions. However, for clinical reporting and management purposes their separation is critical.

Recently, a more generic clinical-based model has been proposed to illustrate the skin response following prolonged exposure to urine and/or stool (Figure 1.11). The model suggested an increase in TEWL and pH values following prolonged and frequent interaction of the skin with irritant substances. In addition, the presence of these irritants induces the release of inflammatory cytokines and histamine, which subject the skin to a vicious cycle of inflammation eventually resolving in skin breakdown (Gray et al. 2012).

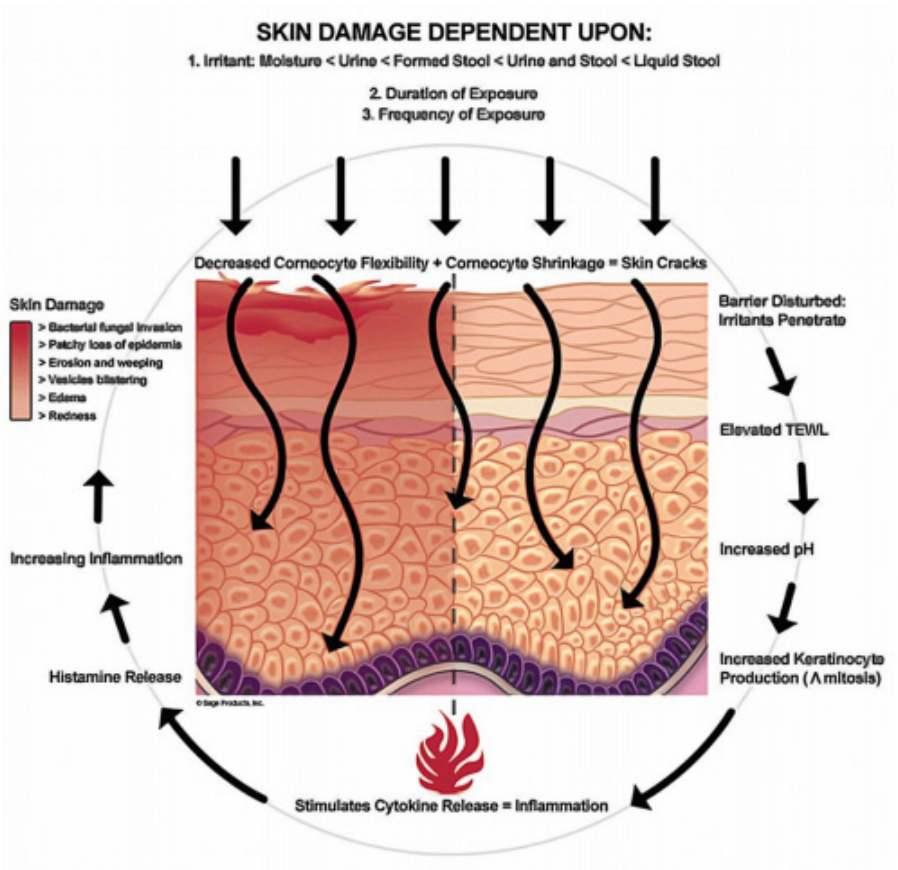


Figure 1.11. A clinical theoretical model of the aetiology and pathophysiology of IAD. Image from (Koudounas 2019)

1.3.3 THE DIFFERENT MECHANISMS OF ACTION OF PRESSURE ULCERS AND INCONTINENCE ASSOCIATED DERMATITIS

The mechanisms leading to the development of PUs and IAD has been comprehensively discussed in sections 1.3.1.3 and 1.3.2.3, respectively. Both conditions have elements of similarities and differences detailed in the below table. Indeed, it has often been reported that, clinically, IAD can be confused in most occasions with category I and/or II PUs (Mahoney et al., 2013). This is not surprising as both conditions present a number of common risk factors, in addition to IAD being a risk factor for PU development. However, it is crucial to differentiate between the two conditions in order to enable healthcare professionals implement adequate preventive strategies, as well as for accurate documentation and quality reporting (Beeckman et al., 2015). From a clinical perspective, it is obvious to state that if the patient is continent, it is unlikely that the condition manifested to be IAD. Although there is ongoing debate whether we can actually differentiate these two conditions when the patient is both incontinent and present with limited mobility (e.g. bedridden). However, it is worth noting that in IAD the damage begins on the surface of moisture-exposed skin, while in PUs the damage can initiated in the underlying soft tissue (Kottner et al., 2009). Histological examination of biopsies harvested from patients presenting with category I PU

and patients manifesting IAD, revealed that pressure ulcers were associated with an ischaemic pattern while in IAD samples an inflammatory pattern was evident, characterized by partial loss of epidermis, dilated vessels with some swelling of the endothelium, oedema of the dermis and presence of inflammatory cells (Houwing et al., 2007).

Table 1.4. Similarities and differences of pressure ulcers and incontinence associated dermatitis characterisation

	Pressure Ulcer	Incontinence Associated Dermatitis
Location	Over bony prominence or under medical device	Perineum; perianal area; inner thighs; buttocks
Associated factors	Reduced mobility; May have reduced sensory awareness	Urinary and/or faecal incontinence
Depth	Initially may present as stage I or DTI; ultimately usually full thickness (III/IV)	Usually partial thickness of epidermal and dermal structures
Shape/distribution	Typically round, if shear involved, may be oval /elongated; distinct borders	Irregular and indistinct borders
Associated findings	May have necrotic tissue; may have undermining or tunnelling	Surrounding skin typically macerated
colour	If redness is non-blanchable, most likely to be a stage I	Blanchable or non-blanchable erythema; pink or white surrounding skin due to maceration
Necrosis	Stage III and IV can present with a black necrotic scab over bony prominences	No necrosis

1.4 PREVENTION OF PRESSURE ULCERS AND INCONTINENCE-ASSOCIATED DERMATITIS

Prevention of PUs and IAD can be very challenging and intensive for HCWs, carers and patients. With regards to PU, various studies have suggested that its formation can be influenced by the shortage of registered nurses and the time they spend at the patient bedside (Qaddumi et al. 2014). However, effective prevention in various care settings involves the implementation of strategies designed to limit PU risk factors. These include protocols involving effective skincare, pressure reduction, the use of appropriate support surfaces, and nutritional considerations. This concept has been recently emphasised within the NHS improvement guidelines, which detailed a model, namely aSSKINg (Figure 1.12), which claims to enable clinical practitioners to recognise early signs of PUs and avoid the deterioration of the patient's skin health (McCoulough 2019).

Figure 1.12. NHS Improvement aSSKINg principles of pressure ulcer prevention. Image from (McCoulough 2019)

Regardless of the clinical tools developed by the various healthcare systems worldwide to reduce the escalating PU incidence, one of the most common measures of prevention of PU is to reduce prolonged and high pressures at the body-support surface. This can be achieved by frequent turning and repositioning of the patient. There is, however, limited published evidence related to optimal repositioning schedules. An observational seminal study, which employed three different turning regimes, every 2-3 hours, every 4 hours, and 2-4 times a day, in elderly patient groups, reported a decrease in PU development when patients were turned every 2-3 hours (Marsden et al. 2015). Although some studies have suggested that critically ill patients should be repositioned more frequently, the findings from Norton et al. have remained the gold standard for the regularity of turning in acute settings. Indeed, the 2 hour-turning regimes have been extensively implemented in clinical practice as a consensus. A more recent study has proposed that the turning schedule should be prescribed individually depending on the patient's risk factors and needs (Källman et al. 2016).

With regards to IAD, the strategy for the prevention of this condition involves the removal of causative elements, skin cleaning and protection, and the application of moisturizers and/or barrier creams (Kottner et al. 2015). Skin cleaning is critical to remove urine, stool, debris, and microorganisms. This should be followed by the application of moisturizers to repair the disrupted skin barrier, aid in the increase of the water content, and promote the reduction of TEWL. A wide array of studies has investigated the relationship between IAD and the use of appropriate skin-cleaning regimes. Indeed, cleaning procedures involving soap and water, in addition to strict infection control of the bath basin, have been widely accepted in the literature (Wilson 2017; Beeckman et al. 2016). The application of skin barrier products is important as it creates a permeable or semi-permeable barrier that avoids the skin from inquiring damage (Beeckman et al. 2015; Beeckman et al. 2016). Barrier products such as ointments, pastes, creams, polymeric film formers, and cyanoacrylates have been under investigation in relation to their capability to protect the skin from IAD causatives. However, there is still limited evidence to suggest which of these products displays outstanding performance, hence their implementation in preventive protocols is difficult and subjective (Beeckman et al. 2015).

Furthermore, it is also imperative to avoid prolonged exposure of the skin to body waste materials. This can be achieved via the use of containment products. The latter are either engineered to keep wetness away from the skin, thereby enabling the improvement of moisture management, or manufactured to decrease the pH of the skin in presence of incontinence (Bliss et al. 2017; Gray et al. 2018)

Nevertheless, various studies have reported the detrimental consequences following the use of body-worn incontinence products, namely, changes in the microclimate due to the occlusive effects to which the skin is subjected (Koudounas et al. 2020). Indeed, occlusion impedes the outward flow of heat and water vapour from the skin into the atmosphere and shields the skin from air currents, which can regulate the skin humidity and temperature (Gray et al. 2012). Hence, there is a compelling need for more research in order to develop smart incontinent products that exercise less occlusive effects and ensure the reduction of IAD prevalence.

1.5 RISK ASSESSMENT OF PRESSURE ULCERS AND INCONTINENCE-ASSOCIATED DERMATITIS

Risk assessment of PUs commences with a thorough examination of the skin and mental status of the patient. This is generally performed in hospitals using risk assessment tools. The most employed risk assessment scales include the Braden, Norton, and Waterlow scales, with Braden and Waterlow being the most widely adopted in the USA and UK, respectively.

Braden scale, structured to be used with adults, is composed of six subscales namely shear and friction, moisture, nutrition, sensory perception, activity, and mobility (Huang et al. 2021). It links the clinical situations with the intensity, duration, and skin tolerance to pressure prior to the damage. The scores are within the range of 6 (low risk) to 23 (high risk), with 16 representing the cut-off score for the prediction of early skin damage. The Norton scale includes five subscales associated with activity, mobility, mental condition, physical condition and incontinence (Šateková et al. 2017). Historically, its cut-off score for the development of PU was 14, although, this has been subsequently amended to 16 (Källman et al. 2014). The Waterlow scale was designed to serve three purposes, namely, standard risk assessment, PU grading, and planning strategies for prevention and treatment. The risk of developing PU is obtained by combining the scores of each subscale including body mass index, continence, type of skin, gender, age, neurological deficit, and malnutrition. It is considered a more advanced and comprehensive predictive tool compared to Norton (Papanikolaou et al. 2007). Although these scales represent the gold standard for PU risk assessment, they are subjective in nature and demonstrate limited positive predictive values, particularly when employed across different care settings and patient populations (Moore et al. 2019; Kottner et al. 2010). This has encouraged the development of new predictive tools, based on the knowledge of healthcare professionals and derived from modifications of existing scales in order to match the requirements of specific patient cohorts. Examples of these scales include PURPOSE T, FRAGMMENT Score and Schoonhoven Prediction Rule, each of which lack sufficient

evidence to confirm their predictive validity (Nixon et al. 2015; Aloweni et al. 2019). However, it is important to note that owing to the challenges of managing such a complex condition (pressure ulcers) on a heterogeneous group of at risk individuals, there are no GOLD standards in our practice. Indeed, in the latest EPUAP, NPIAP and PPPIA guidelines, released in November 2019, evidence-based recommendations for preventing and treating pressure injuries were put in place to support health professionals worldwide reduce the incidence of PUs (EPUAP, NPIAP, PPPIA. 2019). The guidelines outlined an array of care strategies that a clinician should consider once a patient has been admitted to an acute care facility. Relevant aspects of these guidelines are discussed in the sections below.

The risk assessment of IAD involves a visual observation of the skin areas commonly exposed to urine and faeces. However, this can prove difficult due to a lack of standardized definition of this condition and differences in terminology, due to the complexity of this skin condition as it exhibits different stages of severity and damage (Beeckman et al. 2015). In addition, the skin tone of the patient can further complicate the diagnostic process as it is easier to detect early signs of erythema and maceration in lighter skin patients (Beeckman 2017). Nevertheless, different evaluation and scoring tools have been developed for clinicians (Payne 2016). However, these tools have been reported to be time-consuming and linguistically complex when implemented in acute and community care settings. In addition, these tools lack images of the anatomical skin sites, raising difficulties in terms of highlighting severely affected regions (Clarke-O'Neill et al. 2015). Recently, in 2017, a novel IAD diagnostic instrument, termed GLOBIAD, was introduced and internationally validated. It matches the requirements of ease of use with the aid of skin photographic images, clear documentation, and clinical decision-making (Beeckman et al. 2018). GLOBIAD differentiates IAD into 2 categories (1 and 2), each divided into two sub-categories (A and B). Category 1 indicates patients with intact skin and visible erythema, while category 2 represents individuals with erythema and skin loss. The letters A and B are used to identify the absence (A) or presence (B) of infection. This tool has been employed worldwide with reported high sensitivity and high specificity. However, it has proved difficult in assessing clinical signs of infection. To overcome this issue, the developers have proposed using GLOBIAD in combination with microbiological testing (Beeckman et al. 2018).

1.6 SKIN ASSESSMENT OF PRESSURE ULCERS AND INCONTINENCE-ASSOCIATED DERMATITIS

As part of assessment strategies, guidelines require caregivers to perform a regular examination of the skin, particularly on the areas over bony prominences and/or exposure to moisture, to identify

possible skin damage, i.e., current PUs, variation in skin moisture and colour changes (Mervis et al. 2019). Indeed, skin assessment of IAD can be difficult for clinicians as this condition is complex in nature and exhibits different stages of severity and damage. Features of IAD range from different levels of erythema of intact skin to various skin maceration, excoriation and open wounds (Figure 1.13) (Beeckman et al. 2015). In addition, the skin tone of the patient can further complicate the assessment process as it is easier to detect early signs of erythema in patients with a lighter skin tone (Beeckman 2017). Other characteristic signs of IAD consist of poorly demarcated edges and increased temperature of the site affected compared to surrounding areas, as a result of underlying inflammation. In addition, if the skin is lesioned, it can present vesicles and pustules, and in some cases, the severity of the damage can compromise the entire epidermis, exposing an eroded, moist and weeping dermis (Ichikawa-Shigeta et al. 2014). Bacterial infections are also among the signs that could be detected when examining the skin of an IAD patient. Indeed, primary and secondary bacterial infections associated with IAD have been emphasised and represent the primary outcome of interest of a wide array of research (Campbell et al. 2016; Junkin et al. 2007).

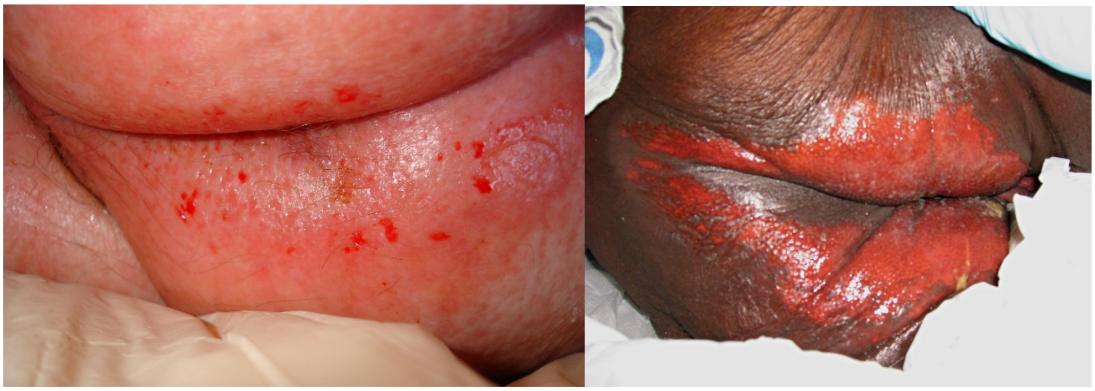


Figure 1.13. Clinical signs of incontinence-associated dermatitis of patients with lighter (left) and darker (right) skin tone. Image courtesy of NHS patients (with permission)

Early identification of PU focuses on detecting changes in skin surface redness. Indeed, skin erythema is one of the earliest signs of a pathological tissue reaction to pressure and shearing forces and its timely detection, with subsequent implementation of preventive strategies, could avoid the development of PUs (Shi et al. 2018). When surface redness is detected, clinicians will perform further skin tolerance assessments to evaluate whether it is blanchable or non-blanchable erythema – discoloration of the skin that does not turn white when pressed – normally termed stage I PU (Figure 1.14) (Whitlock 2013). One recent study has reported that patients presenting with skin erythema defined as non-blanchable were at higher risk of developing PUs of stage II or greater within 28 days compared to patients with blanching erythema (Shi et al. 2020). As such, the authors suggested adopting non-blanching erythema as a parameter to distinguish those patients who are at high risk of incurring PUs from those who are not. The use of non-blanchable erythema

as a threshold for the implementation of PU preventive strategies on patients was formally evaluated for the first time in a randomised clinical trial (RCT) (Vanderwee et al. 2007). Contrary to applying preventions following standard risk assessment methods, the study aimed at evaluating whether there could be a possible increase in the incidence of greater stages of PU (stage II and above) if preventive measures were applied only after the observation of non-blanchable erythema. The results of the study highlighted no significant differences in PU incidence when the control group (patients receiving preventive measures following traditional risk assessment) was compared to the intervention group (patients receiving prevention following the manifestation of non-blanchable erythema). Nevertheless, it is worth noting that the outcome of the study might have been influenced by various factors namely:-

- Inconsistent randomisation protocol – while the intervention group received prevention after the appearance of non-blanchable erythema, the control group consisted of patients which either received preventive measures or did not.
- Inconsistent pressure redistribution strategy – for some patients in the intervention group, pressure offloading consisted of manual repositioning performed by the nurses every 4 hours, while for the others no repositioning was carried out.
- Different support surfaces – patients were either assigned to polyethylene–urethane mattresses (PUM) or alternating pressure air mattresses (APAM) while in a lying posture, with only patients using PUM entitled to manual repositioning.
- Variation of hospital stay – although the median stay per patient was 16 days, nonetheless there was a considerable variation in the length of individual hospital stay, which ranged between 8 – 29 days.
- Incomplete risk assessment – although patients were risk assessed on a weekly basis using the Braden scale, however, this was not performed on all patients but on a randomly selected sub-cohort of participants.
- Lack of consideration of patients' intrinsic factors – the study did not evaluate the implications of patients' intrinsic factors such as mobility status, incontinence, comorbidities, etc.
- Limited diverse patient population – the study did not include patients with darker skin tones.

Regardless of the various limitations, the application in acute care settings of the approach suggested within the study might result in a reduction of patients requiring pressure redistributing measures without increasing the number of individuals developing greater stages of PUs. Nevertheless, both skin and risk assessment remain necessary and practical requirements before any prevention strategy can be introduced. Furthermore, given that the non-blanchable erythema

approach cannot be used in patients with darker pigmentation, there is a need to find alternative strategies to identify early signs of skin deterioration in these individuals.

Recently, a novel point-of-care technology has been developed for the early detection of skin damage, which will be fully discussed in chapter 2, section 2.1.2.3. It is of note that there has been a growing number of clinical studies which have demonstrated high sensitivity to identify earlier signs of skin damage of the device but with corresponding positive predictive values of just 14%, which is indicative of a high number of false positives (Okonkwo et al. 2020). Although considerable effort has been invested by prominent researchers in the tissue viability community in evaluating the device, nevertheless, it is imperative to acquire a better knowledge of the mechanism behind the measurement technique and data interpretation before recommending its use in acute care facilities.

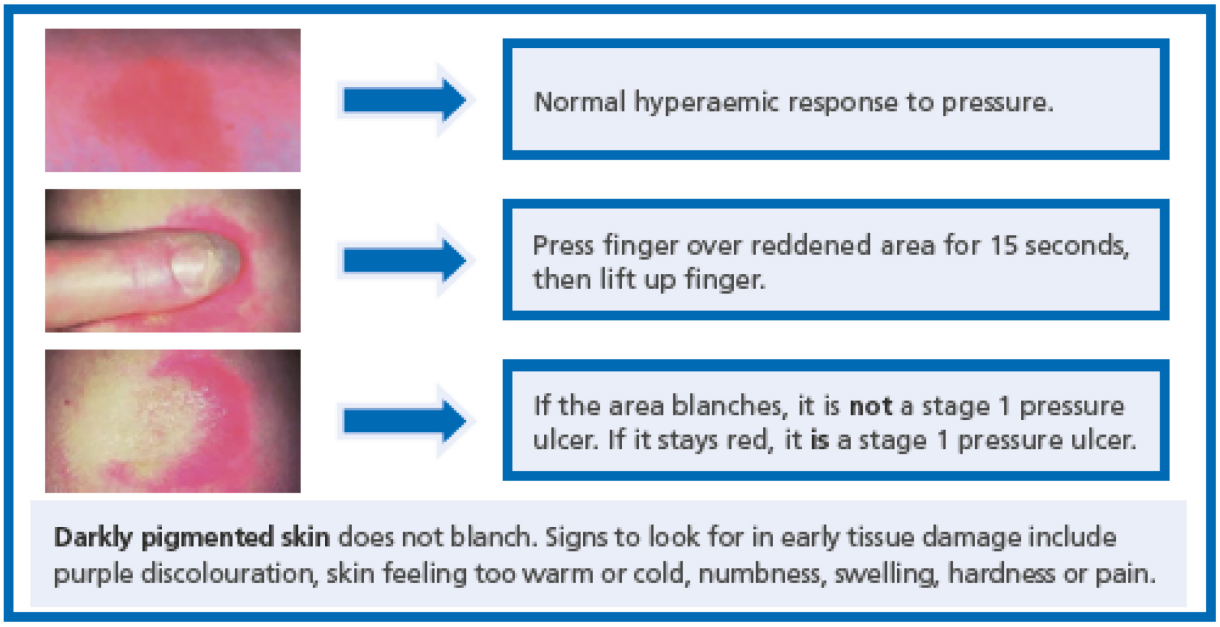


Figure 1.14. Skin tolerance test used for identification of non-blanchable erythema. Image from the NHS Stop the pressure SSKIN bundle assessment.

1.6.1 RISK ASSESSMENT VS EARLY DETECTION: THE QUEST FOR THE OPTIMAL SOLUTION

Risk assessment is the first process, which should be performed to identify patients who might be at high risk of incurring skin damage, and as such require additional monitoring and intervention to avoid escalating their condition. Therefore, risk assessment is an important part of the care model and is routinely undertaken in health and social care settings. However, studies have shown that the current strategies employed in acute and long-term care facilities have proven to lack the adequate sensitivity necessary to distinguish individuals requiring further skin monitoring from those not. This results in many patients being categorised as being at risk, even though they are

not, in order to try to decrease the escalating of PUs. Furthermore, there is a high degree of uncertainty in the academic literature regarding the true benefit of RAS, with several studies demonstrating limitations in the approach. New scales have been recently published, for example, PURPOSE-T, which aims to make the process aligned with the most recent evidence base. The limitations of risk assessment scales can be mitigated by implementing early detection as part of the care model strategy. The latter combines risk assessment with skin assessment, involving not only the visual observation of the patient's at-risk skin sites but also integrating tactile examination and the use of biophysical tools, with associated parameters, to identify minimal, invisible changes in the skin barrier. An example of biophysical tool, which has been proposed for the early detection of PU is the SEM scanner. The latter claims to detect the development of PUs 4 to 5 days prior to gross damage. Indeed, there is a growing body of evidence in relation to the use of biophysical tools for early detection. Nevertheless, their efficacy and cost-effectiveness are not fully understood.

Therefore, both risk assessment and early detection are equally important as the former identifies potential individuals at risk, while the latter screen these individuals in a more exhaustive manner to reduce the number of patients categorised as 'at risk', allowing for preventive strategies to be implemented only on those who most need them. This is also important in terms of the reduction of the associated costs of prevention.

1.6.2 THE COST OF RISK ASSESSMENT AND EARLY DETECTION

Detailing the expenditure incurred by healthcare systems worldwide for the risk assessment of patients can be complicated due to the inability to differentiate between tangible and intangible costs. Nevertheless, some studies were able to provide a rough estimate of the finances associated with PU risk assessment and prevention. Indeed, Padula and co-workers estimated the cost of nursing time to perform a visual assessment of at-risk skin to be $8 per patient (Padula, W. V. et al., 2019). Another study reported that the expenses associated with the risk assessment and prevention of PU development per patient per day ranged between 2.65 € to 87.57 € across all the departments of an acute care setting (Demarre et al. 2015).

With respect to early detection, there is limited available literature on the costs associated with this model of care. Nonetheless, there is an ongoing debate that, given that early detection strategies rely on the employment of novel bioengineering devices, which are associated with elevated costs, it is likely that this might result in an increase in the overall financial expenditure. However, a recent study evaluated the cost-effectiveness of a point-of-care device, SEM Scanner, which has been gaining increasing attention in the tissue viability community. The study reported that the adoption of SEM scanner, in conjunction with clinical judgment, yielded cost-savings of

$4054 per acute care admission, suggesting that sub-epidermal moisture scanners possess the potential to be a better strategy compared to standard care (Padula, William V. et al., 2020). Hence, it is obvious to state that if the cost of early detection methodologies exceeds the costs currently incurred by healthcare systems with traditional risk assessment strategies, the likelihood of implementing this new care model might be limited. In addition, as a consequence of needing to be consolidated in clinical practices, the initial costs associated with early detection might be considerable due to the need to educate healthcare professionals.

1.6.3 LIMITATIONS OF CURRENT SKIN DAMAGE ASSESSMENT METHODS

To reduce the burden of chronic skin damage to the healthcare systems, thereby ensuring that the individual quality of life is not compromised, it is of critical importance to detect early signs of skin damage to avoid wounds from developing. Implementation of preventive strategies continues to rely on risk assessments carried out via visual skin assessments and patients' medical histories. Their limitations have been highlighted in the previous sub-sections with wide-reaching consequences (Payne 2016; Clarke-O'Neill et al. 2015). As an example, in the USA acute settings, incorrect diagnoses i.e. false negatives, which subsequently develop into wounds have resulted in litigation and penalties to the cash-strapped healthcare system. Indeed, more than 17000 PU-related law cases have been reported to be filed annually, equivalent to an extra average burden of $250000 (Bauer et al. 2016). In addition, inaccurate diagnosis of the presence of erythema is common in individuals with darker skin. As an example, in a prevalence survey study of acute and long-term care patients, stage I PU was only identified in 13% of African-American patients as compared to 38% of Caucasian patients (VanGilder et al. 2008). In addition, studies have highlighted higher PU mortality rates for patients with darker skin colour, as a consequence of delayed diagnosis, which, inevitably, results in delayed interventions (Black et al. 2016).

Furthermore, the introduction in clinical acute settings of risk assessment tools, derived from adapting existing tools, was reported to demonstrate no additional value above that of the experienced judgement of a nurse and/or clinician (Chou et al. 2013). This was emphasised by the UK guidelines (Stansby et al. 2014) which highlighted that the tools should only be used as an adjunct to clinical judgement and should not replace it. In addition, many of these skin assessment strategies were developed based on results from subjective interviews of HCWs, patients, and caregivers, with their findings biased due to the large acute and long-term facilities' patient populations diversity and the inability to extrapolate meaningful data from these qualitative research (Campbell et al. 2022). Accordingly, there is a compelling need for an objective number of skin parameters, which are able to detect early signs of loss of skin integrity in all patients, in order to aid clinicians to adopt effective preventative strategies.

1.7 THE RESEARCH PROBLEM AND CLINICAL CHALLENGES

The inability to detect early signs of skin compromise and current limitations in differentiating various types of skin damage represents a significant challenge to healthcare systems worldwide. With respect to PU, although there is extensive literature pertaining to the causes of this condition and international guidelines to assist clinicians to assess the skin of patients in order to prevent PUs from manifesting, nevertheless, the prevalence of this type of skin damage in acute care settings remains high. The reasons for this high prevalence can be attributed to different factors, the most prominent of which consists of:-

- The challenges encountered by caregivers in the attempt to identify early signs of PU
- The ineffective and incomplete use of preventive measures
- The inability to distinguish between patients who require preventive measures from those who are not at risk of incurring PU.

Indeed, as a consequence of the limitation of skin assessment strategies and in order to avoid the development of PUs in patients who are deemed not at risk, clinicians are often forced to implement preventive measures for a large number of patients. This results in an inevitable increment in costs associated with PU prevention and management.

With reference to IAD, the volume of research investigating the pathways leading to the development of this chronic condition is comparatively thin. Although there is an increasing awareness of the role of urine and stool in the development of IAD, nonetheless, there are still challenges and questions which warrant answers namely:-

- The time of exposure to urine and stool after which the skin barrier properties are compromised.
- The vulnerability of the skin to damage following occlusion due to the use of incontinence pads.
- Skin healthy recovery characteristics following exposure to moisture alone or in combination with pressure
- The impact of functional loading in combination with pads on skin parameters,
- Time dependent changes in skin hydration, barrier function and inflammatory response triggered following exposure to urine and faeces

Therefore, the current thesis has been designed to get a better understanding of these issues to bridge the gaps in the knowledge of PU and IAD manifestation and early indicators of changes in skin viability, in addition to providing directions for future research.

Chapter 2 TECHNOLOGIES TO QUANTIFY CHANGES IN SKIN HEALTH

2.1 NON-INVASIVE IN VIVO TECHNIQUES TO INVESTIGATE THE SKIN STATUS

An array of non-invasive *in vivo* techniques has been proposed over the years for use in the monitoring of skin structure and function (Bader et al. 2018). These techniques present an objective and potentially more accurate determination of skin properties and risk of damage in vulnerable patients than that of conventional risk assessments performed by nurses. Indeed, the former is reported to have limited specificity and sensitivity and is highly influenced by the expertise of the clinician performing the assessment (chapter 1, section 1.5) (Walsh et al. 2011). Different healthcare professionals will offer different opinion based on their level of competency, years in profession and knowledge of the skin behaviour of the patient. This can lead to inconsistencies in observation, creating potential for adverse events (e.g. development of PUs), which can affect the psychology of the patient, as well as the self-esteem of the clinician who previously performed the assessment.

Medical devices have been suggested to offer a better solution to overcome these limitations (Ielapi et al., 2020). The numeric nature of their outputs and the use of established thresholds could be used as an adjunct to clinical decision making. Nevertheless, performing clinical activities based solely on the outputs of these devices can still lead to misdiagnoses and inappropriate interventions. The causes of these are variegated ranging from lack of staff training on the interpretation of the results displayed by the device, to incorrect use of the device (Ho et al., 2018). In addition, due to the shortage of healthcare professionals and the need to perform increasingly complex interventions in a fast-paced environment, there is an increase in pressure to which clinical staff are subjected to, which, in turns, increases the opportunity for user error.

Regardless of the device being able to operate correctly and reliably, they might still contribute to medical error if, for example, they operate in a counter-intuitive manner (Money et al., 2011). In addition, healthcare workers are more often required to operated devices of greater complexity, which further challenges safe and efficient diagnoses (Fries, 2012). Furthermore, the device might not be able to detect the correct changes in the parameters for which it was developed, as such leading to erroneous interpretations or the output of the device can be influenced by

environmental factors. It is also important to acknowledge that some device might have their outputs expressed in arbitrary units, enhancing the difficulties of data interpretation. All these factors acan lead users to be less tolerant towards poor design, with greater emphasis placed on manufacturers to design fit-for-purpose devices and prove their efficiency before these devices can become readily available for use. This has motivated the subject of recent interest in the scientific literature and from commercial systems such as the sub-epidermal moisture (SEM) scanner (BBI and Provizio®, UK).

It is generally accepted, however, that a single parameter derived from one biophysical or imaging technique will not prove sufficient to encompass all the complex features of the skin barrier and its associated status (sections 1.1 and 1.3). Hence, a multi-parameter approach is required. Indeed, several non-invasive *in vivo* approaches, involving trans-epidermal water loss (TEWL), pH, stratum corneum (SC) hydration, sub-epidermal moisture (SEM), and erythema scoring have all been employed to examine the altered skin barrier function caused by repetitive mechanical loading and/or change in microclimate (Elban et al. 2020; Gefen et al. 2020; Kottner et al. 2021). In addition, a recent review identified other approaches involving biomechanical, microclimate and biochemical measurements, imaging, and computational modeling of loaded skin and underlying soft tissue in order to monitor compromise to structural and functional skin and sub-dermal integrity (Bader et al. 2018). Given the scope of the current thesis, this chapter will focus on the use of biophysical measures able to identify changes in skin status.

2.1.1 TRANSEPIDERMAL WATER LOSS (TEWL)

The use of TEWL to interrogate the integrity of the skin barrier was first reported over 50 years ago (Spruit et al. 1965). TEWL is defined as the amount of water that diffuses passively from the layers of the dermis and the living epidermis towards the superficial layer of the SC, as a result of the vapor pressure gradient (Honari et al. 2014). Indeed, as the amount of water loss increases, the skin surface humidity rises above ambient humidity, creating a gradient. TEWL values are normally expressed in grams of water per square meter of skin per hour ($g/h/m^2$). This parameter has been extensively employed in both the field of cosmetics and dermatological research to describe variations in skin barrier integrity. The average amount of water passively loss through the human skin has been reported to range from 300 to 500 mL/day (4.0-10.0 $g/h/m^2$) (Boer et al. 2016). Although there is no optimal TEWL value for healthy skin, elevated values are associated with an impairment in the skin barrier integrity (Akdeniz et al. 2018). Various commercial systems are available for TEWL measurement, as critiqued in Table 2.1. These include three different methods,

namely, open-chamber, unventilated-chamber, and Condenser-chamber, which represent the most commonly adopted during *in vivo* experiments (Figure 2.1) (Alexander et al. 2018).

Table 2.1. Commercially available devices for the measurements of trans-epidermal water loss. Table adapted from (du Plessis et al. 2013)

INSTRUMENT	METHOD	MANUFACTURER	ADVANTAGE	DISADVANTAGE	REFERENCE
AquaFlux	Condenser-chamber	Biox Systems Ltd, London, U.K.	Ensures a special environment against air turbulence; Provides continuous measurement without purging, no recovery time needed; Enhanced sensitivity	Not extensively adopted in the scientific community	(Farahmand et al. 2009; Elkeeb et al. 2010; Imhof et al. 2002)
DermaLab	Open-chamber	Cortex Technology, Hadsund, Denmark	Provides continuous measurement	Diffusion zone influenced by the ambient, underestimates high evaporation rates	Cohen et al. 2009; Fluhr et al. 2006
Evaporimeter	Open-chamber	Servo Med AB, Varberg, Sweden	Allows continuous measurement; Extensively used in research to permit comparisons	Must be held in a vertical position, long recording time required, readings affected by air currents	Barel et al. 1995; Cohen et al. 2009
H4300	Unventilated-chamber	Nikkiso-YSI Co Ltd, Tokyo, Japan	A good reported sensitivity; TEWL values could be compared to those obtained with DermaLab; Low price	Unable to sustain continuous readings, TEWL baseline values lower than other devices, product discontinued	(Serup et al. 2006; Tagami et al. 2002; Kikuchi et al. 2017)
MEECO	Unventilated-chamber	Warrington, PA, USA	Protects diffusion zone from the ambient air fluctuations; Short recording time	Unable to sustain a continuous recording	Fluhr et al. 2006
Tewameter	Open-chamber	Courage & Khazaka GmbH, Köln, Germany	Enables continuous measurements; Readings achieved quickly; Small probe minimises the influence of air turbulence; Does not require frequent calibration	Requires a temperature and humidity standardized environment in order to minimise the reading errors	(Steiner et al. 2011; Barel et al. 1995; Fluhr et al. 2006)
VapoMeter	Unventilated-chamber	Delfin Technology, Kuopio, Finland	Short reading times; Insensitivity to air currents; Measurements can be conducted at various contact angles	Does not allow prolonged measurements, unable to discriminate for the effects induced by diurnal rhythm and fluctuations as a function of time	(Steiner et al. 2011; Fluhr et al. 2006; De Paepe et al. 2005)

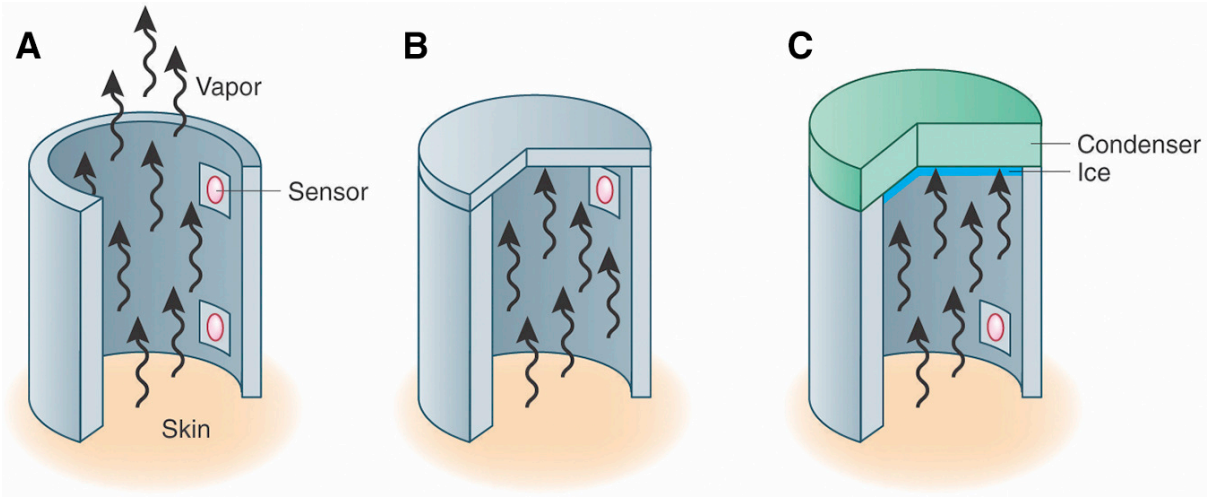


Figure 2.1. Principles of Trans-epidermal water loss measurement. A) Open-chamber B) Unventilated-chamber and C) Condenser-chamber methods. Image from (Alexander et al. 2018)

2.1.1.1 OPEN CHAMBER METHOD

This technique involves a hollow cylinder with open ends which is placed in light contact with the skin. The diffusion of the vapour occurs from the skin through the chamber and out into the surrounding environment (Figure 3.1A). The chamber is composed of two sensors, fixed at an established distance from the skin, which measure the humidity gradient based on Fick's law of diffusion (Scheuplein 1967), following measurements of both temperature and relative humidity (Nilsson 1977). The approach benefits from conducting continuous readings with no occlusion of the skin nor interference with its microclimate. However, it is limited by any environmental influences, such as ambient air movement (Alexander et al. 2018).

Historically, one of the most commonly adopted open chamber devices was the Tewameter TM210 manufactured by Courage and Khazaka. It did, however, present with some limitations including the need for frequent and time-consuming calibrations which involved a cumbersome portable calibration box. This version of the Tewameter was replaced with a modern wireless probe (TM300) of a length of 153 mm, cylinder height of 20 mm, and weight of 90 g. The measurements are transmitted via a small receiver, attached to a computer at an acquisition frequency of 1Hz. The reported uncertainty of the measurements from the manufacturer is ± 0.5 g/h/m² and ± 1.0 g/hm² for the relative humidity above and below 30%, respectively. Recently a newer model, TM Hex, has been introduced into the scientific community. This version incorporates 30 sensors arranged in 6 columns with 5 sensor pairs each inside its chamber, enabling highly accurate readings of temperature and humidity variation. This results in a considerable volume of data acquired within seconds, with very low variations and measurement uncertainty, as stated by the manufacturer.

2.1.1.2 UNVENTILATED CHAMBER METHOD

In this approach, the upper end of the device chamber is closed, protecting the diffusion zone from external disturbances in air movement (Figure 2.1B). The amount of water vapour lost through the skin is accumulated in the chamber with time, resulting in an increase in the humidity, which is recorded. The variations of the temperature and the relative humidity enable the calculation of the flux density, defined as the amount of water diffusing through the SC per unit distance and time (Klotz et al. 2022). Due to the accumulation of humidity in the chamber, this method does not allow for continuous measurements and requires purging after each measurement (Fluhr et al. 2014). The purging process is controlled by the instrument and can require from 20 to 90 seconds. However, the unventilated-chamber method does provide rapid measurements in less than 15 seconds (Salvo et al. 2014).

2.1.1.3 CONDENSER CHAMBER METHOD

An alternative system involving the condenser-chamber method has been introduced to provide continuous measurement while protecting the site from the influence of ambient air movements. This is achieved by presenting its upper edge closed by a condenser, whose temperature is controlled below the freezing point of water (Figure 2.1C). This results in the creation of a humidity gradient which induces water to diffuse away from the surface of the skin. TEWL is calculated from the output of two sensors, placed on the chamber wall and the condenser (Imhof et al. 2014). In addition, the condenser removes incoming water vapor by condensing it to ice and as such allows continuous measurements.

Various studies have compared the results from the different TEWL devices (Kikuchi et al. 2017; Fluhr et al. 2014) and have generally reported no significant differences in their output parameters. By contrast, one study did demonstrate some differences, in particular, the condenser chamber device, which produced remarkable changes in TEWL values following skin disruption with the tape stripping technique (Farahmand et al. 2009). It is generally agreed, however, that where possible the same device should be employed for the duration of a single research study to provide consistency in the reported TEWL values (Rosado et al. 2005).

2.1.1.4 FACTORS INFLUENCING TRANSEPIDERMAL WATER LOSS MEASUREMENT

There are many established factors which influence the accuracy of the TEWL parameter (Table 2.2). These include *environmental factors*, namely temperature, humidity, and ventilation, *endogenous* factors such as anatomical locations, age and skin conditions, and *exogenous factors* (Young et al. 2019; Kottner et al. 2013). The impact of several of these factors is still in question. As an example, while several studies have reported a negative correlation between baseline TEWL

values and skin ageing (Farage et al. 2013; Kottner et al. 2013), other studies have reported no such decrease in TEWL with age(Hon et al. 2020; Firooz et al. 2012). In addition, while some studies have reported a correlation with gender, with a higher TEWL baseline associated with males (Akdeniz et al. 2018), this difference was not supported in other studies (Rougier et al. 2021). In terms of ethnicity, some reports suggest elevated TEWL levels with black skin (Machado et al. 2010), which was not observed in other studies (Yosipovitch et al. 2002; Grimes et al. 2004).

In order to mitigate the impact of these factors, it is important to conduct TEWL measurements employing well-established and standardized protocols (du Plessis et al. 2013), reported guidelines include:

- in a controlled environment, i.e. temperature of 18–21 °C and relative humidity of 40%–60%,
- allow at least 20- 30 minutes for the participants' acclimatization period prior to skin assessments
- measurements to be taken at the same time of day and preferably during the same season
- Gloves should be used to hold the TEWL probe, which should be applied at a constant pressure

In addition, to minimise the interferences due to perspiration and/or skin surface water loss (SSWL), as well as to obtain accurate output, the investigational sites should be pat dried with filter paper and subjects should avoid the application of moisturizers and/or cosmetics prior to measurements (Lodén 2012).

Table 2.2. Diverse factors influencing trans-epidermal water loss and stratum corneum hydration output. Table adapted from (du Plessis et al. 2013)

	TRANSEPIDERMAL WATER LOSS		STRATUM CORNEUM HYDRATION	
	Influence	References	Influence	References
Endogenous Factors				
Age	Yes	(Kottner et al. 2013; Darlenski et al. 2009)	Yes	(Darlenski et al. 2009; Barel et al. 1995)
	No	(Zouboulis et al. 2018; Marrakchi et al. 2007)	No	(Maibach 1988)
Gender	Yes	(Conti et al. 1995; Chilcott et al. 2000)	Yes	(Plewig et al. 1970)
	No	(Zouboulis et al. 2018; Darlenski et al. 2009)	No	(Serup et al. 2006; Darlenski et al. 2009)
Ethnicity	Yes	(Kompaore et al. 1993)	Yes	(Berardesca et al. 2003)
	No	(Grimes et al. 2004)	No	(Machado et al. 2010)
Anatomical sites	Yes	(Kottner et al. 2013; Maibach 1988)	Yes	(Kleesz et al. 2012; Black et al. 2000)
Skin temperature	Yes	(Darlenski et al. 2009)	Yes	(Darlenski et al. 2009)
Sweating	Yes	(Serup et al. 2006; Darlenski et al. 2009)	Yes	(Darlenski et al. 2009; Serup et al. 2006)
Circadian rhythm	Yes	(Le Fur et al. 2001)	Yes	(Le Fur et al. 2001)
	No	(Rogiers 1995)	No	(Yosipovitch et al. 1998)
Skin health	Yes	(Proksch et al. 2008)	Yes	(Proksch et al. 2008)
Exogenous Factors				
Skin washing and wet work	Yes	(Voegeli 2008; Korting et al. 1991)	Yes	(Kezic et al. 2009)
Skin products	Yes	(Brandner et al. 2006)	Yes	(Kezic et al. 2009)
Occlusion	Yes	(Jungersted et al. 2010; Wetzky et al. 2009)	Yes	(Zhai et al. 2002; Kezic et al. 2009)
Skin damage	Yes	(Fluhr et al. 2006)	Yes	(Wetzky et al. 2009)
Smoking	Yes	(Chou et al. 2008)	Yes	(Wolf et al. 1992)
Environmental Factors				
Air convection/movement	Yes	(Darlenski et al. 2009; Serup et al. 2006)	Yes	(Darlenski et al. 2009)
Ambient temperature	Yes	(Darlenski et al. 2009; Serup et al. 2006)	Yes	(Darlenski et al. 2009)
Relative humidity	Yes	(Darlenski et al. 2009; Serup et al. 2006)	Yes	(Black et al. 2000; Serup et al. 2006)
Season	Yes	(Darlenski et al. 2009; Serup et al. 2006)	Yes	(Qiu et al. 2011; Black et al. 2000)

2.1.1.5 TRANSEPIDERMAL WATER LOSS AS A PARAMETER FOR THE DETECTION OF SKIN DAMAGE

TEWL has been employed in various studies to assess the response of the skin following various insults, including tape stripping (Danby et al. 2016), exposure to Sodium Lauryl Sulphate (SLS) (Leoty-Okombi et al. 2021) and moisture (Firooz et al. 2015; Bostan et al. 2019). These studies generally reported significant increases in TEWL values when compared to baseline following the insults. One study of note reported a remarkable increase in TEWL values following repeated exposure of SLS to the dominant mid-volar forearm of participants over the course of three weeks (De Jongh et al. 2006). The study also highlighted that changes in TEWL after a single disruptive event cannot be used to predict the change in TEWL after repeated exposure. The findings of this research were supported by other studies (Khosrowpour et al. 2019; Chan et al. 2019) TEWL was one of the parameters evaluated in one study exploring skin response after exposure to mechanical loading. The study suggested an increase in TEWL levels of 15 healthy subjects after 2 hours of lying in a supine position on a support surface (Kottner et al. 2021).

Research in the host lab has employed TEWL to interrogate early signs of loss of skin integrity following prolonged exposure to mechanical loads and moisture-soaked pads. They reported that the presence of moisture in combination with load promoted an increase in TEWL (Bostan et al. 2019). However, the values reversed back to baseline following 30 minutes of a refractory period. In related studies, the implication of incontinent products moistened or saturated with synthetic urine was evaluated (Koudounas 2019, Koudounas et al. 2020). The studies reported an increase in TEWL as a result of the disruption of the skin barrier properties due to exposure to synthetic urine at pH 7.9.

With respect to medical devices, one study reported the impact of the application of continuous positive airway pressure (CPAP) non-invasive ventilation masks on facial skin health. The crossover cohort study, involving 15 able-bodied volunteers, revealed a statistically significant increase in TEWL following 30 minutes of humidified CPAP application (Alqahtani et al. 2018). Furthermore, in the context of the outbreak of the COVID-19 pandemic, the impact of the prolonged use of respiratory protective equipment (RPE) has also been the recent focus of research. As an example, one study examined the short-term effects of respirators (type N95) on skin physiology following their application for 4 hours. The results revealed a significant increase in TEWL values in association with the devices when compared with the response of unloaded sites on the face (Hua et al. 2020)

2.1.2 STRATUM CORNEUM (SC) HYDRATION

SC hydration is an alternative parameter to examine human skin barrier properties. Again several factors influence this parameter, as highlighted in *Table 2.2*, and guidelines have been produced for the accurate interpretation and comparison of measured values (Berardesca et al. 2018). Increased SC hydration has been associated with enhanced absorption and penetration of potential irritants, with particular reference to the development of contact dermatitis, which is relevant in individuals suffering from incontinence (Kottner et al. 2015). In addition, elevated SC hydration has been suggested to increase the coefficient of friction between the skin and the support surface, which has been implicated in the development of PUs (Derler et al. 2012). By contrast, a decreased SC hydration could lead to skin dryness, which has been reported to be associated with structural stiffness of the epidermis, increased susceptibility to mechanical loads, and the presence of cracks, fissures, and inflammation of the skin (Engebretsen et al. 2016).

Skin hydration is determined by measuring the amount of water present in the SC. This is achieved using different methods, namely, electrical measurement, near-infrared (NIR) multispectral imaging, silicon image sensor technology, optical coherence tomography, nuclear magnetic resonance spectroscopy, and transient thermal transfer (Qassem et al. 2019). The principles and advantages/ disadvantages of each method are detailed in Table 2.3. Focus will be given to electrical methods, which represent those readily available in the current project.

Table 2.3. Different methodologies employed for the assessment of skin hydration with relative advantages and disadvantages.

METHOD	PRINCIPLE	ADVANTAGE	DISADVANTAGE	REFERENCE
Electrical	Measures either the Capacitance, Conductance, or Impedance of the skin	Relatively low cost and short measurement time; Well established in the literature; Allows comparisons; Easy measurement	Influenced by the amount of electrolytes and applied contact pressure; Sensitive to microclimate	(Li et al. 2001; Berardesca 1997)
Near-infrared Spectroscopy	Measures the absorption of NIR light by water in living tissue from its reflectance spectrum	Linear correlation between the NIR absorption intensity and the concentration of water in the skin; Relatively insensitive to interfering factors; Provides a visualization	Influenced by wavelength-dependent scattering effects, and by the presence of other chromophores	(De Rigal et al. 1993; Martin 1993; Walling et al. 1989; Zhang et al. 2005)
Silicon Image Sensor Technology	Detects discrete focal variations in skin surface hydration	Provides information about SC surface topography; Produces images which can be mapped to the skin surface capacitance.	Not widely adopted and, as such, limits direct comparisons	(Leveque et al. 2003; Piérard et al. 2004; Xhauflaire-Uhoda et al. 2006)
Optical Coherence Tomography	Quantifies the epidermal hydration by measuring spatial refractive indices of the skin	Resolves upper skin structures with a high depth resolution; Widely adopted in various medical disciplines.	Indirect method, its reflective index, requires validation, expensive	(Sand et al. 2006; Tearney et al. 1995)
Nuclear Magnetic Resonance Spectroscopy	Measures the protons of the water molecules in a magnetic field, which is converted using the field-fast Fourier transform.	A precise method for the determination of skin hydration, considered as a reference technique;	Inaccessible for some anatomical sites; Required special magnetic coils; Very expensive	(Sand et al. 2006; Girard et al. 2000; Sotoodian et al. 2012)
Transient Thermal Transfer	Measures skin temperature, during thermal stimulation. SC hydration is proportional to temperature changes	Measures hydration at different epidermal depths; Can be used at various anatomical site; unaffected by the application of skin products	Indirect technique; requires longer recording time of approx. 10 minutes	(Girard et al. 2000; Sotoodian et al. 2012)

2.1.2.1 ELECTRICAL MEASUREMENT OF THE STRATUM CORNEUM HYDRATION

Various studies have established that the electrical properties of the skin depend on the SC water content (Lu et al. 2018). Accordingly, skin hydration may be assessed by measuring either the capacitance (the amount of electric charge stored by the body), conductance (the ease of flow of electric current through the layers of skin), or impedance (the resistance of the body to the flow of charge). The measurement principle adopted by different commercially available electrical devices to assess SC hydration is detailed in *Table 2.4*.

Table 2.4. Electrical devices for the measurement of stratum corneum hydration. Table from (du Plessis et al. 2013)

MODEL SYSTEM	MEASUREMENT PRINCIPLE	MANUFACTURER
ASA-M2	Conductance	Asahi Biomed Company Ltd, Yokohama, Japan
Corneometer	Capacitance	Courage & Khazaka, Cologne, Germany
Dermalab Moisture Unit	Impedance	Cortex Technology, Hadsund, Denmark
MoistureMeter SC	Capacitance	Delfin Technologies, Kuopio, Finland
Nova Dermal Phase Meter DPM 9003	Impedance	Nova Technology Corporation, Portsmouth, NH, USA
Skicon 200 and 200 EX	Conductance	ISBS Co Ltd, Hamamatsu, Japan

2.1.2.2 CORNEOMETER®

The Corneometer, manufactured by Courage and Khazaha, is the most widely adopted biophysical device in clinical settings due to its high reproducibility (ICC between 0.92-0.98), easy handling, short measuring time, and financial cost (Kottner et al. 2021). Based on the measurement of the skin capacitance, the device is constructed of gold tracks in the probe head which comes in contact with the dielectric medium of the skin SC, generating an electric field as the result of the presence and absence of electrons within the tracks. The dielectric constant of the skin will change with the water content of the SC. This allows for any changes in skin hydration to be measured by the precision measuring capacitor, with an output measure in arbitrary units (A.U.) (Figure 2.2A) (Ye et al. 2019).

Composed of a spring in the probe head, which ensures constant pressure on the skin during readings, the latest Corneometer model, CM 825w (Figure 3.2B), enables continuous measurements over prolonged periods as well as high sampling frequency (1s). The limited depth resolution (10-20 μm) allows for the exclusion of influences from the deeper skin layers and no galvanic contact is required between the probe and the investigational sites (Heinrich et al. 2003). The probe measures a skin area of 49mm^2, at a frequency of 0.9-1.2 MHz, with a reported accuracy of ±3%. The measured values are transmitted via a small receiver unit, which connects to a computer.

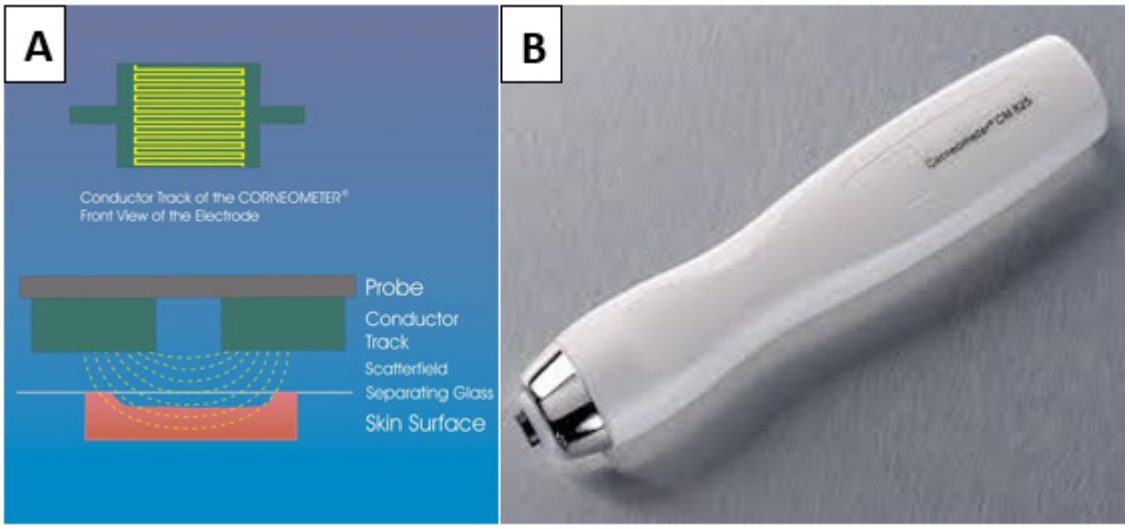


Figure 2.2. A) Principle underpinning the Corneometer ability to measure SC hydration, B) Latest corneometer model CM 825w.

Corneometer has been adopted in various research fields ranging from dermatological research in humans and animals to safety testing of cosmetics and skincare products. As an example, it was used to assess the skin response in a cohort of healthy volunteers lying on both conventional and specialised mattress systems (Denzinger et al. 2020). In a related study, there was a reported increase in SC hydration level following 2 hours of lying in a supine position on a basic foam mattress. The authors hypothesised that the increment in SC levels was a product of occlusion which lead to a variety of structural skin and soft tissue changes (Kottner et al. 2021). An increase in SC hydration levels has also been reported when exploring variations in skin microclimate in relation to PU development due to long-term loading (Scheel-Sailer et al. 2017; Kottner et al. 2015).

The reliability of the Corneometer output to measure SC hydration has been evaluated in a number of studies (Kottner et al. 2021; Elban et al. 2020). These authors have reported a good agreement (ICC ranging from 0.92-0.98) between 4 different researchers when measuring at both the heel and

sacrum prior to and following mechanical loading. Accordingly, the authors suggested the use of single measurements of skin SC hydration in clinical research and practice (Kottner et al. 2021).

2.1.2.3 SUBEPIDERMAL MOISTURE (SEM)

Prior to the presence of detectable skin damage resulting from external challenges an acute inflammatory reaction occurs. The resulting disruption to the microcirculatory vessels and tissue cells causes an increase in both vasodilation and the permeability of blood vessels. Plasma leaks into the interstitial space creating localized oedema and the presence of an increased amount of sub-epidermal moisture (Bates-Jensen et al. 2017). Recently, a handheld non-invasive device, termed the subepidermal moisture (SEM) meter (Bruin Biometrics LLC, Los Angeles, California) (Figure 2.3), has been developed, which purports to be capable of detecting oedema, as well as being able to detect changes in fluid content at the skin and subdermal level (Gefen et al. 2020). The device measures the bio-capacitance of the local tissue, which is highly influenced by its water content, with an output reading in arbitrary units (A.U.). In the clinical setting, measurements involve the local site of interest as well as four adjacent sites, with the difference in values calculated as a so-called “delta value”. The manufacturers recommend that a threshold delta value of 0.6 and above is indicative of increased local oedema, erythema, and heat sensation, which are all symptomatic of early signs of skin damage (Budri et al. 2020). In a recent study, the authors reported that SEM was able to identify pressure-induced skin damage 4 days prior to its identification from the visual assessment by nurses (O'Brien et al. 2018; Moore et al. 2022). In another study using the latest model of the SEM scanner, Provizio® (Figure 3.3), was able to predict the risk of PU occurring at an average of 5 days prior to the current standard of care. However, the positive predictive value was only 14%, owing to a very large number of false-positive readings (Okonkwo et al. 2020). Earlier studies have highlighted the benefits of SEM scanner in differentiating between erythema and stage I PUs (Guihan et al. 2012; Harrow et al. 2014) and identifying stage I PUs in individuals with dark skin (Bates-Jensen et al. 2009). The use of SEM for the detection of early signs of skin damage has been reported to be time-saving and as such has been advocated for use in clinical settings (Budri et al. 2020). However, there is still little evidence to clarify how the electrical bioimpedance measurements of biological tissues relate specifically to the measure in the intracellular fluid. In addition, the considerable variation across anatomical sites, and the use of a delta threshold in arbitrary units, still need to be validated with research-based and clinical studies. Indeed, high inter-individual variability in certain anatomical sites, for example the heels, with SEM basal values ranging from 1.3-3.4 AUs, have been recently reported in a study (Jayabal et al. 2021).

Figure 2.3. SEM hand-held wireless devices. SEM scanner (left), new model Provizio scanner (right). Image from Bruin Biometrics

2.1.3 SKIN ACIDIC MANTLE (pH)

The pH of the skin is a product of both endogenous, i.e. phospholipid-free fatty acids, and exogenous i.e. sweat and sebum, factors. The main function of skin surface pH is to assist in the maintenance of the SC cohesion and integrity, participate in the regulation of epidermal barrier homeostasis and maintain microbial flora balance (Proksch 2018). The skin pH of able-bodied volunteers, which ranges from 4.0 to 6.0 (Ali et al. 2013), follows a sharp gradient across the SC, which is critical for skin renewal and enzymatic activities. As the skin acidic mantle is maintained via the cooperation of various systems, such as sweat production, degradation of sebum and cellular metabolism, pH at the skin surface is a result of the combination of the materials derived from these systems (Mauro et al. 2006). Variations in the surface pH are generally associated with the pathogenesis of skin diseases including irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and Candida albicans infections (Ali et al. 2013). Indeed, an increase in skin pH causes the swelling of the SC, thereby increasing both its permeability and the risk of bacterial colonisation, and subsequent infections (Schneider et al. 2007).

Skin pH can be measured using a universally accepted method, which consists of a glass planar electrode connected to a voltage meter. There are 4 common models of glass planar electrode devices commercially available, including models 900 and 905, manufactured by Courage & Khazaka (Köln, Germany). Each of the systems involves a probe incorporating two electrodes, a glass hydrogen electrode and a reference electrode. When the probe is applied to the skin, the concentration of hydrogen ions released from amphiphilic-free fatty acid lipids into the water is

captured (Figure 2.4). The pH detected at the skin surface differs from the intracellular pH, as the skin liquids are not purely aqueous solutions (Stefaniak et al. 2013).

The output of the skin pH-meter model 905 (Courage & Khazaka) is expressed in units of pH. The device with a planar head of probes has been reported to be reliable (estimated ICC of 0.87) (Kottner et al. 2021), with an uncertainty of ± 0.1 pH units, and allows continuous measurements without occluding the skin(du Plessis et al. 2018).

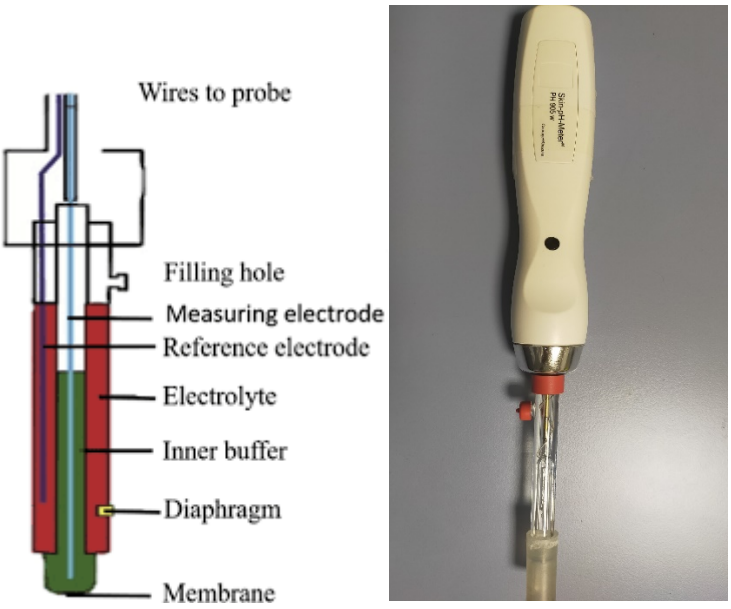


Figure 2.4. Skin pH-meter composition (left) and wireless battery-operated skin pH-meter model 905 (right). Image from Courage & Khazaha and host lab.

Various factors influence the measurement of skin pH (Ali et al. 2013; Barel et al. 2014; Man et al. 2009). These include:

- Endogenous factors, such as age, gender, ethnicity, anatomical sites, and skin health
- Exogenous factors include washing and occlusion and
- Environmental factors, such as temperature, humidity, and season

Therefore, it is necessary to follow the measurement guidelines when assessing the parameter of skin pH in clinical settings (Stefaniak et al. 2013).

2.1.3.1 THE ROLE OF THE ACIDIC MANTLE AS A PARAMETER IN SKIN HEALTH

Changes in the skin surface pH have been the focus of many studies examining the skin response to insults and diseases. As an example, the effects of the application of various structured skincare regimens were compared to routine skincare on nursing home residents affected by xerosis cutis (Hahnel et al. 2017). No significant differences in skin pH were reported between the range of the

skincare regimes. Research has shown that cleansing products can induce an increase in the pH of the skin which affects the epidermal barrier function and skin microflora (Blaak et al. 2018). In a separate study, the skin pH of able-bodied volunteers was challenged with the use of respirator protective equipment (RPEs). The authors highlighted a significant increase in skin pH following prolonged (~4 hours) usage (Hua et al. 2020)

Urinary incontinence, representing the main cause of IAD, has been reported to induce skin surface pH variations, with the presence of ammonia often resulting in a shift to alkaline levels. This inevitably disrupts the acid mantle, thereby impairing the skin barrier function by altering the local microflora and activating disruptive enzymes (Beele et al. 2018). These damaging processes will only be enhanced with the additional presence of liquid faeces containing more active enzymes active at higher pH levels (Beeckman et al. 2015; Beeckman et al. 2016). One study investigated the impact of incontinence on epithelial-moisture barrier function and the subsequent risk for IAD following the exposure of synthetic urine saturated into incontinence pads to two local areas, the perineal and perigenital, in 30 healthy volunteers. Results confirmed an increase in pH values compared to untreated baseline at all time points (Phipps et al. 2019). In addition, individuals suffering from incontinence are likely to use incontinent pads in order to wick away urine from the skin surface. However, the presence of pads creates an occlusive environment, which could result in degeneration of the SC and infiltration by other irritants and microorganisms, leading to significant changes in the skin microclimate and surface pH (Beeckman 2017). Nevertheless, a study investigating the effects of two pad regimen change on skin surface pH reported no differences when a frequent pad-changing regime was compared to a less frequent alternative (Fader et al. 2003).

2.1.4 STRATEGIES FOR THE DETECTION OF SKIN INFLAMMATION

2.1.4.1 MEASUREMENT OF MICROCIRCULATION

Inflammation represents a complex process which involves the mobilization of various immune cells, chemotactic and chemotaxis agents in order to remove pathogens and restore tissue homeostasis. The recruitment of these factors is enabled via the blood flow in the microcirculation, and thus monitoring the microcirculation is crucial for the detection of early signs of inflammation, as well as systemic pathologies and specific skin disorders (Yang et al. 2014). Various non-invasive techniques for skin microcirculation have been used in clinical research, including capillaroscopy and video microscopy, laser Doppler flowmetry, thermography, and indirect measurements of transcutaneous gas tensions (Neubauer-Geryk et al. 2019). The measurement principles, as well as the advantages and disadvantages of these techniques, are presented in *Table 2.5.*

Table 2.5. Non-invasive strategies for the assessment of microcirculation. Table adapted from (Neubauer-Geryk et al. 2019)

METHOD	MEASUREMENT PRINCIPLE	MEASURED PARAMETERS	ADVANTAGE	DISADVANTAGE
Capillaroscopy	Allows for the analysis of the morphology of capillaries and microcirculation of the nail fold	Visualization of microcirculation, for example of the nail fold	Examination in real time, non-invasive, simple and reproducible	Requires experience for image interpretation; Does not consider medications that can cause changes to the vessel
Video microscopy	Depolarization of a light during tissue penetration is collected by an orthogonal polarizer and transformed into an image	Visualization of microcirculation and evaluation of morphology and function	Image of microcirculation obtained in real time	Increased sensitivity to motion and pressure artifacts; unable to measure high blood flow velocities; Time consuming
Laser Doppler Flowmetry	Monochromatic narrow-band light backscattered due to the presence of erythrocytes	Speed and concentration of erythrocytes in the blood stream	Measures the microcirculatory flux of the tissue and fast changes of perfusion; Measures perfusion quantitatively in real time	Influence of the tissue's optical properties on the perfusion signal; Motion artifact noise; Lack of quantitative units for perfusion; Light penetrates only to a depth of 1–1.5 mm
Thermography	Correlation of body temperature and blood flow	Temperature map	Ease of measurement, low cost	Excessive pressure from the sensor can alter the blood flow
Transcutaneous oximetry	A polarographic method using electrode is heated to 44°-45°C to induce local hyperaemia	Tension of local oxygen equivalent to that in skin vessels, measures carbon dioxide tensions	The ease of measurement;	It is time-consuming taking 15 min before measurement conditions are set, needs to be repeatedly calibrated, expensive system

2.1.4.2 QUANTIFICATION OF SKIN COLOUR

The monitoring of skin colour in response to challenges has been widely adopted as an indirect indicator to evaluate the level of skin inflammation. Indeed, an increase in skin erythema or redness

is among the clinical signs which characterize the early stages of an inflammatory process (Ciaccia 2011). Accordingly, in addition to different types of smartphone-based applications (Uthoff et al. 2020) and skin imaging and analysis equipment (Anqi et al. 2022), various non-invasive colorimetric devices have been developed for the monitoring of skin colour and its modulation under the influence of various types of stimuli. The technology underpinning the functioning of these colorimeters is based on scanning reflectance spectrophotometry, while others rely on tristimulus colorimetry and on narrow-band simple reflectance colorimetry (Ly et al. 2020). This colorimetric technique forms the basis of a number of commercial systems, which have been widely used in skin health research.

2.1.4.3 MEXAMETER®

An example of a widely adopted colorimeter is the Mexameter system manufactured by Courage & Khazaha (Germany), whose latest model (MX 18) has been used to measure skin erythema as well as the melanin content. Its measurement principle is based on the absorption and reflection of three specific light wavelengths emitted by the device in contact with the skin. The light reflected by the skin is collected by a detector, which can calculate the amount of light absorbed as a result of the difference between emitted and reflected light (Figure 2.5). Two specific wavelengths (green: 568 nm, red: 660 nm) are used, corresponding to the spectral absorption peak of haemoglobin. Analysis of reflected light from wavelengths in the red and near-infrared provides values of skin melanin content (Clarys et al. 2000; Baquié et al. 2014). The probe has been reported to allow easy and quick measurements. It can be employed on all body sites with a measurement uncertainty of ±5% according to the manufacturer. Its outputs are expressed in arbitrary units (A.U.). The device, however, has been reported to be easily influenced by the skin pigmentation status and its output less reproducible compared to other colorimetric devices (Baquié et al. 2014)

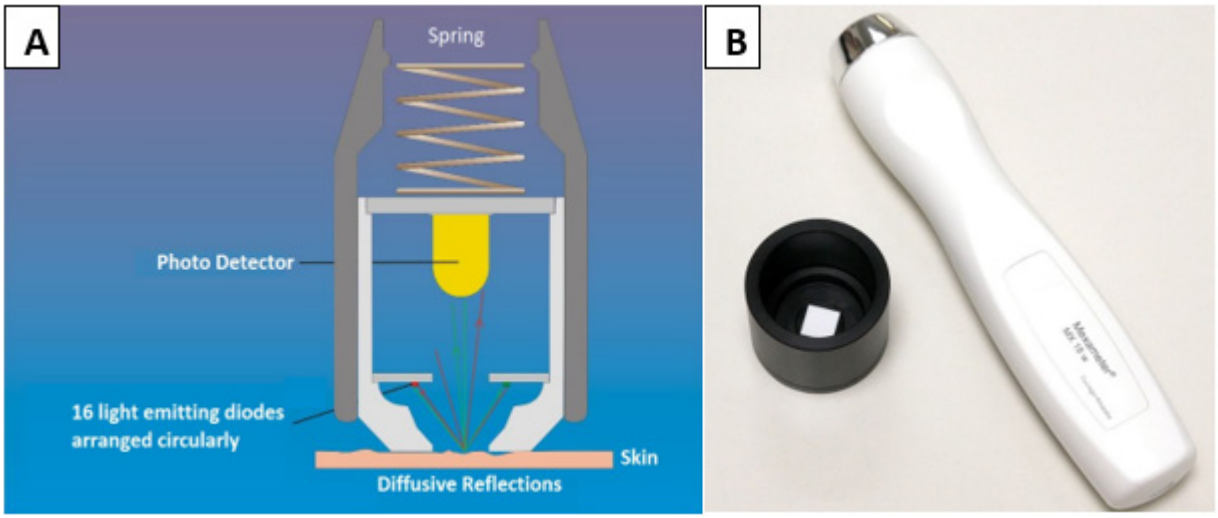


Figure 2.5. A) Measuring principle of Mexameter and B) wireless Mexameter MX 18. Image from Courage & Khazaha.

Mexameter has been employed in several studies to examine changes in skin colour following external insults. As an example, it was used in a randomised trial to investigate the treatment efficacy of two different creams in the treatment of acanthosis nigricans (Treesirichod et al. 2021). It has also been used to determine the anti-erythema effects of aloe vera solutions on irritated skin areas (Fox et al. 2014).

The measurement of skin redness by the Mexameter system has also been used in the diagnosis and classification of PUs. For example, its measure of skin erythema was compared to both the consolidated Norton risk assessment scale and objective biophysical parameters at PU-affected areas in a cohort of geriatric participants. The study showed a statistically significant correlation of erythema with PUs at the sacrum ($p < 0{\cdot}05$) when this parameter was assessed objectively (Borzdynski et al. 2016). In a separate study, small but reproducible changes in skin redness were reported at the sacrum following 30 minutes of lying in a supine position by healthy volunteers on a standard hospital bed (Scheel-Sailer et al. 2015). Enhanced skin erythema levels, measured with Mexameter, have also been reported in a study evaluating the impact of N95 face protectors on facial skin health (Hua et al. 2020).

Although it is encouraging to note the increasing number of healthcare professionals and industries engaging in the development of new technologies for assessing the skin integrity of at-risk patients, nonetheless, it is imperative to perform evidence-based research pertaining to acquiring in-depth knowledge of these devices. Indeed, as warned by Professor Bader in his last editorial, “The last hurrah”, it is critically important to assess the robustness, accuracy, and sensitivity of the threshold values of novel technologies prior to their acceptance in clinical practice (Bader 2022).

2.2 GENERAL RESEARCH QUESTIONS

The research questions addressed in this PhD project are as follows:

1. Can parameters from biophysical measurements, differentiate between different skin damage mechanisms?
2. Do extrinsic and intrinsic factors affect the output of biophysical skin parameters?
3. Are biophysical parameters sensitive in differentiating spatial changes in skin health?
4. Can biophysical parameters detect temporal changes in skin health?

2.3 AIMS OF THE RESEARCH

This PhD programme adopted an exploratory research approach with the aim to evaluate the ability of biophysical tools to detect early signs of skin damage, achieved through a series of studies including analysis of secondary data, lab-based controlled studies, longitudinal evaluation of skin changes during COVID-19, and a cohort patient study in individuals presenting with stage I PUs. To achieve this aim, a series of objectives have been defined to guide parallel studies on a range of healthy cohorts, HCWs and patient groups. Given the exploratory nature of the thesis, hypothesis will not be stated but developed following the outcome of this exploration.

2.4 RESEARCH OBJECTIVES

The objectives of this research project are:

1. Analyse retrospective data sets of biophysical skin parameters exploring different skin insults and anatomical locations on healthy volunteers (Chapter 4).
2. Design and implement lab-based studies to explore the effects of incontinence pads and pressure exposure on the biophysical skin response during damage and recovery (Chapter 5).
3. Survey healthcare workers who are at risk of skin damage from personal protective equipment to evaluate the frequency of reported skin reactions (Chapter 6).
4. Recruit a cohort of healthcare workers to translate biophysical tools to monitor temporal changes in skin health following prolonged respirator protective equipment application (Chapter 7).
5. Design and implement a longitudinal cohort study of patients with grade I pressure ulcers, to examine the spatial and temporal changes in skin health (Chapter 8).
6. Collaborate with other doctoral fellows to explore other analytical techniques to characterise skin health including non-invasive biochemical markers (inflammatory proteins) and skin cell changes (Chapter 9, discussion).

Chapter 3 GENERAL METHODOLOGY

One of the primary goals of this thesis was to identify non-invasive methods to detect the early signs of skin damage indicative of PUs or IAD. This required the adoption of a multiphase approach, which included retrospective analysis of established data sets, subjective qualitative surveys, lab-based experiments on healthy cohorts and a patient study exploring skin parameters over the site of damage. This approach was employed to examine the skin response to a number of different external insults including prescribed mechanical loading, exposure to moisture irritants and following the application of functional medical devices attached to the skin. Subsequently, the spatial and temporal changes in the skin status of individuals presenting with early signs of damage e.g. non-blanching erythema were assessed. In order to translate the measurements to a clinical setting, the biophysical parameters were taken from CE-marked dermatological tools, which were non-invasive in nature, and enabled the estimation of a range of skin parameters. These included trans-epidermal water loss, erythema, skin acidity, and skin hydration levels.

The original plan for the project was to perform a series of lab-based and clinical studies, pertaining solely to PU and IAD detection. However, due to the occurrence of the Coronavirus Pandemic, several government-driven restrictions on human testing resulted in a modified research plan to accommodate available data as well as research projects allied to those working in healthcare settings during the pandemic. For example, it soon became apparent that the continuous and prolonged PPE usage by healthcare workers (HCWs) in managing Covid-19 patients resulted in adverse reactions to facial skin sites, thus providing the motivation to monitor early signs of skin damage following periods of exposure to PPE devices. Therein, as part of the PhD programme, the methodologies were also translated to examine skin changes in HCWs.

Accordingly, distinct studies were designed to establish the sensitivity and specificity of these tools, with their associated objective parameters, to detect early changes in skin health over a variety of pre-and post-skin damage models. These are described separately in chapters 4 – 8 and summarised in Figure 3.1. Prior to commencing with the prospective studies, analysis of the results from retrospective data was performed to better understand skin responses following a series of standardised skin insults namely;

- mechanical loading,
- tape-stripping,
- overhydration and
- chemical irritation.

Data collection for this study was conducted prior to the Pandemic by an external researcher (Professor Barbara Bates-Jensen). Subsequent data analysis and manuscript drafting were, however, performed by the doctoral fellow together with another colleague from the host lab. The results from which informed the design of future studies.

To address some of the limitations of the retrospective data, further lab-based studies within the host facility were performed monitoring skin parameters from able-bodied volunteers following more clinically relevant insults including moisture exposure, occlusion with incontinence pads and mechanical loading in the form of high sitting (Chapter 5).

The lab-based project was followed by a pre-clinical study of PPE-related skin reactions in HCWs. This involved a sequential design, which started with a survey pertaining to the nature, frequency and anatomical sites of adverse skin reactions following the use of PPE (chapter 6). The questionnaires were disseminated to HCWs at three different UK NHS Hospitals Foundation Trusts, in collaboration with NHS England and Improvement. Following the survey, which confirmed a high prevalence of skin reactions under PPE, the facial skin health of healthcare professionals was assessed using the array of biophysical tools, to evaluate skin response pre- and post-RPE application. This included measurements of skin biophysical, biomarker and cell parameters. The latter two elements of the study were conducted with colleagues from the European Network 'STINTS'. The final clinical study was conducted on patients admitted to the University Hospital Southampton NHS Foundation Trust, who presented with signs of skin damage identified as stage I PUs (i.e., non-blanchable erythema). This study examined the potential of biophysical tools to monitor the spatial and temporal changes in both damaged and adjacent skin sites. Relevant findings from the study are reported according to the STROBE method statement. The knowledge acquired from the findings of these studies was necessary to establish sensitive skin parameters, with the potential to detect early signs of skin damage and provide clinicians with optimal prevention strategies that match individual requirements i.e. a personalised preventative approach.

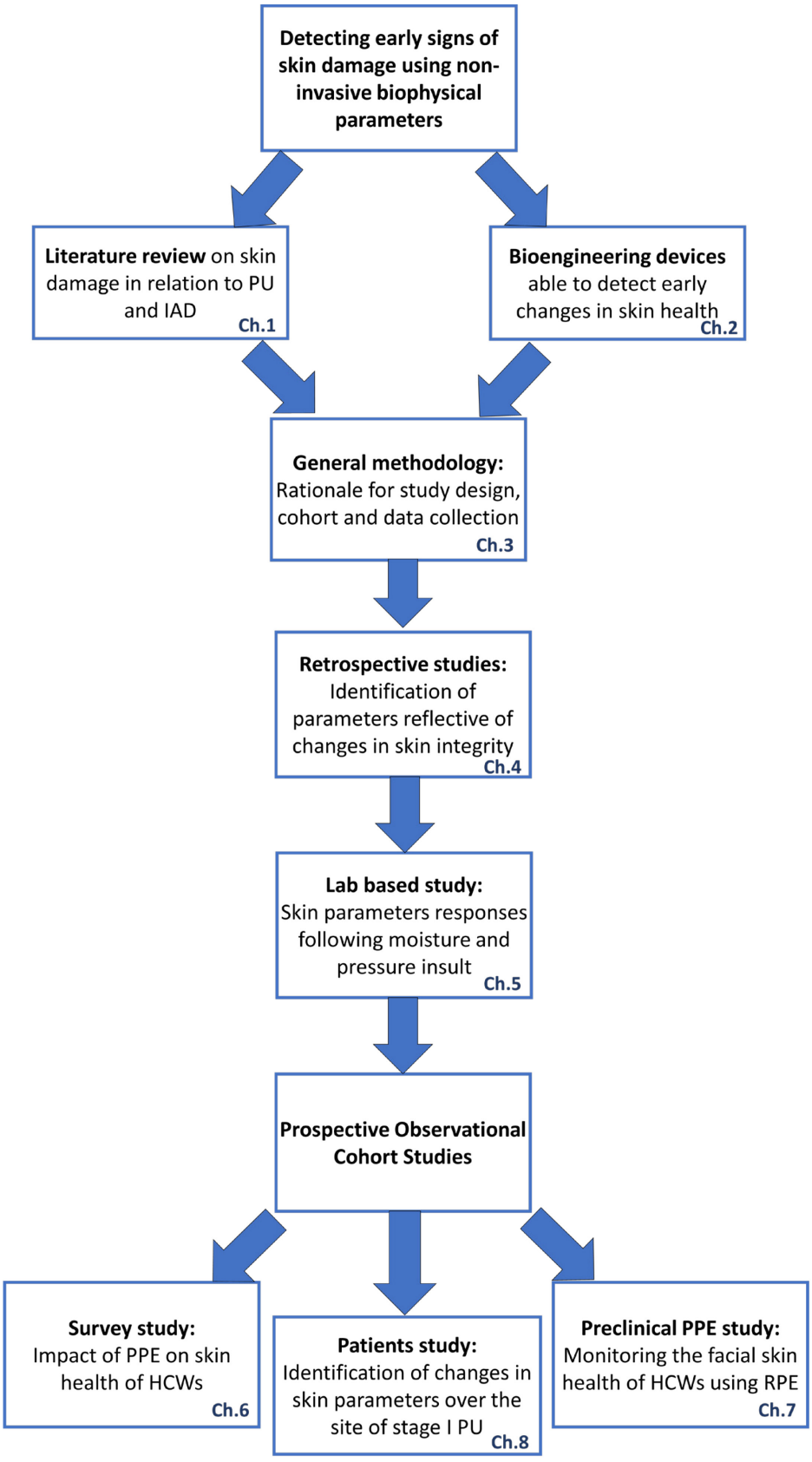


Figure 3.1. Schematic of studies aligned with the research questions and objectives of the current thesis.

3.1 FACTORS CAUSING CHANGES IN SKIN HEALTH AND RATIONALE FOR INVESTIGATION

Different individuals, presenting with a range of ages, genders, ethnicities and body types were recruited into the studies and their skin either was manifesting signs of early damage i.e., stage I PU, or was challenged with external stimuli, such as exposure to chemical substances and/or mechanical loading, and occlusion with medical devices (Figure 3.2). Indeed, this range of challenges has been reported to compromise skin health following different pathways (Bouten et al. 2003; Hoogendoorn et al. 2017). Among these pathways, ischaemia, generated as a consequence of impaired blood flow due to prolonged application of pressure, has been well-established to be responsible for the development of PUs (chapter 1, section 1.3.1.3). An approach involving the use of weighted-circular cylinders as a source of pressure on the skin has been employed in the host lab to explore changes in skin parameters (Figure 3.2 A) (Bostan et al. 2019; Soetens et al. 2019). Nonetheless, more research is needed to elucidate changes in biophysical parameters when the skin is subjected to clinically relevant pressures. Another source of pressure can result from the prolonged application of medical devices (chapter 1, section 1.2.1.3). This was evident with HCWs wearing respirator protective equipment (RPE), in the form of FFP3 respirators, during the pandemic as a protective measure against the COVID-19 virus. Indeed, such devices need to be tightly attached to the skin surface, which compresses the skin at the device interface. Under this condition, the skin is prone to breakdown due to various activities occurring at the epidermal-dermal level, including poor local blood circulation, tissue ischemia and hypoxia (Zhou et al. 2020). In addition, the skin is further compromised by moisture as a result of a large amount of water vapor exhaled from the mouth and nose, which keeps the facial skin in a moist environment for a long period and reduces the ability of the stratum corneum to resist external pressure and shear forces (Kottner et al. 2018). Furthermore, the frictional forces between the skin and the respirator device can induce the development of surface erythema, blisters, and skin tears. As such, HCWs employing face protectors are at high risk of acquiring PUs and moisture-related skin damage, hence changes in their skin properties need to be investigated (chapter 7).

When skin is exposed to moisture in the form of urine, IAD can develop (chapter 1, section 1.2.2). This is mostly observed in the geriatric population, who suffer from incontinence episodes and might present with mobility issues, which require them to sit or lie for a prolonged time (Beeckman 2017). In order to maintain skin health, it is imperative to transport moisture away particularly from loaded skin sites with the use of absorbent products. The latter, however, can enhance the risk of developing PUs and IAD as a result of occlusion, and variation in the microclimate at the skin-product interface (Falloon et al. 2018). In recent years, Industries have invested in the innovation of the design of incontinence pads to achieve a “breathable” skin-friendly interface. These products

claim to maintain the skin in a healthy state while transporting moisture away from loaded skin sites. Therefore, evidence-based research is needed to examine skin responses to these pads both in moistened and dry conditions, as well as in the presence and absence of applied pressure (chapter 5).

In addition to pressure and moisture, shear and frictional forces can also be applied to the skin of patients who are required to sit or lie for prolonged periods due to limited mobility. These forces are known to contribute to changes in the skin barrier properties. Common strategies used in a lab setting to mimic friction and altered barrier functions are tape stripping and exposure to Sodium lauryl sulfate (SLS). The former is a minimally invasive procedure, which has been universally validated for the subsequent removal of the SC via the adoption of adhesive tapes. The latter is known for causing skin discomfort, which can lead to irritation and skin water loss. These strategies provided means to evaluate variations in skin parameters in a cohort of healthy volunteers (chapter 4). Furthermore, regardless of the different skin assessment tools used in acute care settings, clinicians still face various difficulties in identifying patients at high risk of developing pressure ulcers. Many reasons could be associated with this phenomenon, the most prominent of which include lack of sensitivity and specificity of the assessment tools employed, the subjective nature of the assessment scales and the inability to clearly identify skin surface redness in patients with darker pigmentation. It is therefore critical to offer caregivers objective parameters able to differentiate, at an early stage, between healthy and damaged skin in hospitalised patients (chapter 8).

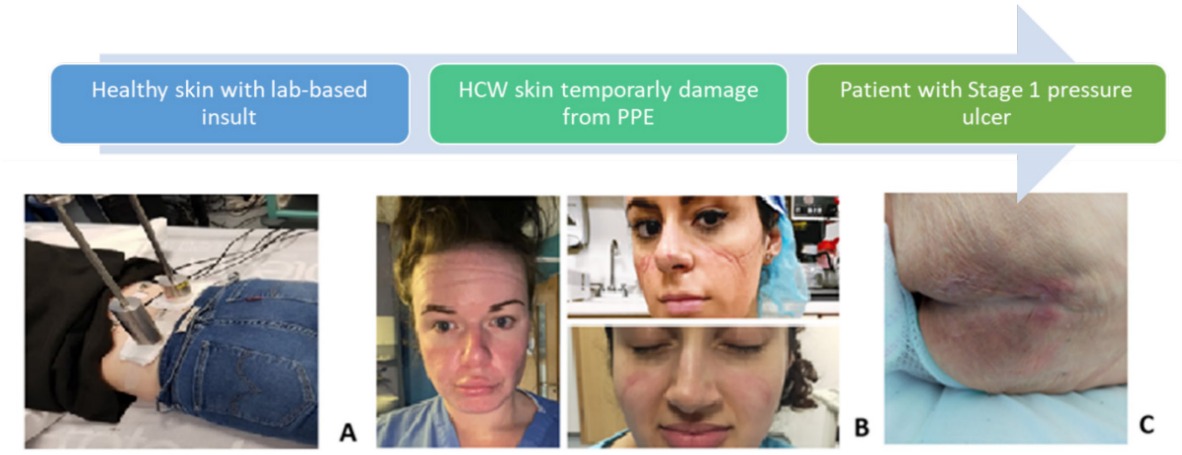


Figure 3.2. Examples of skin damage and factors responsible for skin compromise. (A) Cylindric weights applied to the sacral skin of a participant to mimic pressure by a support surface. (B) redness blanching and indentation mark caused by prolonged wearing of RPE. (C). stage I PU (non-blanchable erythema) as a result of prolonged lying in bed.

3.2 JUSTIFICATION OF STUDY DESIGNS AND COHORTS

Except for the retrospective study, observational cohort studies were used as an established method in the present thesis. Although clinical research has been long dominated by randomised control trials (RCTs), considered to be the gold standard for effective research (Hariton et al. 2018), it was not appropriate for the aims of the thesis (section 2.3). Indeed, well-designed observational studies have been shown to provide reliable results which are similar to those of RCTs (Benson et al. 2000). Observational studies present different advantages compared to RCTs namely, lower cost, greater timeliness and a wider population of participants. These studies are particularly useful for the identification of risk factors and prognostic indicators, as well as utilised in studies where it would be impossible to employ RCTs (Benson et al. 2000). In addition, with observational studies, the researcher does not intervene, and rather "observes", records and investigates the strength of the relationship of the exposure with the disease variable (Timmreck 2002). In addition, the prospective nature employed in this thesis together with case-controlled inputs confer the studies the ability to demonstrate temporality and therefore identify true risk factors and not associated factors with the condition investigated (Maclure et al. 2000). This was supported by a case-controlled trial design, where individuals acted as their own controls for spatial and temporal changes in skin health (McCalden et al. 2011; Björnsdóttir et al. 2005). Observational studies have been adopted also for the development of diagnostic and monitoring tools (Colli et al. 2014), as well as to examine the accuracy of diagnostic procedures and the comparisons between different diagnostic measures (Takwoingi et al. 2013; Wade et al. 2013). For example, this study design approach was used for the design and validation of a diagnostic tool used for a systematic assessment of IAD in clinical practice (Beeckman et al. 2018).

Nevertheless, findings from observational research have been often criticised for being easily influenced by unpredictable confounding factors, defining this research method among the lower positions in the hierarchy of evidence-based research strategies (Song et al. 2010).

In the present thesis, an observational cohort approach was selected as the optimal study strategy to meet the aims and objectives, as well as answer adequately to the research questions presented in chapter 2, section 2.2

With respect to the cohort of participants enrolled in the various studies, these were recruited by employing strategies involving the use of poster advertisements and gatekeepers. In addition, given the practical considerations of recruitment and the conditions surrounding the Pandemic, no sample size calculation was performed for all the studies, except for the study involving hospitalised patients (chapter 8), and each individual served as their own control. However, a convenient sample size of between 10 to 20 participants was selected to ensure that each study had acquired sufficient

and meaningful data. Similar cohort sizes have been previously used in other research to provide data with statistical significance (Hua et al. 2020; Kottner et al. 2015)

Differently from the other studies, the patients' study (chapter 8) was designed to recruit a sample size of 50 participants over a 12-month period, with the assumptions of a 10% recruitment rate and a 10-15 sample withdrawal to follow-up. In addition, a further sample size power calculation was performed based on a 50% change in transepidermal water loss between skin sites representing an indicator of a clinically meaningful effect (chapter 8, section 8.3.3).

In all the studies, a standardised governance process was followed through institutional ethics and/or HRA approvals. Details of the different ethics are present in each results chapter with corresponding approval letters in the appendix. Recruitment was undertaken at a local institutional level, at hospital adopter sites and through word of mouth. Each individual who expressed an interest in participation was provided with a Participant Information Sheet (PIS) via email or by the study coordinators, allowing them at least a period of 24 hours to make a decision about their participation in the study. Informed consent was received directly from the participant via email or by the researcher on the first screening assessment, and a copy of the signed informed consent sheet was provided to the participant. It was made clear to all participants that participation was entirely voluntary, and that each participant had the right to opt-out of the study at any time without giving any reason.

3.3 DATA COLLECTION AND PROTECTION

Participant data were anonymised, via a unique identifier in order to remove any traceable information. Demographic and anthropometric data were also collected from all the participants. These included age, gender, ethnicity, height, weight, body mass index (BMI), and any other information pertaining to factors which were of relevance to the specific study e.g. working hours of HCWs, type of PPE kit used, location of erythema, etc. In some studies, photographic images of the skin sites under investigation were also collected, with appropriate permission. All acquired data were protected in compliance with the General Data Protection Regulation (GDPR) 2018. Data collection for the studies involving biophysical assessment of the skin ranged approximately between 10 to 60 minutes for each session, depending on the number of skin sites investigated.

3.3.1 BIOPHYSICAL MEASUREMENTS OF SKIN INTEGRITY

To investigate the integrity of the skin barrier at the investigation sites, various non-invasive biophysical techniques were adopted (chapter 2, section 2.1). An array of parameters was estimated from the measurements of Transepidermal water loss (TEWL), skin surface pH, erythema level, Stratum Corneum (SC) hydration, sub-epidermal moisture and the sampling of skin sebum

(which is beyond the scope of the current thesis). Each technique was adopted according to published guidelines (du Plessis et al. 2013; Stefaniak et al. 2013) and used following specific assessment orders (Kottner et al. 2014). Each parameter will be discussed separately.

Transepidermal Water Loss (TEWL) (Figure 2.1) was assessed using an open-chamber method (Tewameter TM 300, Courage & Khazaka, Germany), measuring the amount of water that passively diffuses through the skin. TEWL was measured by placing the device in gentle contact with the skin for 1 minute and the parameter was determined as the mean of the last 10 TEWL measurements, recorded when a period of equilibrium was achieved. TEWL values are expressed in grams of water per square meter of skin per hour ($g/h/m^2$).

Studies have reported that TEWL values can be influenced by various factors (Table 2.2) including the ambient condition. In the thesis, humidity and the temperature of the testing environment were controlled for the majority of the study (except for the patient's study described in chapter 8). However, the temperature of the skin surface during the assessments was not measured. It is worth noting that the Tewameter TM300 is capable of measuring skin surface temperature during measurements. However, these values were not taken into account during data analysis. Indeed, in this thesis, a pragmatic approach was used to minimise the influences of external factors. It is of note, that for all study designs, multiple measurements were taken at each time points (on two or more sites of the skin), thus making values comparable and equivalent in environmental conditions. This case controlled, time dependent approach mitigated any effect of temperature or humidity.

Skin surface pH & Erythema (Figures 2.4 & 2.5) were measured using two distinct probes, namely, a pH meter (PH 905, Courage & Khazaka, Germany) and Mexameter (MX18, Courage & Khazaka, Germany), respectively. As previously reported in chapter 2, the pH meter measurement is based on a combined electrode, where both glass H^+ ion-sensitive electrode and additional reference electrode are located in one housing. The Mexameter is based on the principle of absorption/reflection of different wavelengths of light. The outputs from both devices were obtained with gentle contact on the skin, and an average of 5 repeated measures was recorded. Both parameters are reported in arbitrary units (A.U.).

Stratum corneum (SC) hydration (Figure 2.2) was determined using a Corneometer (CM 825, Courage & Khazaka, Germany). The device measures the variations in the dielectric properties of the skin due to changes in surface hydration. SC hydration is represented as the average of 5 repeated measures when the device is placed in gentle contact with the skin and is expressed in A.Us.

Sub-epidermal moisture (SEM) (Figure 2.3) was measured using a SEM scanner (Bruin Biometrics LLC, Los Angeles, California). SEM was recorded at five points on and around each test site using a standard protocol (Gefen et al. 2020). This included one located directly over the test site and four other sites ~10 mm from the test site at 90° intervals aligned with the body axis (cranial, right, caudal and left). Pressure was applied at an optimal level indicated by the SEM device, prior to taking a reading. This device converts the bio-capacitance of soft tissues into arbitrary units (AUs) to establish a delta between adjacent skin sites. In particular, an estimated SEM delta was calculated by subtracting the average of four circumferential measurements from the value at the test site. A SEM delta value of 0.6 has been recommended by the manufacturer as a threshold indicative of tissue damage. It is of note that SEM was only adopted with only retrospective studies (chapter 4).

Scanning Laser Doppler Imaging (LDI) (SLDI, Moor Instruments Ltd, Axminster, UK) incorporating a helium-neon red gas laser (wavelength, 635 nm) was utilised to measure skin blood flow. The device was implemented over the local test sites, corresponding to 100 × 150 mm (252 × 368 pixels) scans, sampled at 4 ms/pixel. Both greyscale and flux images were stored to identify the test sites, and the LDI data were analysed offline using the manufacturer's software package. Key parameters from the images included the mean and standard deviation of the flux values recorded in arbitrary units (A.U.) Similar to SEM device, LDI was employed only in one of the retrospective studies (chapter 4).

Interface pressure

The monitoring of the distribution of pressures has become a common practice in both clinical and lab-based settings. Different commercial sensors have been developed to estimate these distributions at the interface between the individual and the support surface. The functioning of these sensors is based on the principles, including electronic, which involve capacitive and resistive sensors, pneumatic and electro-pneumatic (Gyi et al. 1998). These systems have been employed in a large number of studies to assess the effects of positioning and the performance of support surfaces, with the outcome parameters generally involving peak pressures, peak pressure index (PPI), defined as the peak pressure values averaged over a limited number of sensors, mean or median pressure values and contact area in specific regions of interest. An advantage of these systems is the provision of real-time images of the pressure distribution which is displayed in the form of a colour map, where low pressures are represented by cold (or blue) tones and higher pressure values by warm (or red) tones. In this thesis, interface pressure measurements were recorded using a full-body pressure monitoring system (ForeSite PT, XSENSOR Technology Corporation, Canada), in the form of a fitted mattress cover. It incorporates 5664 pressure

measuring sensor cells, with a spatial resolution of 15.9 mm, covering a sensing area of 762 mm x 1880 mm. Each sensor operates within a pressure range of 5 – 200 mmHg (0.7 – 26.6kPa), with an accuracy ± 2 mmHg and an acquisition rate of 1 Hz. The system provides a series of parameters which are exported using dedicated software (ForeSite PT Analyser and ProV7, Xsensor, Canada). For each frame of the acquisition, specific parameters included the mean and peak pressures, contact area and coordinates of the centre of pressure with respect to a direction both parallel and perpendicular to the long axis of the mat.

3.4 PRINCIPLES OF STATISTICAL STRATEGIES

Given that one of the main objectives of the thesis is to explore the potential of biophysical tools to detect minimal changes in skin parameters both in lab-based environmental and in acute care facilities following various challenges, specific statistics were employed to address spatial and temporal trends in skin health. Indeed, this strategy enabled the use of different research methodologies within the thesis, consisting of primary (chapters 5, 6, 7 and 8) and secondary (chapters 1, 2 and 4) research. There are several advantages of exploratory research which can make their results more compelling than those of confirmatory studies. Indeed, this strategy presents with numerous advantages, namely: avoidance of researcher's prophecy biases, facilitate inference to the best explanation, and allows peer reviewers to make additional contributions at the data analysis stage. In addition, an exploratory research, if conducted in an adequate manner, possesses the ability to lay a strong foundation for future studies (Rubin et al., 2022).

In the present thesis, given the exploratory nature of the studies, the doctoral fellow was mainly interested in finding and analysing trends within the data, supported, but not defined, by the p values from statistical tests. Indeed, p value is formulated based on the concept of probability and it measures the likelihood of any observed difference between two groups to be due to chance. As it is a probability, P is associated with values ranging from 0 to 1. While values close to zero indicate that the observed differences are unlikely to be a result of chances, values close to 1 signify that there are no differences between the groups other than due to chance. In scientific research, it is common for researchers to state whether the tested hypothesis is significant (defined as $p<0.05$) or not significant ($p>0.05$) and as such limit their conclusions based on this dichotomous category. Nevertheless, this approach of whether to decide a test of hypothesis is significant or not based on the p-value has generated an intense debate among statisticians. Thus, in the present thesis the reporting of data were statistical tests yield non-significant p-values was included. This is particularly the case for the lab-based observational studies, where no formal power calculations were included. By contrast, a power calculation was performed for the patient cohort study (chapter 8). This was performed in order to recruit the optimum number of patients for the most

ethical trial design and was a prerequestate to achieving IRAS ethical approval. We chose the most sensitive parameter, TEWL, for this estimation due to a greater confidence in the potential effect size and confidence intervals.

In addition to the statistic test utilised in the thesis, a multiple comparison analytic approach could have been adopted. Indeed, these are statistical tests that are used to compare multiple groups or variables simultaneously. Multiple comparison analyses are also employed to identify differences between multiple groups, to identify which variables are associated with a particular outcome, or to identify patterns or relationships between variables. One common method of multiple comparison analysis is the Bonferroni correction, which adjusts the significance level to account for the increased risk of type I error when multiple tests are performed. The Bonferroni correction involves dividing the significance level (e.g., 0.05) by the number of tests performed, which results in a more stringent threshold for statistical significance. Another method of multiple comparison analysis is Tukey's test, which compares all possible pairwise comparisons between groups and adjusts the significance level based on the number of comparisons performed. Multiple comparison analyses can be a useful tool in research, but it's important to use them appropriately and interpret the results cautiously. Indeed, due to their nature to increase the risk of type I error, researchers are required to adjust the significance level accordingly and consider the practical and clinical significance of the observed differences.

3.5 HEALTH AND SAFETY ISSUES

Given that the clinical studies took place during the outbreak of the Coronavirus pandemic, procedures had to be established to ensure the safety of both the participants and the researcher. Accordingly, during data collection, in order to minimise the contact between participant and researcher, Standard Infection Control policy and institutional method statement for conducting human research were followed. This involved the researcher being regularly tested for COVID-19 for all the studies involving face-to-face contact. In addition, the researcher wore a long-sleeved disposable fluid-repellent gown (covering the arms and body), a filtering face respirator mask, eye protection and disposable gloves. Furthermore, his hands were washed and sanitised before and after assessments following the NHS Hand Hygiene Policy. All devices used during the studies were thoroughly cleaned prior to and post-assessment according to the NHS infection and prevention policies. In addition, if requested by participants, personal protective equipment (PPE) was provided consisting of a pair of disposable nitrile gloves and a surgical face mask. Each participant was also requested to wash or apply alcohol-based hand sanitizer prior- and post-PPE usage. Furthermore, they were assisted to follow the correct donning, doffing and disposal of the PPE. A distance of 2 metres was observed at all times during data collection, with the exception of the period of skin assessment. The setting allocated for data collection was thoroughly cleaned after

each test session to minimise the risk of contamination. Besides the participant and the researcher, no other individual was allowed into the assessment setting during the sessions. Participants were advised that they should be removed from the study if they felt any of the common symptoms, during the period (2020 - 2022), associated with COVID-19, namely, loss of smell and taste, temperature or a continuous dry cough. The health of the researcher was also monitored and was advised to suspend the study immediately if any of the aforementioned symptoms were present.

Chapter 4 SELECTION OF BIOPHYSICAL SKIN PARAMETERS USING RETROSPECTIVE DATA ANALYSIS

Prior to commencing with the lab-based studies (chapter 5), the results of two previous studies using various biophysical tools were comprehensively analysed to determine the value of a selected array of biophysical parameters. Indeed, in the last decade, an increasing volume of literature has adopted non-invasive tools to examine differences in skin responses following various types of external challenges (chapter 2, section 2.1). Despite the claims of being more accurate in detecting small variations in skin status and, as such, more reliable compared to consolidated hospital skin assessment scales (Borzdynski et al. 2016), the translation into acute care settings has been limited. There are many reasons for this including:

- the unknown quantification of these biophysical tools and associated parameters, either alone or in combination, to detect and predict early skin damage
- a lack of understanding regarding the specificity of these parameters to distinguish between various skin insults and damage mechanisms.
- the arbitrary nature of many of their output parameters, with the exception of TEWL
- the various extrinsic, intrinsic and environmental factors which have been suggested to influence their output parameters, but are poorly understood (discussed in Table 2.2)

Some of these issues were highlighted in a recent review, where the authors detailed the need for more evidence-based data to evaluate the ability of bioengineering tools to distinguish between mechanical, chemical and environmental challenges (Bader et al. 2018).

Although there are many studies examining the implication of intrinsic factors (age, gender, anatomical locations, etc) on skin response, there are still uncertainties about their influences on skin parameters. This motivated the design of two studies in the host lab, focused on achieving an improved understanding of skin behaviour following insults. Both studies involving a cohort of healthy participants were granted University of Southampton Institutional ethics (ERGO-FOHS-26040). Informed consent was obtained from each participant in each cohort prior to testing. Data collection was performed by an external researcher, as part of a secondment to the host lab, as previously stated (chapter 3), while analysis and manuscript drafting were carried out by the research fellow together with another early-stage researcher (Hemalatha Jayabal), part of the same doctoral fellowship programme.

4.1 INVESTIGATING THE ABILITY OF BIOPHYSICAL PARAMETERS TO DETECT CHANGES IN SKIN STATUS FOLLOWING MECHANICAL AND CHEMICAL INSULTS.

The results of this study have been published in the journal of Clinical Physiology and Functional Imaging:

*Jayabal, H., Bates-Jensen, B.M., **Abiakam, N.S.**, Worsley, P.R. and Bader, D.L. (2021). The identification of biophysical parameters which reflect skin status following mechanical and chemical insults. Clin Physiol Funct Imaging, 41: 366-375. https://doi.org/10.1111/cpf.12707*

4.1.1 INTRODUCTION

As previously stated in section 1.2.1.2, during their hospital stay, the skin of patients can be subjected to a multitude of insults which include mechanical i.e. pressure, friction and shear forces and non-mechanical e.g. moisture, chemicals and enzymes, in the form of body waste products. The prolonged presence of these insulting agents will inevitably lead to the development of skin damage. Indeed, individuals with impaired mobility and incontinence are more likely to develop chronic wounds such as pressure ulcers (PUs) and incontinence-associated dermatitis (IAD). These wounds represent a major financial burden to healthcare providers (Table 1.2), in addition to their detrimental effects on individual quality of life. In order to mitigate the formation of chronic wounds, individuals are regularly examined and evaluated, and the necessary preventive measures are implemented in a range of acute and community care settings (section 1.4). As conventional skin assessment strategies do not offer reliable means of identifying the risk of acquiring skin damage at an early stage, there is a compelling need to identify a set of robust parameters that clearly differentiate the variable nature of insults.

4.1.2 AIM OF THE STUDY

Accordingly, this study was designed to examine the tissue response using an array of biophysical techniques, following exposure to a range of insults mimicking clinical situations in a cohort of able-bodied volunteers.

4.1.2.1 STUDY OBJECTIVES

The objectives of the study include the followings: -

1. Recruit a number of able-bodied volunteers cohort, of different ages, gender and body sizes.
2. Challenge participants' forearm skin with different insults ranging from mechanical loading to chemical irritation.

3. Perform data collection at different intervals involving biophysical measurements
4. Identify temporal changes in skin physiology, indicative of changes in barrier function both during insult and subsequent recovery.
5. Examine possible associations between participants' intrinsic factors with the parameters under investigation.

4.1.3 MATERIALS AND METHODS

All the measurements were carried out in the Biomechanics Testing Laboratory in the Clinical Academic Facility in Southampton General Hospital, with the environment controlled at a temperature of 23± 2°C and a relative humidity of 42 ± 6%. Exclusion criteria included a history of skin-related conditions or neurological or vascular pathologies that could affect tissue health. The study received the University of Southampton ethics approval, as stated above and participants were required to sign and date a consent form prior to commencing the assessments.

4.1.3.1 TEST PROTOCOL

Participants were requested to wear comfortable loose-fitting clothing and attend data collection sessions on two consecutive days. Each of the measurements was taken with the participant in a sitting position, with their arms resting at chest height on a table surface. Forearm was chosen as the site of investigation owing to its ease of accessibility. The volar aspect of each forearm was marked with three assessment zones of 20 mm diameter separated by at least 20 mm. Four separate insults were imposed on the test sites to simulate real-life conditions, while two sites were used as negative controls (Figure 4.1a). Tape stripping (n=40), which represents a minimally invasive method that removes the skin layers, was used as a positive control as in previous studies (Koppes et al. 2016; Bostan et al. 2019; Koudounas 2019). Chemically-induced irritation was simulated by applying a surfactant, sodium-lauryl-sulphate (SLS) at 0.5% concentration, to the skin surface. SLS has been used to simulate chemical irritation of the skin, for example, in the case of contact dermatitis (Tupker et al. 1997; Schnetz et al. 2000; Koudounas 2019; di Nardo et al. 1996). Mechanical loading and moisture in the form of a water-saturated pad, simulating sitting/lying and over-hydration conditions, respectively, represented the other two insults. In the first session, the six sites were measured at baseline (-24 h) using the four separate measurement techniques. Following the baseline recordings, insults were applied to two skin sites, namely:

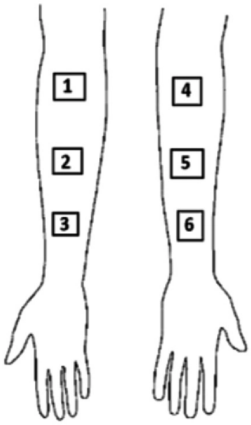
- Site 1 – Saturated water pad (ø 20mm) worn for 24 hours
- Site 4 – Saturated pad (ø 20mm) containing sodium lauryl sulphate (SLS) solution, worn for 24 hours

In the second session, insults were applied to the two other sites, namely:

- Site 3 - Mechanical loading equivalent to a pressure of 11.0 kPa (80 mm Hg) was applied using a circular indenter of diameter 42.4 mm with an integrated load cell which constantly monitored the loading over a period of 30 minutes
- Site 6 - Tape stripping 40 times using medical tape

Immediately following the removal of each skin insult, a second reading was taken for each of the measurement tools (0 h), and subsequent measurements were repeated at 1 hour (1 h) and 3 hours (3 h) to monitor recovery characteristics. A schematic of the test protocol is illustrated in Figure 4.1b

(a)



Site	Insults
1	Saturated water pad (24 hours)
2	Control site 1
3	Mechanical loading (11 kPa – 30 minutes)
4	Saturated pad with 0·5% Sodium lauryl sulfate (SLS) (24 hours)
5	Control site 2
6	Tape-stripping (40x using medical tape)

(b)

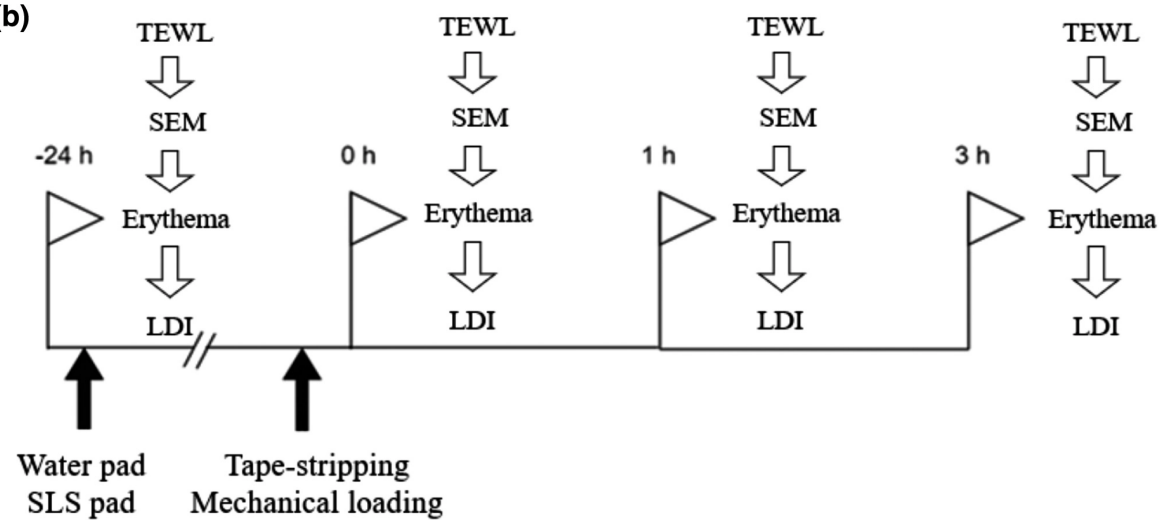


Figure 4.1. a) Schematic representation of the investigational sites on the volar aspect of the forearms in addition to the different skin challenges which were were employed in the study (b) Timeline of the experimental protocol detailing the interval of skin assessment and the various parameters assessed

4.1.3.2 SKIN PARAMETERS MEASUREMENT

Skin response to the challenges was elucidated by interrogating four separate parameters namely:

- TEWL was monitored at the investigational sites over a period of 1 minute using a Tewameter device (CK instruments, Germany)
- Skin blood flow was measured over the local test sites using Scanning Laser Doppler Imaging (SLDI, Moor Instruments Ltd, Axminster, UK)
- Sub-epidermal moisture was assessed at five points on and around each test site using a hand-held, portable, diagnostic device (SEMScanner, Bruin Biometrics LLC, USA)
- Erythema was evaluated by imaging a region of interest (ROI) over the test site along with a coloured reference patch using a mobile imaging application. (ScarletRed Holding, GmbH, Austria)

4.1.3.3 DATA ANALYSIS

Data was assessed for normality using probability plots and the Shapiro-Wilk test. Individual responses were presented to assess response patterns to different insults. To assess sensitivity, appropriate thresholds (mean + 2 SD) were selected for each of the parameters based on the response at control sites. The choice of threshold encompassing the mean ± 2SD is a commonly employed threshold in medical diagnostics to distinguish between findings from healthy and non-healthy cohorts (Indrayan 2012). MATLAB (MathWorks, USA) was used to create bubble plots of the data. For the purpose of three-parameter representation, skin outputs were normalized to their baseline values to account for inter-individual variations, with the exception of the SEM 'delta' parameter, which by its use of a delta accounts for inter-individual variations.

4.1.4 RESULTS

Eleven healthy participants (6 males and 5 females) were recruited from the local community. The participants were aged between 23 and 64 years (mean age 37 years) with a mean height and weight of 1.7±0.1 m and 76±14 kg, respectively, and a corresponding mean BMI of 25±5 kg/m^2. The demographics of the participants are summarized in Table 4.1.

Table 4.1. Summary of participant demographics

Participant ID	BMI	Age	Gender
1	18.2	57	F
2	20.8	28	F
3	21.6	28	F
4	22.7	37	M
5	24.0	28	M
6	24.7	64	M
7	24.8	24	M
8	24.8	23	M
9	27.8	37	F
10	28.4	31	M
11	36.1	52	F

The individual skin responses at each of the six test sites are presented for the four separate biophysical parameters in Figure 4.2. Relevant changes for each parameter are most conveniently described separately.

4.1.4.1 TRANSEPIDERMAL WATER LOSS (TEWL) (Figure 4.2a)

- An elevated increase was observed throughout the 3 h time frame following tape stripping and SLS irritation.
- An increase was detected immediately after the application of the water-saturated pad, however, TEWL values returned to baseline after 1h.
- Minimal variability in 10/11 participants was observed both at the site exposed to mechanical loading, as well as at both control sites.

4.1.4.2 SUBEPIDERMAL MOISTURE (SEM) (Figure 4.2b)

- A small number of participants exceeded the SEM delta threshold value of 0.6 following mechanical loading. However, this increase was not sustained throughout the 3 h time frame.
- A high degree of variability in the delta values was observed in the response of the individuals immediately after exposure to moisture
- Chemical irritation and tape-stripping evoked high response variability, with a few individuals exceeding the 0.6 delta threshold at 0h and subsequent periods following the insults.

4.1.4.3 SCANNING LASER DOPPLER IMAGING (LDI) (Figure 4.2c)

- A small group of individuals showed an elevated response following exposure to the water-saturated pad, which was maintained throughout 3 h time frame
- 6/11 individuals demonstrated an elevated response following exposure to the chemical insult, which was maintained throughout 3 h time frame
- All the participants yielded few changes to basal values at both control sites.

4.1.4.4 ERYTHEMA (Figure 4.2d)

- A high degree of variability was revealed across the cohort when participants were exposed to chemical irritation, water-saturated pads or following tape stripping
- 5/11 participants demonstrated high erythema values following mechanical loading.
- No other consistent and/or remarkable responses were evident following exposure to the other three insults.

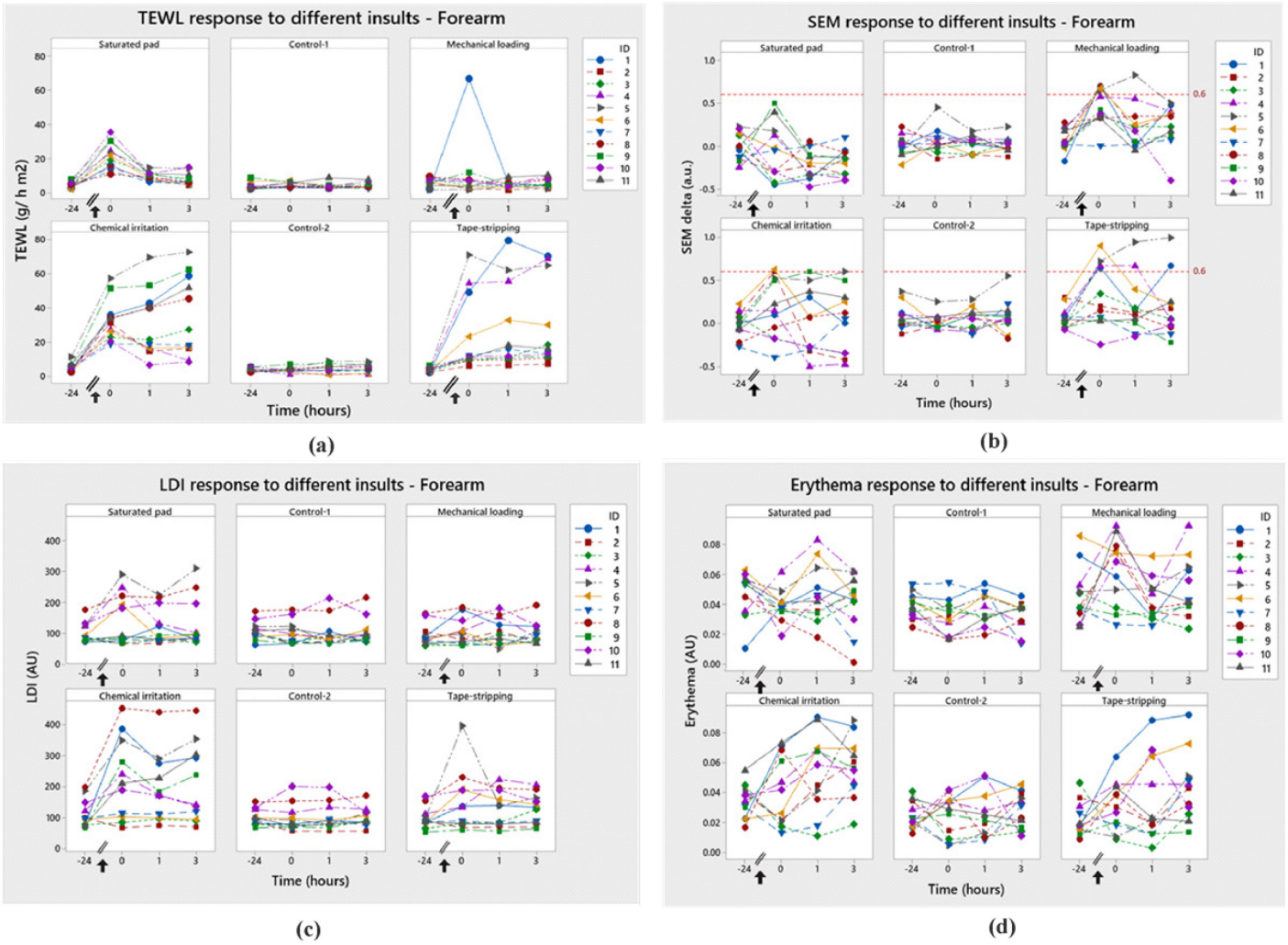


Figure 4.2. Temporal profiles of skin responses to various insults measured as a function of (a) TEWL (b) SEM (c) LDI and (d) Erythema response to different skin insults. The black arrows indicate when the insults were applied. The red dotted lines in Figure b indicates the threshold value recommended by the manufacturer

An alternative analysis of this data involved estimating arbitrary threshold values for the parameters of TEWL, LDI and erythema based on a value of 2 standard deviations above the mean value measured at control sites. With respect to the SEM parameter, the 'delta' threshold of 0.6 was used based on the recommendation from the manufacturers. Table 3.1 details the number of participants whose responses exceeded these derived threshold values. It is clear that no single parameter was sensitive across each of the insults. Many of the parameters revealed a temporal trend following an insult, with the number of participants exceeding the threshold diminishing with time, indicating a degree of skin recovery. Indeed, the derived thresholds for SEM delta, LDI and erythema were exceeded in less than 50% of the cases, at the majority of the time points following all the insults. By contrast, the TEWL response to chemical irritation (CI) and tape stripping (TS) was elevated above its threshold in the majority i.e 8/11 and 11/11, respectively, over the entirety of the 3 hour test period (Table 4.2).

Table 4.2. Descriptive summary of skin output parameters for a range of insults (TS- Tape stripping, ML-Mechanical loading, CI- Chemical irritation, SW – Saturated water pad)

Parameter (Units)	**Range**		**Threshold**	**Immediate response after insult (0h)**				**1 hour post-insult (1h)**				**3 hours post-insult (3h)**			
				No. of participants exceeding the threshold				No. of participants exceeding the threshold				No. of participants exceeding the threshold			
	Control	Insult		TS	ML	CI	SW	TS	ML	CI	SW	TS	ML	CI	SW
TEWL ($g\ m^{-2}\ h^{-1}$)	0.8 — 8.9	1.6 — 79.7	≥ 8	10	2	11	11	10	1	10	8	10	2	9	3
SEM - delta (AU)	-0.28 —0.55	-0.5 — 1.0	≥ 0.6	4	4	2	0	2	1	1	0	2	0	1	0
LDI flux (AU)	52— 216	48 — 453	≥ 177	4	1	7	5	3	1	5	3	2	1	5	3
Erythema (AU)	0.004 — 0.054	0.001 0.093	≥0.06	1	6	4	1	3	1	5	3	2	4	5	2

Further sensitivity analysis was performed to extend SEM 'delta' threshold in order to include a range of values from 0.1 - 0.7 A.U. The relative sensitivity of each threshold was examined for three insults namely mechanical loading, tape stripping and chemical irritation, as highlighted in Figure 4.3. The analysis revealed a decrease in the percentage of participants exceeding each threshold from immediately post-insult (0h) to three hours post-insult (3h). Interestingly, the analysis showed that even at the lowest threshold value of 0.1, skin changes following tape-stripping and chemical irritation were detected in less than 65% of participants. By contrast, the thresholds were more sensitive to mechanical loading, particularly at a delta value ≤0.3, a value which represents 50% of that recommended by the manufacturers (Ross et al. 2019).

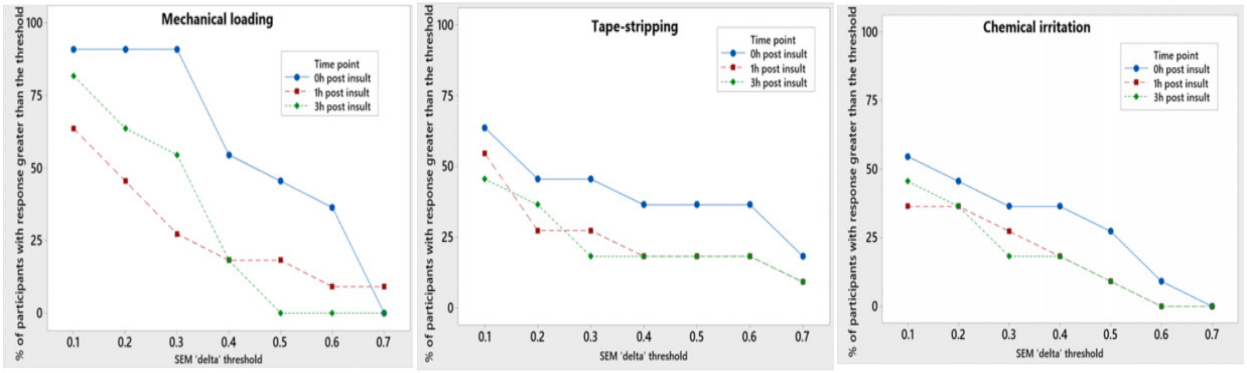


Figure 4.3. Sensitivity analysis of SEM 'delta' parameter following mechanical loading, tape-stripping and chemical irritation. Figure highlights a decrease in the number of individuals exceeding each threshold with time.

Given that thresholds derived from single biophysical parameters were limited in detecting skin changes following insults, as evidenced by Table 4.2, a complementary evaluation using combined parameters might possess the potential to provide a more sensitive assessment. Indeed, three of the parameters, namely TEWL, LDI and SEM were integrated into bubble plots at 3-time points (0h, 1h and 3h) and were used to evaluate the response of tape stripping as a function of the BMI of the participants (Figure 4.4) The size and the colour intensity of each bubble correspond to the normalized LDI and SEM responses, respectively, with the y-axis corresponding to the normalized TEWL response. It is evident that a sub-group of individuals, with a low to normal BMI (<25 kg/m^2) exhibited elevated response throughout the experimental period for each of the three parameters. However, this trend was not detected in another group of individuals with similar BMI.

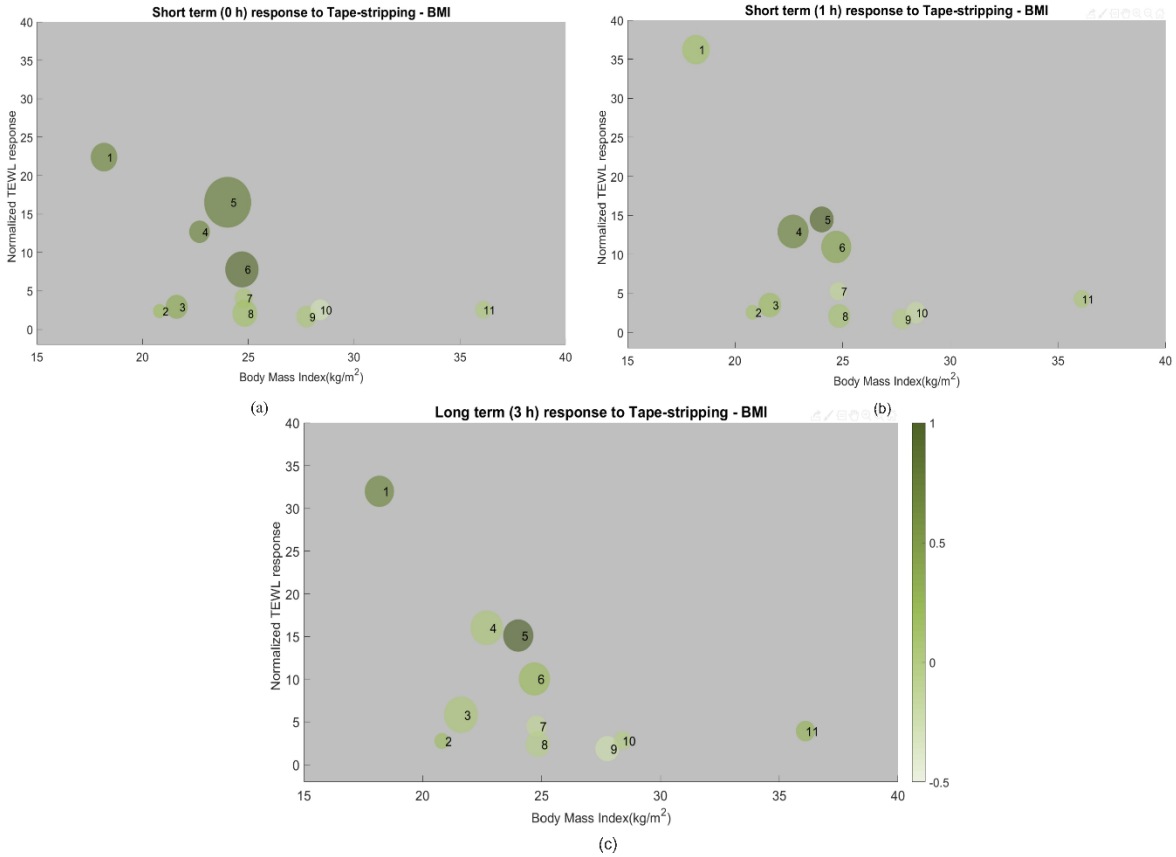


Figure 4.4. Bubble plot of the role of BMI following tape stripping. BMI plotted on the x -axis and normalized TEWL response on y-axis. The size of each bubble is proportional to normalized LDI response and the bubble colour is given by the SEM 'delta' response. The data labels on the bubble indicate the participant IDs. (a) Immediately after the insult (0h) (b) 1h post insult (1h) (c) 3h post insult (3h). Figure highlights a group of individuals with BMI <25 kg/m^2 exhibiting increased skin responses.

4.1.5 DISCUSSION

The primary aim of this study was to examine the skin response to four established insult models in a healthy cohort using established biophysical techniques. The findings highlighted that there were sub-groups of individuals within the cohort who responded distinctly to specific insults. Accordingly, the overall sensitivity of the thresholds derived from the biophysical techniques was limited in detecting temporal changes evoked by the skin insults. The variability of the parametric values associated with erythema and SEM, apparent at both control sites and following insults, limited the potential to identify changes in skin integrity. Nonetheless, there were some significant correlations between the biophysical parameters derived from TEWL, SEM and LDI, which provide some confidence that individual responses to insults could be robustly identified. The skin challenges employed in the present study have been previously used to alter the healthy status of the skin barrier. For example, studies have reported increased TEWL values following exposure of

the skin to moisture (Bostan et al. 2019; Fader et al. 2011). However, these values returned quickly to baseline, which indicates that the impairment was transient in nature.

Sustained pressure in the form of mechanical loading, which is well-established as one of the major factors leading to the development of PUs (see chapter 1, section 1.3.1), has been also the objective of various research, with reported temporal loss in the skin barrier function and development of erythema (Kottner et al. 2015). Indeed, a recent study in the host lab showed a significant increase in TEWL values when the sacral skin of able-bodied volunteers was challenged with 9 kPa of applied pressure (Bostan et al. 2019). Differently, the present study detected minimal variations in TEWL throughout the period of data collection. The inconsistency might be due to the different anatomical locations investigated in both studies (volar forearm vs sacrum). Chemical irritation with SLS and tape-stripping have been investigated in several studies involving TEWL, blood flow, inflammatory biomarkers and erythema response (Fluhr et al. 2001; Angelova-Fischer et al. 2012; Soltanipoor et al. 2018). Indeed, it is well established that both these insults evoke damage to the skin barrier, although the effects have been reported to be reversible with time (Han et al. 2017). Results from the present study indicated a marked increase in the biophysical parameters following chemical irritation to the skin. In particular, a 3-fold increase was observed in TEWL values immediately after the insult (Figure 4.2a). This increase was sustained throughout the test period, signifying the extended effects of chemical irritation. Similarly, on tape-stripping, a pronounced increase in TEWL, erythema and SEM were observed throughout the test period. Although TEWL was able to detect minimal variations for most of the insults, this was not the case for some of the other parameters, namely SEM, LDI and erythema, making the selection of a threshold for these biophysical tools challenging. As an example, although a threshold delta value of 0.6 has been prescribed for SEM, nonetheless, the present study revealed that for a given threshold, there were clear differences in sensitivity with respect to both time and nature of the insult t the delta. Furthermore, as the risk of developing PUs is a function of both extrinsic and intrinsic factors (see section 1.2.1.1), the current study revealed distinct differences in responses following mechanical or chemical insults even in a subgroup of individuals with similar BMI. This reinforces recent work in the host laboratory, which has highlighted the importance of cluster analysis when examining the response of able-bodied cohorts to skin challenges.

The study is clearly limited by the small sample size and the use of the forearm site, thereby precluding the generalizability of its findings to all tissue sites. Moreover, the study is conducted on a healthy cohort and further testing on vulnerable individuals (chapter 8) is critical in order to translate the findings to a clinical setting.

4.1.6 CONCLUSIONS

In this study, the sensitivity of parameters derived from biophysical techniques to monitor skin response following mechanical and chemical challenges was evaluated. A difference in temporal response of the biophysical parameters was observed depending on the type and degree of the insult. With the exception of TEWL, the other parameters, namely SEM, LDI and erythema revealed considerable variability limiting clear delineation of responses. Thresholds indicative of skin changes lacked sensitivity in a number of the parameters, with, for example, the SEM 'delta' being affected by both time and threshold magnitude. It was evident that a single biophysical parameter could not effectively capture the tissue response to a range of insults.

4.2 ANATOMICAL VARIABILITY OF SUBEPIDERMAL MOISTURE AND ITS CLINICAL IMPLICATIONS

*Jayabal, H, Bates Jensen, BM, **Abiakam, NS**, Worsley, PR and Bader, DL (2021). Anatomical variability of sub-epidermal moisture and its clinical implications. Journal of Tissue Viability, 30, 434-438. https://doi.org/10.1016/j.jtv.2021.04.003*

4.2.1 INTRODUCTION

Commercial systems sensitive to tissue capacitance and permittivity have gained attention as a non-invasive biophysical measure of localized oedema in the sub-epidermal tissues (Harrow et al. 2014; Gefen et al. 2020). Any early inflammatory changes coupled with impaired lymphatic drainage, associated with the early stage of tissue damage i.e. PUs, could result in an accumulation of interstitial fluid underneath the skin tissues (Quintavalle et al. 2006). This increase in sub-epidermal moisture levels creates a local change in tissue impedance and/or capacitance (Gefen 2018). This forms the basis of the sub-epidermal moisture (SEM) device (Bruin Biometrics, USA; Figure 2.3), which has been evaluated in both pre-clinical and clinical studies in detecting early signs of skin damage (Bates-Jensen et al. 2017; Moore et al. 2017). In addition, SEM scanner has been claimed to be able to identify skin damage days earlier than standard visual examinations by clinicians using risk assessment scales (O'Brien et al. 2018). However, the device has been reported to have a low specificity of 33%, indicating a high number of false positives, and to present with a 14% positive predictive value (PPV) (Moore et al. 2022). Nevertheless, one blinded longitudinal clinical study involving 189 patients, deemed at risk of PU development on the heel and sacral region, evaluated the sensitivity and the specificity of SEM parameter. The study reported a sensitivity of 87.5% and a specificity was 32.9%, with an area under the receiver operating characteristic curve (AUC) of 0.67, resulting in SEM being able to detect the occurrence of PUs 4.7 days prior to standard skin

assessment strategies (Okonkwo et al. 2020). Although SEM has been validated for use on specific anatomical locations, namely the sacrum and heel, however, clinical data have shown the influence of anatomical morphology on its output (Peko et al. 2020). Indeed, studies have shown distinct differences in the SEM delta between the sacrum and heels (Gershon et al. 2021). Furthermore, PUs are also known to occur in different body sites other than the sacrum and heel (e.g. trochanter, ischial tuberosity, etc..) (see section 1.2.1).

Thus, there is a need for more research to better understand the site-specific i.e. intra-individual and inter-individual variations associated with baseline SEM values. This has motivated, in the host lab, the design of research to get a better understanding of factors which could influence SEM parameter outputs.

4.2.2 AIM OF THE STUDY

The present study aims at investigating SEM readings at a number of different body sites to establish the basal values, as well as explore possible correlations with demographic factors

4.2.2.1 STUDY OBJECTIVES

The objectives of the study include the followings: -

1. Recruit a number of healthy participants cohort.
2. Collect basal SEM values at different anatomical sites of the individuals recruited
3. Investigate possible associations of SEM parameter with the participants' intrinsic factors

4.2.3 MATERIALS AND METHODS

This was a cohort observational study using healthy volunteers. Each individual was measured on multiple anatomical locations in order to derive regional differences in SEM values and variance across the cohort. Exclusion criteria included a history of skin-related conditions or neurological or vascular pathologies that could affect the health of skin tissue. Institutional ethics was granted for the study (ERGO-FOHS-26040) and informed consent was obtained from each participant prior to testing.

4.2.3.1 STUDY PROTOCOL AND EQUIPMENT

Sub-epidermal moisture was measured using a handheld, portable device (SEM Scanner 200 model, Bruin Biometrics LLC, USA). The device measures the bio-capacitance of soft tissues which is converted into arbitrary units (AUs). All measurements were taken according to the manufacturer's guidelines. To review briefly, light skin pressure was applied at an optimal level indicated by the

device prior to each recording. The basal SEM measurements were collected from 27 anatomical sites involving bony prominences, which included the right (R) and left (L) sides of the shoulder, scapula, hip, trochanter, buttocks, ischial tuberosities, medial knee, lateral knee, medial ankle, lateral ankle, lateral heel, posterior heel, as well as the middle of the back, sacrum and coccyx (Figure 4.5). SEM readings were taken in triplicate by an experienced nurse (BBJ) and a mean of the three values was calculated for each body location. Participants either adopted a supine, prone or side-lying posture, ensuring that the sites were unloaded prior to taking the measurements. This was carried out by giving sufficient time for recovery after loading as informed by previous studies (Bostan et al. 2019). All the measurements were performed in a controlled lab environment set at a temperature of 23 ± 2°C and relative humidity of 42 ± 6 %.

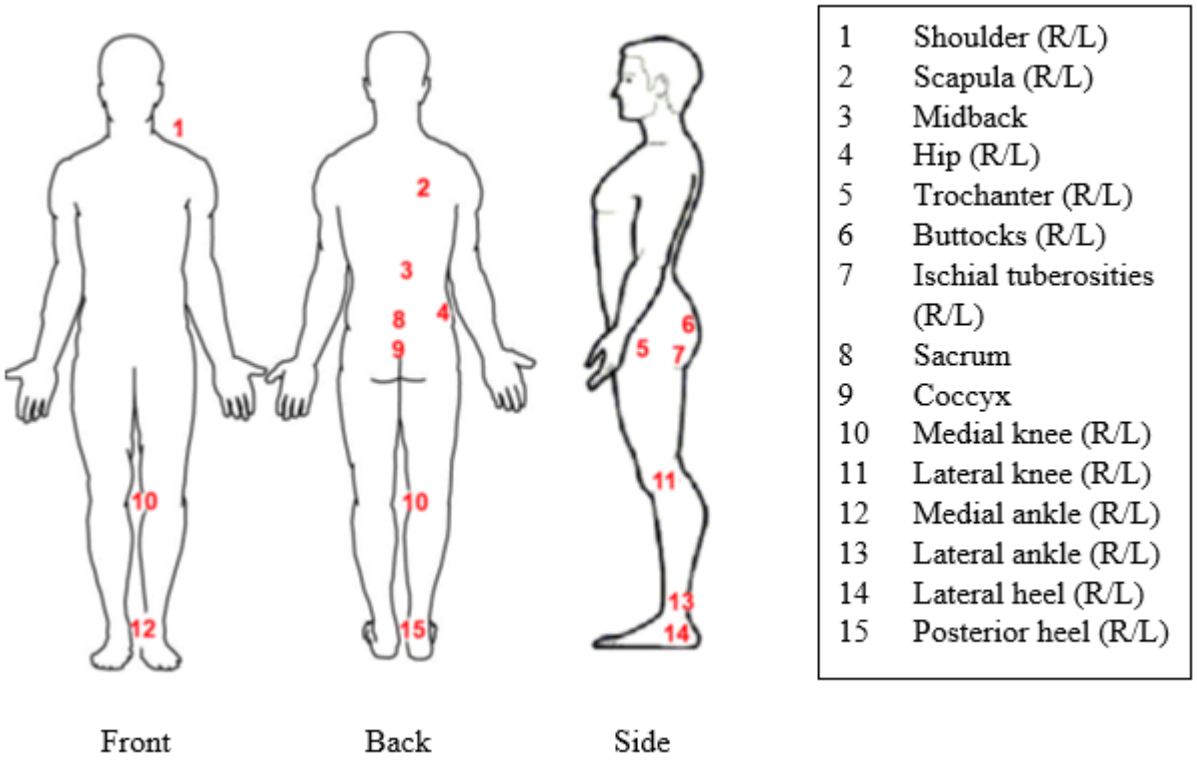


Figure 4.5. Anatomical sites of SEM investigation, which include 27 locations involved with bony prominences

4.2.3.2 STATISTICAL ANALYSIS

Data was imported into Excel 2019 (Microsoft, USA) and MATLAB (MathWorks, USA) was used for creating an appropriate presentation of the results. Data was assessed for normality using a probability plot and Shapiro-Wilk test. Accordingly, data was presented using the mean and coefficient of variation (CV). The relationship between the intrinsic factors, such as BMI, and SEM values was examined using linear regression. A level of 5% ($p \leq 0.05$) was considered statistically significant. Subsequent analysis of the data was performed using a cluster analysis approach employed previously by the authors (Bostan et al. 2019). To review briefly, the ranks were summed

based on SEM values across all the locations for each of the participants and presented according to participant age and BMI.

4.2.4 RESULTS

24 able-bodied volunteers (10 males and 14 females), with no history of skin conditions and with a mean age and BMI of 48±17 years and 25±4 kg/m^2 respectively, were recruited into the study. Examination of the data revealed interesting findings in terms of intra- and inter-individual baseline SEM values as the result of mean and coefficient of variation (CV) (Table 4.3). Indeed, it was observed that each anatomical region presented with similar trends when the right body sites were compared to the left. Nonetheless, it was also observed that certain cephalad anatomical locations, such as shoulder, scapula, and mid-back presented with higher basal values compared to those in caudad (towards the feet) locations. In addition, across the participants, the CV values demonstrated site-specific variability, which ranged from 8% to 27%, with the highest degree of variability associated with the sites adjacent to the heels.

Table 4.3. Mean and coefficient of variation (CV) of baseline SEM values measured rank-ordered to different anatomical sites

Location	Mean	CV (%)
R - Hip	2.8	12.6
L - Shoulder	2.8	12.2
R - Shoulder	2.8	12.0
L - Hip	2.8	10.6
R - Scapula	2.8	8.1
Midback	2.8	9.8
L - Scapula	2.7	10.9
R - Medial knee	2.6	12.4
Coccyx	2.6	16.7
Sacrum	2.6	11.9
L - Medial knee	2.5	14.7
R - Ischial	2.5	11.9
R - Lateral heel	2.5	23.2
L - Lateral heel	2.5	20.2
R - Trochanter	2.4	11.0
L - Trochanter	2.4	8.5
L - Buttock	2.4	11.8
L - Ischial	2.4	13.9
R - Lateral knee	2.4	19.2
R - Medial ankle	2.4	11.3
R - Buttock	2.4	11.1
L - Lateral knee	2.3	20.5
R - Posterior heel	2.3	26.2
L - Medial ankle	2.3	14.7
L - Posterior heel	2.2	22.9
L - Lateral ankle	2.2	14.4
R - Lateral ankle	2.2	19.3

Furthermore, analyses highlighted that the individual baseline SEM values at different anatomical sites ranged varied considerably ranging from 1.1 to 3.7 AUs. When the data were analysed according to the BMI some trends were observed, which were conveniently presented in the form of a heat map (Figure 4.6). Indeed, higher baseline SEM values were generally evident in participants presenting with a lower BMI (< 20 kg/m^2) i.e. subjects #1–4.

Participant ID	BMI (kg/m^2)
1	18.2
2	19.1
3	19.3
4	19.5
5	20.8
6	21.6
7	21.6
8	22.7
9	23.6
10	24.0
11	24.2
12	24.7
13	24.8
14	24.8
15	24.8
16	25.3
17	25.8
18	26.8
19	27.8
20	28.4
21	31.0
22	31.0
23	31.1
24	33.9

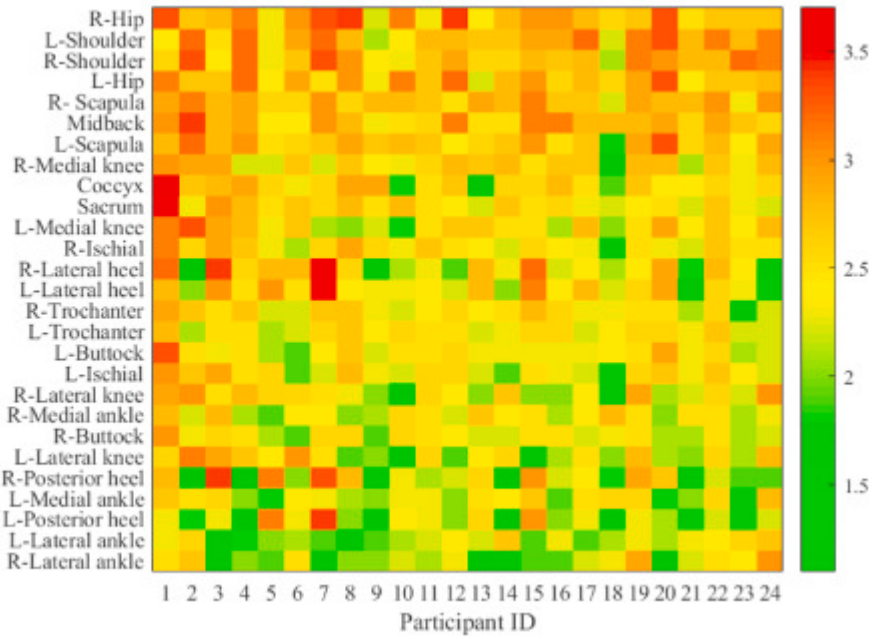


Figure 4.6. Heat map representation of baseline SEM values across 27 anatomical sites for individuals. Participants (1-24) ordered from left to right based on increasing BMI. The figure highlights trends in which individuals with low BMI presented with higher SEM values.

The implication of intrinsic factors, namely age, BMI and gender were also evaluated with respect to SEM values ranked for each location. The ranks were summed across all the locations for each of the genders as indicated in Figure 4.7. As the maximum rank sum value was estimated to be 648, an arbitrary rank-sum threshold value of 325 (half of the maximum rank-sum value) was adopted to observe the influence of these intrinsic factors. Close examination of rank-sum analysis with respect to BMI revealed considerable variability: -

- Out of the nine participants with BMI < 24 kg/m², six presented with elevated SEM rank sum values,
- Two of the eight participants with BMI ranging from 24-26 kg/m² exhibited high SEM values,
- Of the seven participants with BMI > 26 kg/m^2, four of them revealed elevated SEM values.

With respect to age, participants less than 50 years presented with elevated SEM baseline values compared to those above 50 years of age. No interesting trends were observed in relation to gender diversity.

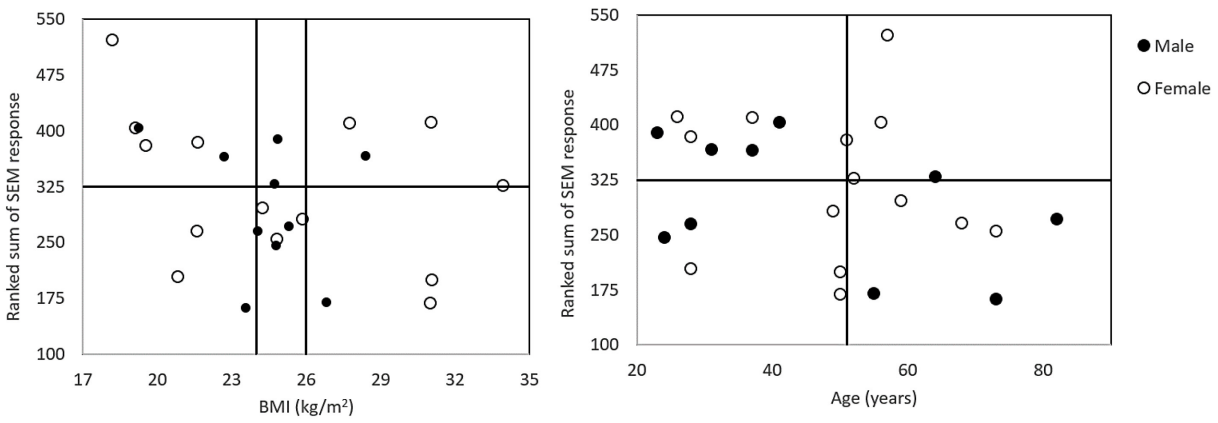


Figure 4.7. Association of SEM rank sum with BMI (left) and Age (right). With respect to BMI, figure highlights that elevated SEM rank values associated with participants with BMI < 24 kg/m² and < 50 years of age.

4.2.5 DISCUSSION

The finding of this study revealed distinct differences in SEM values recorded at 27 different anatomical sites of the body, with a clear trend in decreasing values from the head to the feet. These results are consistent with studies in which different commercial devices able to measure tissue oedema were employed to assess various anatomical locations across the body of healthy volunteers (Mayrovitz 2019; Guihan et al. 2012). In addition, previous studies in which SEM parameter was investigated, reported values of 2.5–2.6 at the heels, which are in close agreement with the present study (lateral heel, mean = 2.5) (Clendenin et al. 2015). Although basal SEM values varied considerably between anatomical sites, nevertheless there was consistency in mean basal SEM values with respect to the sagittal plane, i.e. the right and the left sides of the body, which provides confidence of a reliable measurement at each specific body site. Furthermore, high inter-individual variation was observed at specific anatomical sites, for example, the heels. Indeed, the coefficient of variation (26%) is a result of the cohort SEM recording, which ranged from 1.3 to 3.4 AUs. As such, a 0.6 delta value would represent a relative change of 46% for one individual and 18% for another. This variation could be a result of the influence of participants' intrinsic factors, such as BMI, age and gender. Interestingly the results of the present study contrast with previous reports where a weak correlation was found between SEM values at the heel with age (Bates-Jensen et al. 2018). This study also detected associations between participants' BMI and SEM values, with individuals presenting with low BMI <20 kg/m² expressing elevated baseline values. These findings were incongruent with a recent study reporting no significant differences between cohorts of

different BMI (Mayrovitz et al. 2020). The differences could be attributed to the different commercial devices employed, as well as the cohorts.

Regardless of all conflicting opinions in relation to its use, SEM device has been reported to considerably reduce the time required for the detection of early signs of PUs at the patient's bedside, and as such has been advocated for use in clinical settings (Budri et al. 2020). Indeed, SEM has been recommended as an adjunct measurement to the routine risk assessment scales in relation to PU development (NICE 2020; EPUAP/NPIAP/PPPIA 2019).

4.2.6 CONCLUSIONS

This study evaluated SEM baseline values across the transverse plane of the body, with considerable variability detected. In addition, SEM values seemed to be affected by specific site anatomic location, BMI and age. It is interesting to note that the manufacturer has not detailed any information pertaining to the impact of intrinsic and extrinsic factors on SEM readings. The study highlighted the need to perform more studies in relation to the anatomical variability of SEM before the device could be consolidated in clinical settings.

4.3 SKIN MEASUREMENT SUMMARY

Section 2.1 provided a critique of the array of bioengineering strategies, which had been previously employed to evaluate the integrity of the skin in response to various external challenges. The conclusions in that literature review coupled with the analysis of the findings from the two retrospective studies described in the present chapter informed the selection of the techniques and their associated output parameters, which will be used in the experimental studies in the thesis (Table 4.4). As highlighted in this chapter, except for TEWL, none of the devices presented an outstanding performance in detecting transient changes in the skin barrier function. This was particularly evident for erythema, LDI and SEM. Nevertheless, the device used for erythema detection, ScarletRed Holding (Vienna, Austria), is less used in the literature for the detection of skin surface redness compared to the well-consolidated Mexameter MX18 (Figure 2.5), which offers more studies for comparisons. As such the mexameter device will be employed in the studies in the following chapter.

Table 4.4. Summary of the methods adopted for subsequent studies to assess skin response in healthy and patient cohorts. The main advantages and disadvantages of these devices are highlighted in chapter 2.

METHOD	INSTRUMENT	MANUFACTURER
	Skin Barrier Assessment	
Skin barrier function	Tewameter® TM 300w	Courage & Khazaka, Germany
Stratum corneum hydration	Corneometer® CM 825w	Courage & Khazaka, Germany
Skin erythema	Mexameter® MX18	Courage & Khazaka, Germany
Skin surface pH	Skin-pH-meter® 905w	Courage & Khazaka, Germany

Alongside the issues mentioned in this retrospective study, LDI presents various disadvantages which limited its use in this thesis, the most prominent of which include cost and the inability to provide a continuous measurement. With respect to SEM scanner, although it has growing international interest for its potential as the next-generation tool for the early detection of PU, the literature review and the above studies have identified a number of limitations. Namely, the following can be summarised:

1. there could be some influence of intrinsic factors (age, BMI and gender) in its output,
2. the thresholds provided by the manufacturer may be insufficient to detect early sign damage at the skin surface following mechanical and chemical challenges,
3. Its highly sensitive and poorly specific performance in practice has resulted in a low predictive value.

The researcher and his associates engaged with Bruin Biometrics for an independent evaluation using the device. However, there was an inability to reach a commercial agreement whereby independent research could be conducted prospectively with the device. Therefore, with regret, it will not feature in the corresponding thesis.

Chapter 5 EVALUATING THE CHANGES OF SKIN BIOPHYSICAL PARAMETERS FOLLOWING CHALLENGES WITH MOISTENED INCONTINENCE PADS AND PRESSURE APPLICATION

The results of this study have been submitted for publication in the Journal of Wound, Ostomy and Continence Nursing (JWOCN):

Abiakam, N, *Jayabal, H, Worsley, P, et al. The effects of moistened incontinence pads on loaded skin with reference to biophysical and biochemical parameters. Journal of Wound, Ostomy and Continence Nursing 2022;* ***Accepted in press***

5.1 INTRODUCTION

There are many scenarios where prolonged exposure to moisture can induce variations in the barrier properties of the skin. As extensively described in Chapter 1, section 1.2.2, when moisture is a result of body waste products, namely urine and/or faeces, this could lead to the development of a clinical condition termed incontinence-associated dermatitis (IAD) (Gray et al. 2012). The socioeconomic implications of IAD have been previously reported (section 1.2.2.1), with the condition not only representing a major health challenge to the individuals affected but also to healthcare professionals who are trying to maintain a healthy skin status (Cunich et al. 2022). Clinical manifestation of IAD ranges from mild erythema to more extreme tissue loss and skin breakdown. The perineal, perianal, inner thighs and convex areas of the buttocks are the anatomical locations most affected (Beeckman 2017).

Prolonged exposure to urine will overhydrate the skin, release harmful enzymes when interacting with stool, and decrease tissue tolerance to friction and pressure (Lichterfeld-Kottner et al. 2020; Lumbers 2018). In particular, urine ammonia is capable of shifting the skin pH from acid to neutral or even alkaline levels. This causes a disruption of the skin's acidic mantle, alteration of its flora in relation to high bacterial colonisation and overgrowth, diminished stratum corneum cohesion, and impaired skin barrier function (Fluhr et al. 2002). In addition, prolonged skin contact with urine induces changes in the local microclimate, where heat, humidity and moisture decrease the protective function of the skin (Ichikawa-Shigeta et al. 2014).

Current interventions for the prevention and treatment of IAD are designed to minimise skin exposure to urine and/or stool through the management of incontinence and a structured skincare

regimen. This typically comprises prompt cleansing of the skin to remove irritants, skin protection with barrier products and restoration of skin moisture. However, studies have demonstrated that incontinence pads can contribute to IAD when left in-situ for prolonged periods (Junkin et al. 2007; Gray et al. 2012). Indeed, it has been asserted that prolonged contact between patients and incontinence pads induces occlusion of the skin, changes in microclimate leading to increase temperature and humidity (Falloon et al. 2018), enhanced transepidermal water loss (TEWL), and disruption of the skin-ambient fluid exchange mechanisms (Koudounas et al. 2020).

Changes in the epidermal function due to prolonged exposure to moisture, in the presence or not of incontinence products, have been the subject of various studies. These have focused on the biophysical pathways underpinning the loss of skin integrity (Falloon et al. 2018; Fujimura et al. 2016; Phipps et al. 2019). A study reported compromised barrier properties, as evidenced by a significant increase in transepidermal water loss (TEWL), following skin challenge against moist incontinence pads in combination with mechanical loads (Bostan et al. 2019). However, in this study, the loading of the sacrum was achieved using custom-made cylindrical weights, which are not reflective of the real loading scenarios in acute and long-term care facilities. Another study demonstrated a significant increase in mean skin surface water loss when chemically irritated skin is insulted with synthetic urine (Koudounas et al. 2021). A study also reported variations in skin pH towards the alkaline region, leading to dryness and decreased antibacterial defence of the barrier function (Schmid-Wendtner et al. 2006). In addition, biochemical markers of local inflammation post-moisture exposure have recently been explored, where a significant increase of the pro-inflammatory interleukin 1 alpha (IL-1α) was observed following exposure to saturated incontinence pads and mechanical loads (Bostan et al. 2019; Koudounas et al. 2020).

However, there is still a limited understanding of skin healthy parameters' behaviour in relation to clinical loading scenarios, and recovery characteristics of skin biophysical parameters following damaging insults.

5.2 AIM OF THE STUDY

The present study aims to investigate the temporal changes in skin health parameters following exposure to dry and moist continence pads, presenting with two different buffering capacity, with and without the addition of postural mechanical loads. In addition, the study aims to answer the following research questions:

- Can TEWL and skin hydration vary as a consequence of the application of mechanical load as a function of Peak pressure index and peak pressure gradient?

The study was conducted in collaboration with an industrial partner, Essity AB (Sweden).

5.2.1 STUDY OBJECTIVES

The objectives of the study include the followings: -

1. Recruit a number of healthy participant group, including both young and old individuals.
2. Challenge participants' sacral skin with two different incontinent products loaded with synthetic urine (SU) over a period of time, both with and without mechanical loading.
3. Perform data collection at different intervals involving biophysical measurements
4. Identify temporal changes in skin physiology, indicative of changes in barrier function and hydration both during insult and subsequent recovery.
5. Examine possible associations between participants' intrinsic factors with the parameters under investigation, including interface pressure.

5.3 MATERIALS AND METHODS

5.3.1 STUDY PARTICIPANTS AND SETTING

The study recruited a cohort of 12 healthy volunteers who were subjected to a skin challenge involving saturated and dry incontinence products. The small sample size was deemed appropriate given that each individual would act as its own control, limiting the need for a power calculation. The cohort, recruited during the period of COVID-19 restrictions, included a range of ages and body types. Indeed, in order to examine the implications of age differences on the behaviour of skin parameters following moisture and pressure insults, individuals of two different age ranges were included in the study. The first phase of recruitment was performed in December 2020 and involved six young able-bodied volunteers, whereas the second phase of the recruitment process, between June and September 2021, recruited an older cohort of healthy volunteers. Both cohorts were recruited from the local community and were identified through poster advertisements and local recruitment strategies. Those interested in participating were screened to exclude those presenting with the following conditions: -

- Back pain limiting the time spent in the supine position
- Active skin diseases on one of the test sites
- Diabetes
- An intake of non-steroidal anti-inflammatory drugs in the last 7 days.

All tests were performed in a testing laboratory (Clinical Academic Facility, Southampton General Hospital), which was maintained at a room temperature of 22.5± 0.7 ^{o}C and relative humidity of 42± 6%. Participants were allowed to acclimatize for 15 minutes prior to commencing the study session and their demographic data (gender, ethnicity, and BMI) were recorded. The research project received approval from the University of Southampton Ethics Committee (FOHS-ERGOII

25851.A1). Written, signed and dated consent was received from each participant prior to commencing the test session.

5.3.2 INCONTINENCE PRODUCT PREPARATION

Two different incontinence products namely, products M and T (both provided by Essity AB, Gothenburg, Sweden), were investigated in the study. Both products were presented in circular-shaped specimens with a diameter of 40 mm and prefabricated to include the following sections (Figure 5.1):

Top sheet: Non-woven polypropylene (PP)

Core section: cellulose fibres + superabsorbent polymer (SAP) particles

Back-sheet: Polyethylene (PE) + Non-woven laminated

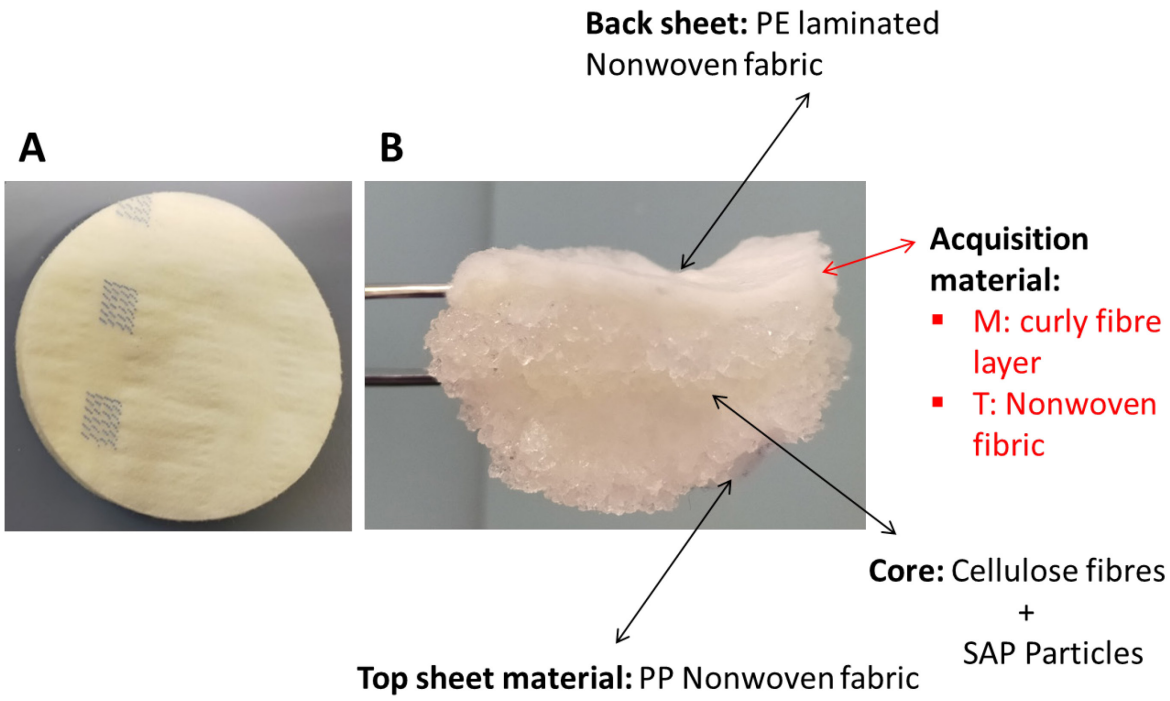


Figure 5.1. (A) Molicare pad in a dry state (B) Different sections of product M and the transformation of SAP particles into hydrogel particles following the dispensing of 15 mL of synthetic urine into the M product sample

The two products differ from each other in terms of the acquisition material component, which consisted of a curly fibre layer for product M and non-woven fabric for product T. Due to intellectual property reasons, as well as a practical strategy to blind the researcher performing the study, the industrial partner refused to disclose detailed information in relation to the difference in the buffering capacities of the two products under investigation. Nevertheless, both pads were

designed to allow the transport of urine through the top sheet, subsequent transport through the acquisition layer and storage in the core layer.

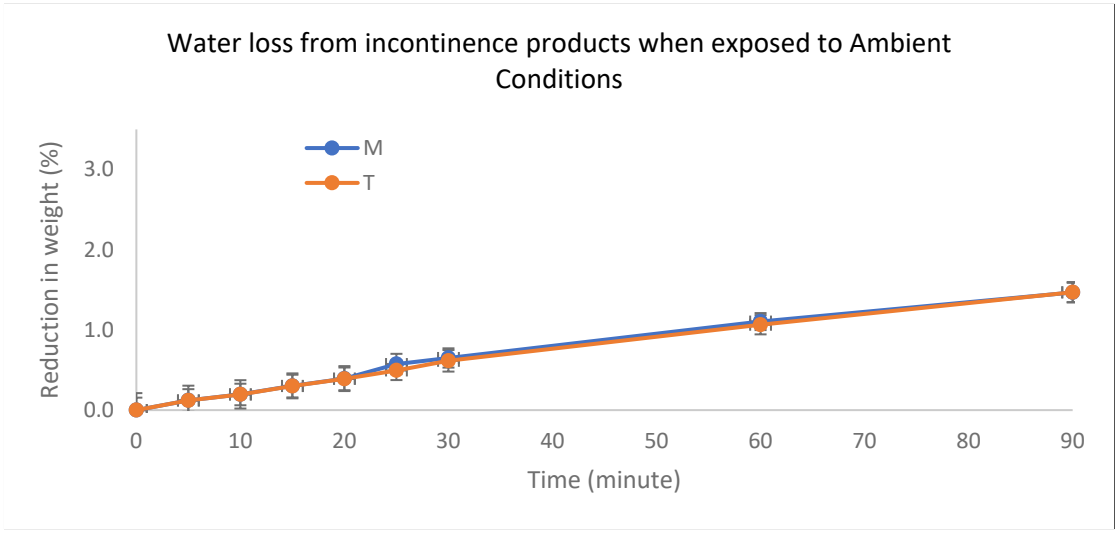
The products were saturated with 15 mL of synthetic urine (SU) (pH= 8), formulated based on the composition and protocol as reported by Mayrovitz and colleagues (2001) and detailed in Table 5.1. The SU formulation was kept refrigerated at 4^{o}C and allowed to acclimatize to room temperature prior to each test session. The pH of the SU was monitored during each session using a pH meter (pH 213 Microprocessor pH Meter, HANNA Instruments, UK) to ensure consistency. SU was dispensed into the pads via serological pipettes until saturation was achieved, as observed by a small leakage of fluid from the pad samples. Each sample was left to equilibrate with the environment for 15 minutes to ensure equal distribution of the liquid within the product. The pH of the surface and core of the products were recorded before and at the end of each test using a pH meter (PH 905, Courage & Khazaka, Germany).

Table 5.1. Composition of synthetic urine in 50 mL of distilled water (Based on Mayrovitz et al. 2001)

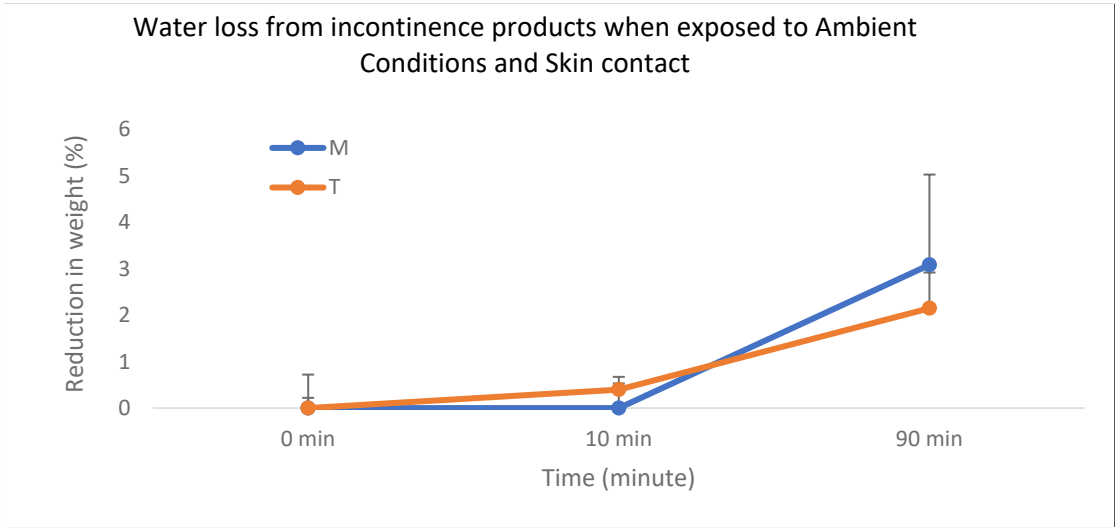
Chemical	Weight (g)	Concentration (w/v)
Urea	1.25	2.5%
Sodium Chloride	0.45	0.9%
Ammonium Chloride	0.15	0.3%
Sodium Sulphite	o.15	0.3%
Anhydrous disodium hydrogen orthophosphate	0.125	0.25%
Creatinine	0.10	0.20%

Gravimetric tests were conducted on the two saturated incontinence products which were exposed to the ambient environment to examine urine loss through evaporation over a 90-minute period. The mean weight of the products in a dry state was 2.86 ± 0.07 g and 2.98 ± 0.07 g for products M and T, respectively. Immediately after saturation, the corresponding weights were 33.06 ± 0.21 and 33.34 ± 0.15 g. The absorbance and swelling capacity of both products was facilitated by the presence of the superabsorbent polymer (SAP) particles, which convert into hydrogel particles (Figure 5.1B). The findings from the gravimetric tests revealed that both products lost less than 1.5% of their saturated weight due to evaporation (Figure 5.2A). It is worth underlining that the increment in weight of the products (as described above) is mostly a result of the increased weight of the SAP particles during their transformation into hydrogels.

In a separate test, samples of the saturated products were attached to the skin to examine the influence of the combination of evaporation and diffusion with time when exposed to the ambient environment. Results indicated that the maximum decrease in weight was 3% for each of the incontinence products (Figure 5.2B). However, product M was more variable in weight compared to product T, as evidenced by the elevated standard deviation.



A



B

Figure 5.2. (A) Estimated amount (%) of urine content lost from incontinence products as a result of evaporation and (B) as a result of the combination of diffusion and evaporation.

5.3.3 ANATOMICAL LOCATIONS OF SKIN CHALLENGE

Five separate sites (A, B, C, D, and E) on the upper buttock and lumbar region of each participant were marked using a non-permanent marker (Figure 5.3). To ensure clear differences between each test site, a 40 mm and 60 mm vertical and horizontal separation, was maintained between sites. Sites A and B were exposed to the dry products M and T, respectively. Sites C and D were

exposed to the corresponding 100% saturated samples. Site E represented the negative control skin site, which remained unchallenged throughout the test period. Samples of Incontinence products were held in place via an impermeable adhesive dressing (3M™ Tegaderm™, United Kingdom).

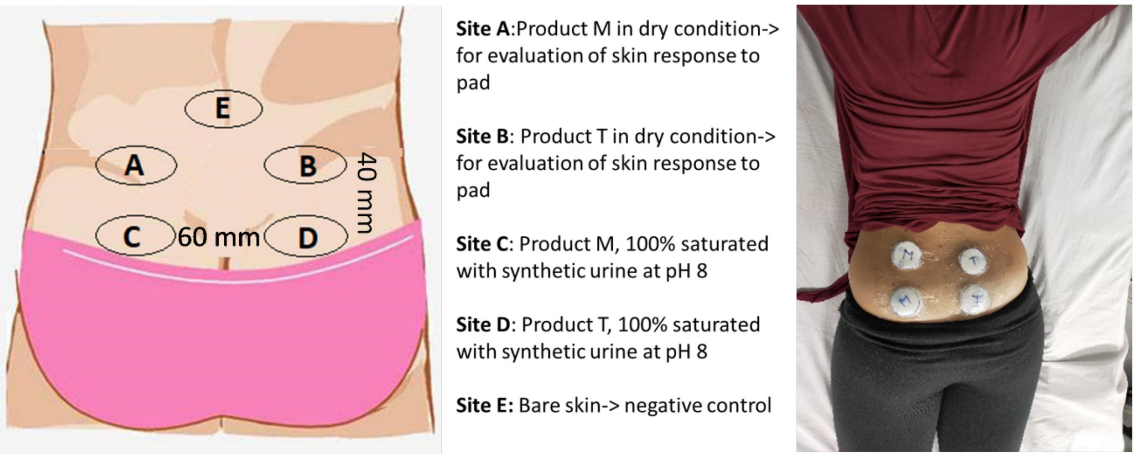


Figure 5.3. Experimental test sites on the sacral region of each participant. Sites separated by a distance of 40 mm vertically and 60 mm horizontally.

5.3.4 EVALUATION OF SKIN BIOPHYSICAL PARAMETERS

To investigate the integrity of the skin barrier, a series of non-invasive biophysical techniques were used, namely, transepidermal water loss (TEWL), Stratum Corneum (SC) hydration, skin surface pH, and erythema. All parameters were assessed following international guidelines (du Plessis et al. 2013; Stefaniak et al. 2013) and data was collected following the protocol described in section 3.3.1. In addition to biophysical parameters, inflammatory biomarkers were sampled in a biofluid namely, sebum, released from the surface of the skin and epidermal corneocytes were also collected from the sites of investigation. The quantification of biomarkers and analysis of corneocytes are beyond the scope of this doctoral thesis. Nonetheless, a brief description of the main findings will be detailed in chapter 9.

5.3.5 TEST PROTOCOL

The skin of the participants was challenged following a standardised protocol, as summarised in Figure 5.4. To review briefly, after 15 minutes of acclimatization, the baseline values for each skin parameter were recorded at each test site. Subsequently, the sacral sites of the participants, who were lying in a prone position, were exposed to samples made from both products i.e. products M or T, in a dry or saturated state for 60 minutes. At the end of this period, the incontinence products were removed for a brief period, the skin was blotted for excess fluid removal and an intermediate skin measurement using the biophysical methods was performed. This was followed by a further

60 minutes of skin exposure to incontinence products with the participants adopting a high sitting position (semi-Fowler's position). This involved the head of the bed being raised at 45 degrees and the foot of the bed flexing the legs, as illustrated in Figure 5.4. Interface pressures were monitored throughout the test period using a high-resolution sensing array (ForeSite PT, XSensor, Canada), at an acquisition frequency of 1Hz. The mat incorporates 5664 pressure measuring sensor cells, with a spatial resolution of 15.9 mm, covering a sensing area of 762 mm x 1880 mm. Each sensor operates within a range of 5 – 200 mmHg (0.7 – 26.6 kPa) and has reported manufacturer accuracy of ± 2 mmHg. At the end of the test period, equivalent to 120 minutes from the beginning of the test session, the individual samples were removed and the skin of the participants blotted to remove an excess of fluid. The skin parameters were again assessed at each site. In addition, the pH values of the saturated pads were also recorded. Participants were allowed a further 30 minutes of recovery in the prone position prior to a final skin assessment (Figure 5.4).

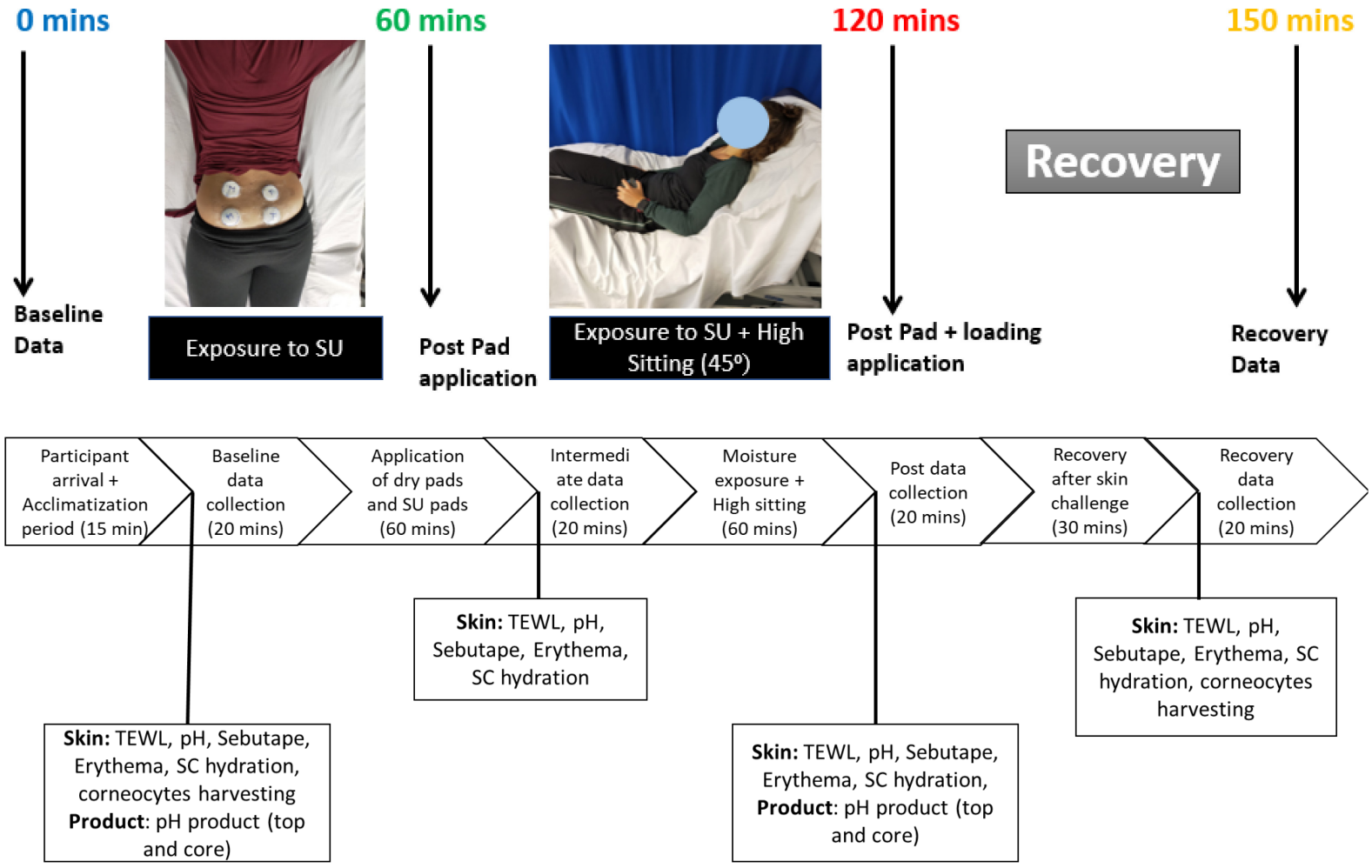


Figure 5.4. Summary of test protocol

5.3.6 INTERFACE PRESSURE MONITORING

Four interface pressure parameters were estimated from the monitoring data which was captured from the participants' sacrum and buttocks areas (Figure 5.13) during the high sitting posture between times 60 and 120 minutes. These included

- peak pressures
- contact area, defined as the area in which individual sensors exceeded 5 mmHg
- peak pressure index defined as the average pressure over a matrix of 3x3 cells
- peak pressure gradient, defined as the maximum change in pressure values between adjacent cells

5.3.7 STATISTICAL ANALYSIS

The data from each skin biophysical parameter were imported into Microsoft Excel (Microsoft 365, USA). In addition, the data were normalised to a baseline value (time point zero) to examine the fold differences in the parameters, as previously described by the authors (Bostan et al. 2019; Henshaw et al. 2020). Closer analysis of the data revealed their distribution was non-normal in nature and, as such, non-parametric analyses were used. Mann–Whitney U test was used to assess the independence of responses at the different time points of data collection. Subsequently, a rank sum test was used to compare the values across the experimental time frame as previously described (Bostan et al. 2019; Jayabal et al. 2021). The Friedman test was employed to investigate whether the challenges to the skin were able to induce temporal variations in parameters. Spearman correlation was employed to evaluate possible associations of the parameters with intrinsic and extrinsic factors. Tests were considered to be statistically significant at the 5% level ($p < 0.05$).

5.4 RESULTS

5.4.1 PARTICIPANTS

Twelve healthy volunteers, four males and eight females with a Body Mass Index (BMI) ranging from 18.5-37.7 kg/m² were recruited into the study. Table 5.2 summarises the anthropometric and demographic information of the participants. Due to the nature of the age, participants were divided into two separate cohorts presenting with two different age ranges namely, from 32 to 39 years old (P1 – P6) and from 50 to 62 years old (P7 – P12). Participants were derived from three ethnic backgrounds, although the vast majority were of a White ethnicity (10/12).

Table 5.2. Demographic and Anthropometric data of study participants

Participant ID	Gender	Age (years)	Ethnicity	BMI kg/m^2
P1	Female	32	White	21.9
P2	Male	36	White	32.1
P3	Female	32	White	19.8
P4	Male	39	Black	23.6
P5	Male	39	White	29.4
P6	Female	35	Mixed Ethnicity	29.8
P7	Female	59	White	24.1
P8	Female	62	White	18.5
P9	Female	54	White	34.7
P10	Male	51	White	25.7
P11	Female	56	White	37.7
P12	Female	50	White	25.1

5.4.2 TRANSEPIDERMAL WATER LOSS (TEWL)

In the present study, several TEWL temporal trends were evident, as illustrated in Figure 5.5, particularly associated with the sites C and D, exposed to the saturated pad samples.

Indeed, a 2.0-5.5 fold increase in absolute TEWL values was evident at these two sites. At site C, after 60 minutes of exposure to moisture, the values increased to a range of 28.2 to 49.5 g/h/m², representing a statistically significant difference from baseline (median = 35.1, interquartile range (IQR) = 6.3) values ($p<0.001$). With the added application of pressure, TEWL values at 120 minutes were in a similar range, namely between 32.2 and 45.8 g/h/m^2 (median = 37.7, IQR = 6.6). The difference between the unloaded (60 minutes) and loaded (120 minutes) states was not statistically significant.

A similar statistically significant difference ($p<0.001$) was revealed at site D immediately after exposure to saturated samples of the incontinence products T. However, contrary to site C, the application of pressure in the period 60 – 120 minutes, generated a further increase of this parameter, which was statistically significant ($p< 0.05$). Indeed, 10 out of 12 participants exhibited higher TEWL responses following skin loading, which ranged from 33.3 to 47.7 g/h/m^2 (Figure 5.5).

By contrast, minimal variations were detected at the dry sites (A and B) with some participants exhibiting significant increase ($p<0.05$) in TEWL following the application of pressure, i.e., 120 minutes, at site A, although the absolute TEWL values did not exceed 30 g/h/m^2 for any of the participants across the sites.

Figure 5.5 also revealed that, although there were still significant differences ($p<0.05$), however, absolute TEWL values decreased at sites C and D towards a return to baseline values across the cohort following the 30-minute recovery period. In addition, there were no time-dependent changes at the control site, E, at any time point over the test session.

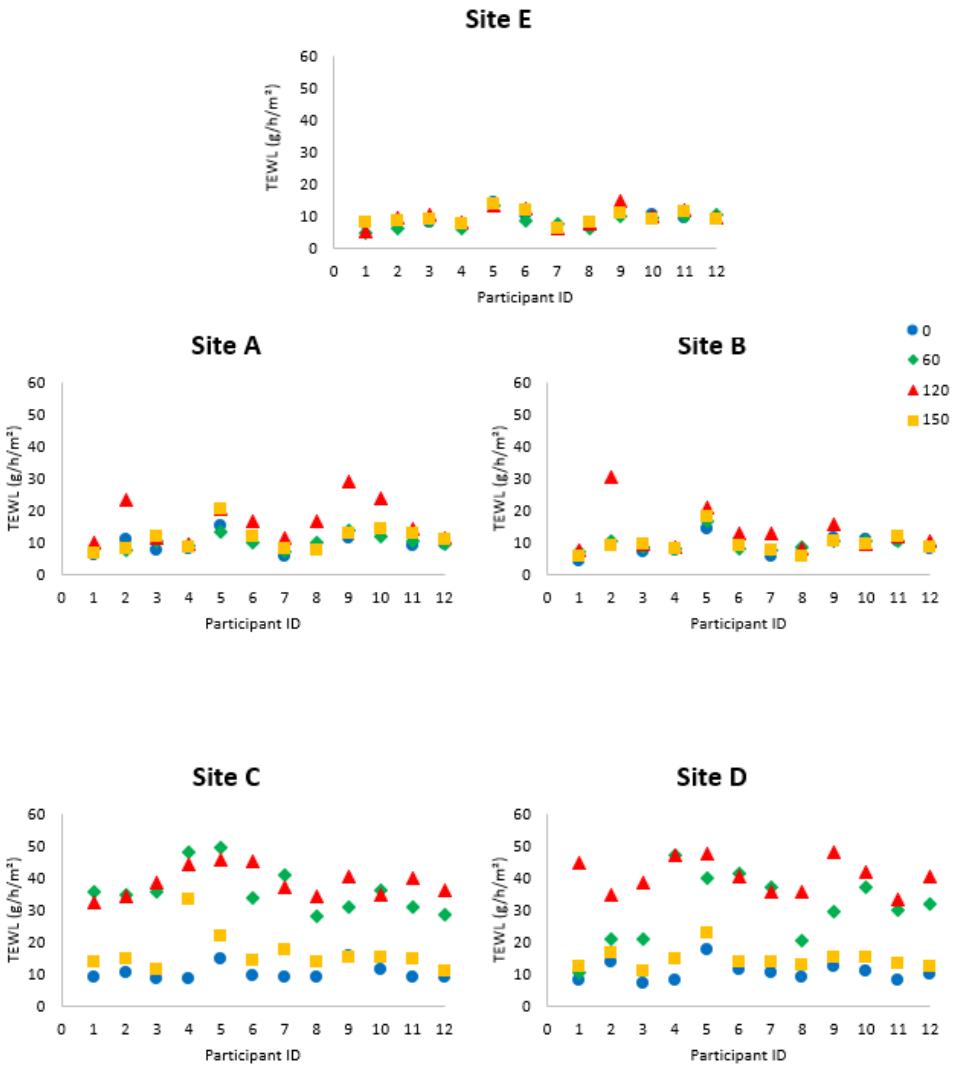


Figure 5.5. Changes in absolute TEWL values at the five sacral sites of each participant over the test session. While a significant increase in TEWL is detected at sites C and D, minimal variations are observed at the dry and control sites

5.4.2.1 RANKING OF TEWL PARAMETER OUTPUTS

In an alternative presentation, the individual values for absolute TEWL for each time point were ranked from 1 to 4 at each site. The results for sites C and D, as presented in Table 5.3, revealed that the highest total rank sums were clearly associated with time points 60 and 120 minutes. However, there were small differences between these sites, with site D having the highest rank associated with pressure and moisture exposure (120 min) for 10/12 of the participants.

When examining the total ranks associated with sites A and B, the maximum values generally occurred at 120 minutes, following the application of pressure. It is interesting to note that the total ranks following the recovery period were always higher when compared to the initial baseline values at each test site (Table 5.3).

Table 5.3. Rank sum of absolute TEWL values at the five sacral sites over the test session.

	SITE A (TIME mins)				SITE B (TIME mins)				SITE C (TIME mins)				SITE D (TIME mins)				SITE E (TIME mins)			
ID	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150
P1	1	3	4	2	1	3	4	2	1	4	3	2	1	2	4	3	3	1	2	4
P2	3	1	4	2	2	3	4	1	1	4	3	2	1	3	4	2	2	1	4	3
P3	1	2	3	4	1	2	4	3	1	3	4	2	1	3	4	2	1	2	4	3
P4	1	3	4	2	1	3	4	2	1	4	3	2	1	3	4	2	2	1	4	3
P5	2	1	3	4	1	2	4	3	1	4	3	2	1	3	4	2	4	2	1	3
P6	2	1	4	3	3	1	4	2	1	3	4	2	1	4	3	2	2	1	4	3
P7	1	2	4	3	1	3	4	2	1	4	3	2	1	4	3	2	2	4	1	3
P8	2	3	4	1	2	4	3	1	1	3	4	2	1	3	4	2	2	1	3	4
P9	1	3	4	2	3	2	4	1	2	3	4	1	1	3	4	2	3	1	4	2
P10	2	1	4	3	4	3	1	2	1	4	3	2	1	3	4	2	4	2	3	1
P11	1	2	4	3	2	1	4	3	1	3	4	2	1	3	4	2	1	2	4	3
P12	2	1	4	3	1	3	4	2	1	3	4	2	1	3	4	2	2	4	3	1
Total	**19**	**23**	**46**	**32**	**22**	**30**	**44**	**24**	**13**	**42**	**42**	**23**	**12**	**37**	**46**	**25**	**28**	**22**	**37**	**33**

5.4.3 STRATUM CORNEUM HYDRATION

The temporal profiles of SC hydration values at the five different locations are presented in Figure 5.6. The findings indicate: -

- Sites A and B show no consistent trend in skin hydration throughout the time course of the experiment. However, there were increases for some participants, i.e. P7, P9, P10, which was statistically significant from baseline ($p< 0.05$).
- There were no remarkable changes in hydration levels on the control site (site E), which presented a high degree of inter-subject variability.
- Values at site C increased significantly ($p< 0.001$) after 60 minutes of exposure to moisture followed by a slight decrease following the application of pressure. There was a general decrease to baseline for all participants, except P4, after the recovery period.
- Values at site D demonstrated an increase in skin hydration after 60 minutes of exposure to moisture ($p< 0.001$). Subsequent application of pressure yielded a small increase or decrease in values. There was a general decrease towards baseline for all participants, except P4 and P5, after the recovery period.
- It was interesting to note that SC hydration presented an improved recovery process compared to TEWL

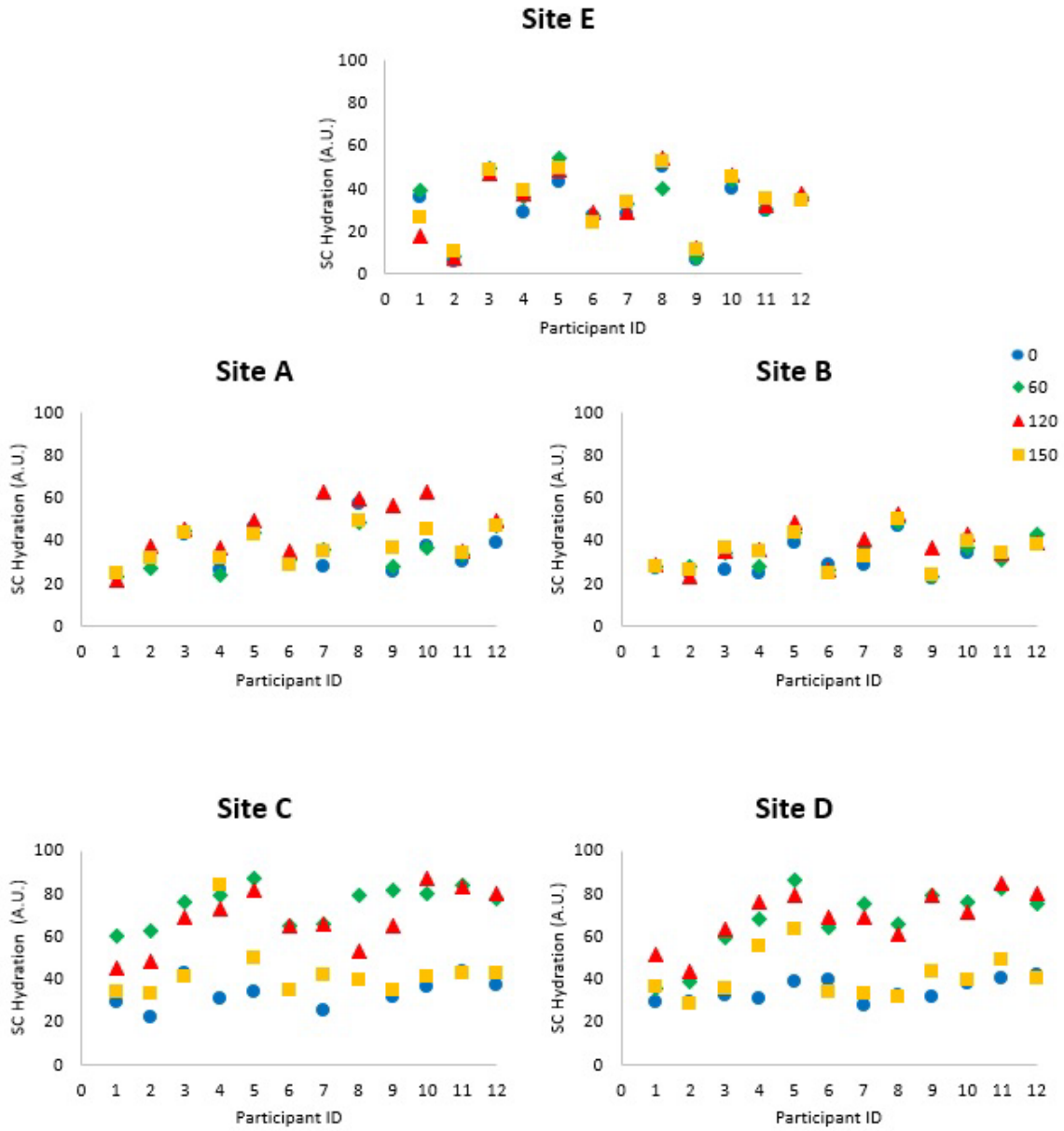


Figure 5.6. Changes in absolute skin hydration values at the five sacral sites of each participant over the test session. Significant increase at sites C and D following moisture exposure, exacerbated at site D with the application of pressure. Minimal changes observed at the control and dry sites.

5.4.3.1 RANKING OF STRATUM CORNEUM HYDRATION PARAMETER OUTPUTS

The ranking for SC hydration values for each time point is presented in Table 5.4. When examining the results associated with sites A and B, the maximum rank values generally occurred at 120 minutes, following the application of pressure after exposure to the two dry samples of incontinence products.

By contrast, data revealed the highest SC hydration values in site C were associated with exposure to moisture in the form of synthetic urine-saturated M pads in the absence of load. Indeed, there was a small decrease following the period of pressure application (Table 5.4). For site D, however, the application of pressure was more likely to maintain the high skin hydration response.

Skin hydration at control site E also increased during moisture and pressure application period, although in limited manner.

Table 5.4. Rank sum of absolute Stratum corneum hydration values at the five sacral sites over the test session.

	SITE A (TIME mins)				SITE B (TIME mins)				SITE C (TIME mins)				SITE D (TIME mins)				SITE E (TIME mins)			
ID	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150
P1	3	2	1	4	1	2	4	3	1	4	3	2	1	2	4	3	3	4	1	2
P2	2	1	4	3	2	4	1	3	1	4	3	2	2	3	4	1	1	3	2	4
P3	1	3	4	2	1	2	3	4	2	4	3	1	1	3	4	2	1	4	2	3
P4	2	1	4	3	1	2	4	3	1	3	2	4	1	3	4	2	1	2	3	4
P5	3	2	4	1	1	3	4	2	1	4	3	2	1	4	3	2	1	4	2	3
P6	1	3	4	2	4	2	3	1	1	3	4	2	2	3	4	1	2	3	4	1
P7	1	3	4	2	1	3	4	2	1	4	3	2	1	4	3	2	1	3	2	4
P8	3	1	4	2	1	2	4	3	2	4	3	1	2	4	3	1	2	1	4	3
P9	1	2	4	3	1	2	4	3	1	4	3	2	1	4	3	2	1	2	4	3
P10	2	1	4	3	1	2	4	3	1	3	4	2	1	4	3	2	1	2	4	3
P11	1	2	4	3	2	1	4	3	2	4	3	1	1	3	4	2	1	2	3	4
P12	1	3	4	2	3	4	2	1	1	3	4	2	2	3	4	1	2	3	4	1
Total	**21**	**24**	**45**	**30**	**19**	**29**	**41**	**31**	**15**	**44**	**38**	**23**	**16**	**40**	**43**	**21**	**17**	**33**	**35**	**35**

5.4.4 ERYTHEMA

The temporal profiles of the erythema values at the five different locations are presented in Figure 5.7. The findings indicate: -

- Participants presented with different levels of erythema across the time course of the experiment. Three participants i.e., P4, P5, and P6 exhibited higher values for each of the five test sites.
- Sites A and B exposed to the dry sample pads demonstrated a small significant ($p< 0.05$) increase in erythema value after the application of pressure at 120 minutes, particularly participants P10 and P11.
- It was interesting to note that Sites C and D exhibited a significant ($P< 0.05$) decrease in erythema values below those at baseline values for all participants after moisture exposure at 60 minutes, followed by a slight increase following the period of applied pressure.
- After the recovery period of 150 minutes, erythema values were generally similar or greater than baseline values at all challenged sites.
- Few time-dependent changes were detected at site E, with the exception of participants P8, P10, and P12, who displayed a slight increase at 120 minutes.

Given the unremarkable findings with erythema, rank analyses similar to those of TEWL and SC hydration were not performed.

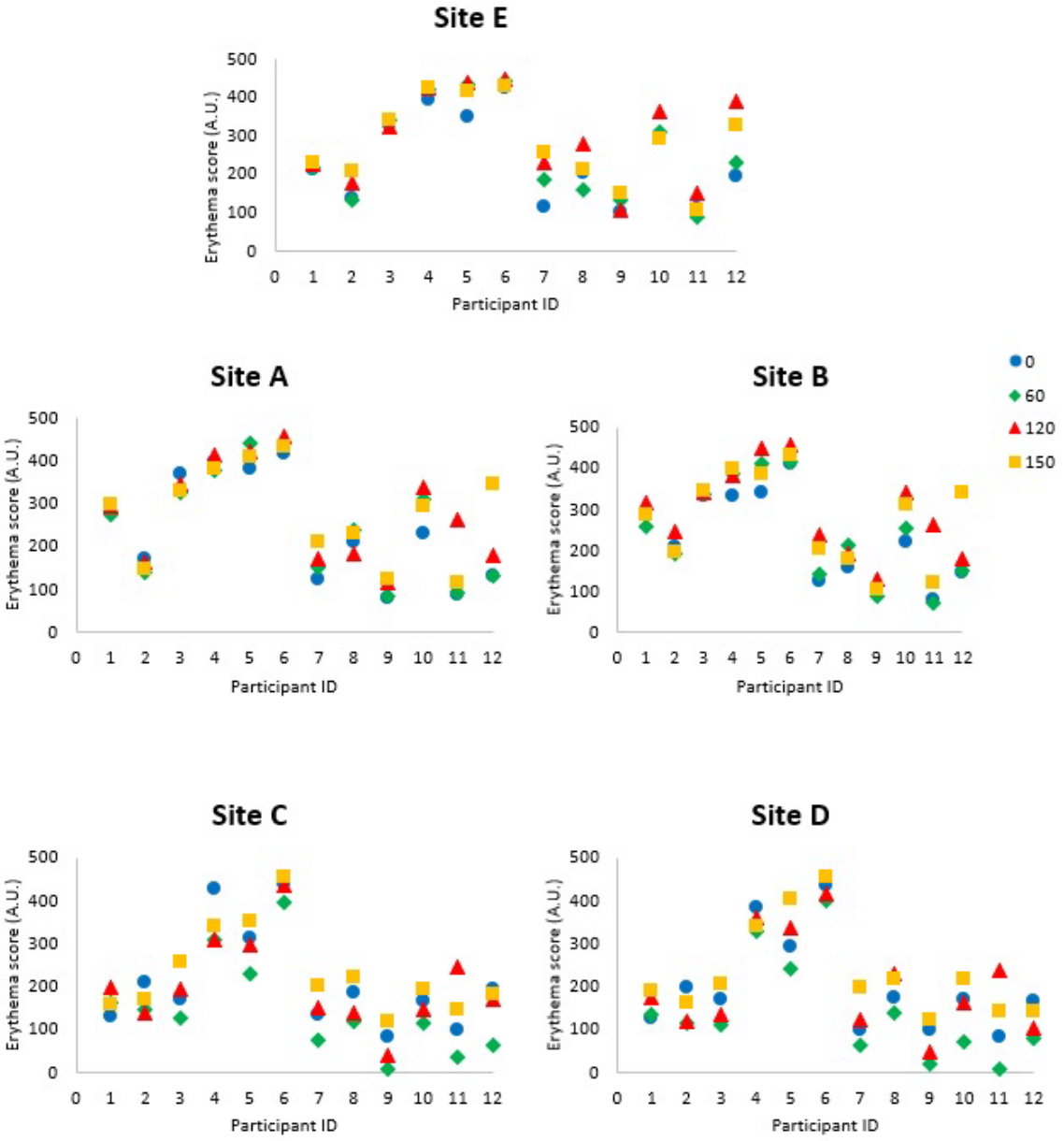


Figure 5.7. Changes in absolute erythema values at the five sacral sites of each participant over the test session. Significant inter-subject variation observed, with sites C and D exhibiting a decrease in erythema values following exposure to moisture

5.4.5 pH

The skin pH at each of the test sites throughout the test session is detailed in Table 5.5. The main findings are summarised as follows:

- The pH of all participants was maintained at a slightly acidic level throughout the test session
- Participants, P2 and P8, consistently displayed lower pH values at all test sites compared to the other participants
- No remarkable changes were evident at any of the test sites at the different time points of the data collection.

Table 5.5. Temporal pH profile at the five different sacral sites for all participants

	SITE A (TIME mins)				SITE B (TIME mins)				SITE C (TIME mins)				SITE D (TIME mins)				SITE E (TIME mins)			
ID	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150	0	60	120	150
P1	6.3	6.0	6.0	6.1	6.2	6.1	6.2	6.0	6.4	5.9	6.0	5.9	6.4	5.8	5.9	5.8	6.3	6.2	6.1	6.0
P2	4.8	4.8	4.9	4.8	4.7	4.7	4.8	4.9	5.3	6.0	5.9	5.6	5.2	5.6	5.9	5.7	4.8	4.7	5.2	5.1
P3	5.9	5.8	6.0	5.7	5.8	5.8	5.9	5.5	6.1	5.9	6.0	5.9	5.7	5.8	5.8	5.8	5.7	5.7	5.5	5.5
P4	6.2	6.0	6.1	6.2	6.4	6.2	6.3	6.2	6.7	6.1	6.3	6.3	6.7	6.2	6.2	6.0	5.7	5.9	5.9	5.9
P5	5.2	5.2	5.4	5.6	5.4	5.4	5.5	5.6	5.7	6.2	6.1	6.0	5.8	5.9	6.0	5.9	5.5	5.4	5.7	5.8
P6	6.2	6.0	5.9	6.1	6.0	5.9	5.9	6.1	6.8	6.0	6.2	6.1	6.4	6.0	6.1	5.9	5.7	5.7	5.9	5.9
P7	6.2	5.9	5.7	5.7	6.4	6.3	5.8	5.9	6.7	6.2	6.0	5.9	5.7	6.2	6.1	6.1	6.1	6.2	6.0	5.7
P8	5.5	5.6	5.2	5.2	5.5	5.6	5.7	5.5	5.6	5.7	5.8	5.8	5.6	5.8	5.9	5.8	5.3	5.4	5.4	5.5
P9	6.7	6.2	6.1	5.8	6.8	6.4	6.1	5.9	6.2	6.3	6.1	6.0	6.9	5.9	6.2	6.0	6.5	6.3	6.3	6.2
P10	5.5	5.3	5.3	5.4	5.7	5.4	5.6	5.6	5.8	5.9	5.9	5.8	5.9	6.0	6.0	5.9	5.2	5.9	5.5	5.5
P11	7.0	6.7	6.4	6.6	6.7	6.8	6.7	6.8	6.9	6.1	6.3	6.0	6.8	6.2	6.0	5.9	6.4	6.9	6.6	6.6
P12	6.1	6.0	5.8	5.8	6.1	6.0	6.0	6.0	6.0	6.0	6.0	5.9	6.1	6.1	6.1	6.1	5.9	5.9	6.0	6.0
Median	6.2	6.0	5.9	5.8	6.1	6.0	5.9	5.9	6.2	6.0	6.0	5.9	6.0	6.0	6.0	5.9	5.7	5.9	5.9	5.9
Range	2.2	1.9	1.5	1.8	2.1	2.1	1.9	1.9	1.6	0.6	0.5	0.7	1.7	0.6	0.4	0.4	1.7	2.2	1.4	1.5

5.4.6 pH OF INCONTINENCE PRODUCTS

pH values at the surface and core of the two different incontinence products were analysed before and after skin challenge at sites C and D. The findings are illustrated in Figure 5.8.

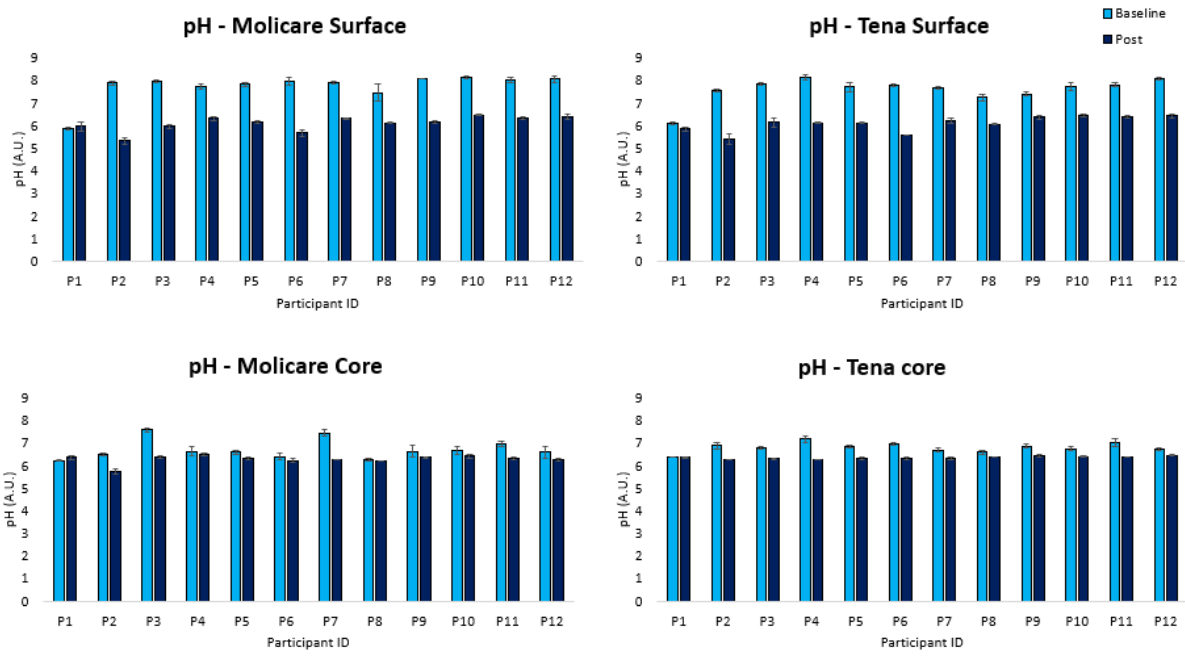


Figure 5.8. pH variation measured at both the surface and the core of Molicare and Tena sample pads before and after skin challenge.

The results revealed that both surface and core pH values of the two products were all in the acidic range (≤6.5) at the end of the test session. In 11 out of 12 participants, the baseline pH values at the surface of both types of sample pads were higher than the post-test session values by a difference ranging from 0.2 to 2.6 AU.s. This trend was not evident with P1 where the values were similar, approximately pH 6.0 for both pads.

In the majority of participants, the baseline pH values at the core of both types of sample pads were higher than the post-test session values by a difference ranging from 0.2 to 1.2 pH units. This trend was not evident with P1. Post application pH values ranged from 5.3 (product M) to 6.5 (product T) on the surface for both incontinence pads. The corresponding core values were 5.74 (product M) to 6.52 (product M).

5.4.6.1 INCONTINENCE PRODUCTS BUFFERING CAPACITY

Further investigation evaluated the buffering capacity on the skin underneath the products saturated with SU, namely, at sacral sites C and D. The pH values at baseline and post-challenge on the surface of both the skin and the incontinence pads are summarised in Table 5.6.

It was evident that at baseline the skin pH values were in the acidic range at both sites, whereas the surface basal values of the samples of the products, immediately after saturation with SU, were in the neutral or alkaline pH range. By contrast, at the end of the test session, the pH values of the surface of both products were acidic in the range of 5.3 to 6.5 and 5.4 to 6.5 for products M and T, respectively. The skin pH was maintained within the acidic range throughout the session for both test sites.

Table 5.6. Buffering capacity effect of incontinence products on the skin of participants

	Skin (site C)			*Product M (site C)*			*Skin (site D)*			*Product T (site D)*		
ID	*Baseline*	*Post*	Δ	*Baseline*	*Post*	Δ	*Baseline*	*Post*	Δ	*Baseline*	*Post*	Δ
P1	6.4	6.0	0.4	5.9	6.0	-0.1	6.4	5.9	0.4	6.1	5.9	0.2
P2	5.3	5.9	-0.6	7.9	5.3	2.6	5.2	5.9	-0.6	7.6	5.4	2.1
P3	6.1	6.0	0.1	8.0	6.0	2.0	5.7	5.8	-0.1	7.9	6.2	1.7
P4	6.7	6.3	0.5	7.8	6.3	1.4	6.7	6.2	0.4	8.1	6.1	2.0
P5	5.7	6.1	-0.4	7.8	6.2	1.7	5.8	6.0	-0.3	7.7	6.1	1.6
P6	6.8	6.2	0.5	8.0	5.7	2.3	6.4	6.1	0.3	7.8	5.6	2.2
P7	6.7	5.9	0.7	7.9	6.3	1.6	5.7	6.1	-0.4	7.7	6.2	1.5
P8	5.6	5.8	-0.2	7.5	6.1	1.3	5.6	5.8	-0.3	7.3	6.1	1.2
P9	6.2	6.0	0.1	8.1	6.2	1.9	6.9	6.0	0.8	7.4	6.4	1.0
P10	5.8	5.8	-0.1	8.2	6.5	1.7	5.9	5.9	-0.1	7.8	6.5	1.3
P11	6.9	6.0	0.7	8.0	6.4	1.7	6.8	5.9	0.8	7.8	6.4	1.4
P12	6.0	5.9	0.0	8.1	6.4	1.7	6.1	6.1	0.0	8.1	6.4	1.6

5.4.7 CORRELATIONS WITH INTRINSIC FACTORS

Given that SC hydration and TEWL were the only parameters which highlighted significant variations following challenges, they were selected for further analysis pertaining to their associations with selected intrinsic factors, namely, BMI and age. As an example, when the SC hydration at the negative control site (E) was examined with respect to the BMI of the participants a negative trend was observed, as illustrated in Figure 5.9. The correlations were found to be statistically significant at time points 0 minutes (r_s= -0.65, p= 0.02) and 60 minutes (r_s= -0.62, p= 0.03), while a non-statistically significant trend was observed at 120 minutes (r_s= -0.49, p= 0.11) and 150 minutes (r_s= -0.53, p= 0.08). Examination for each of the four other test sites (A – D), revealed no further significant correlations between SC hydration and BMI.

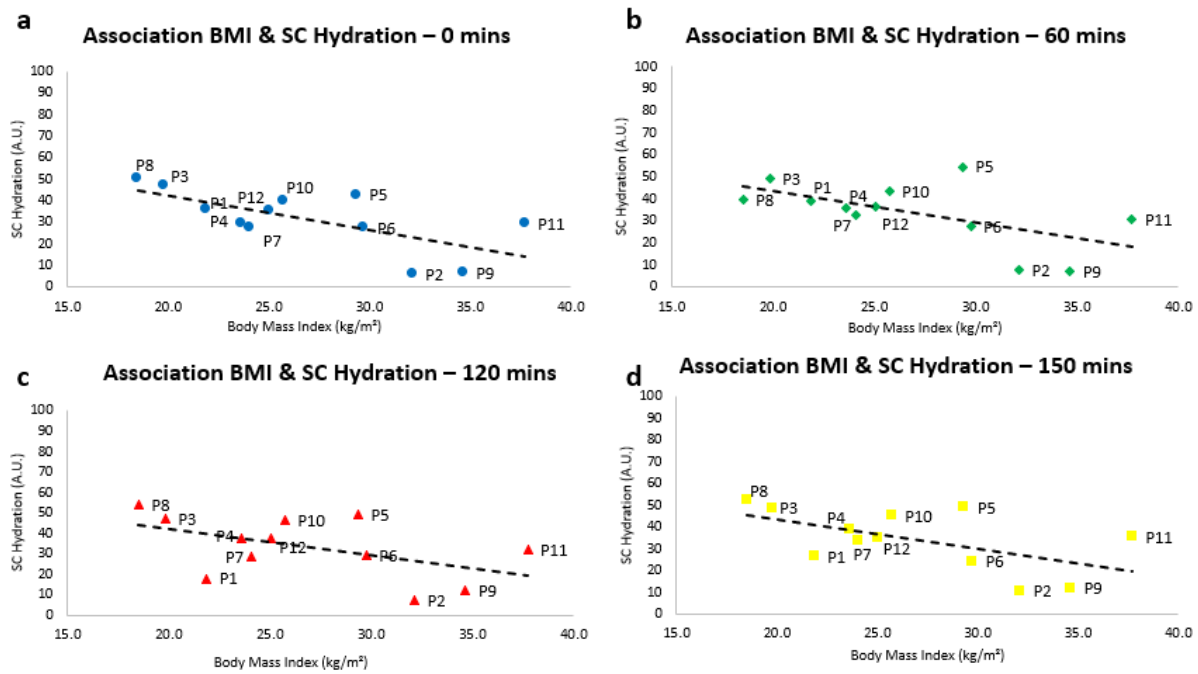


Figure 5.9. Correlation between individual body mass index and SC hydration at control site E. An inverse relationship was evident between the two variables which were statistically significant i.e. p<0.05, at time points 0 and 60 minutes.

A similar approach was employed to examine the relationship between BMI and TEWL values. In this case, there was no significant correlation at any of the test sites, as illustrated at 60 minutes in Figure 5.10.

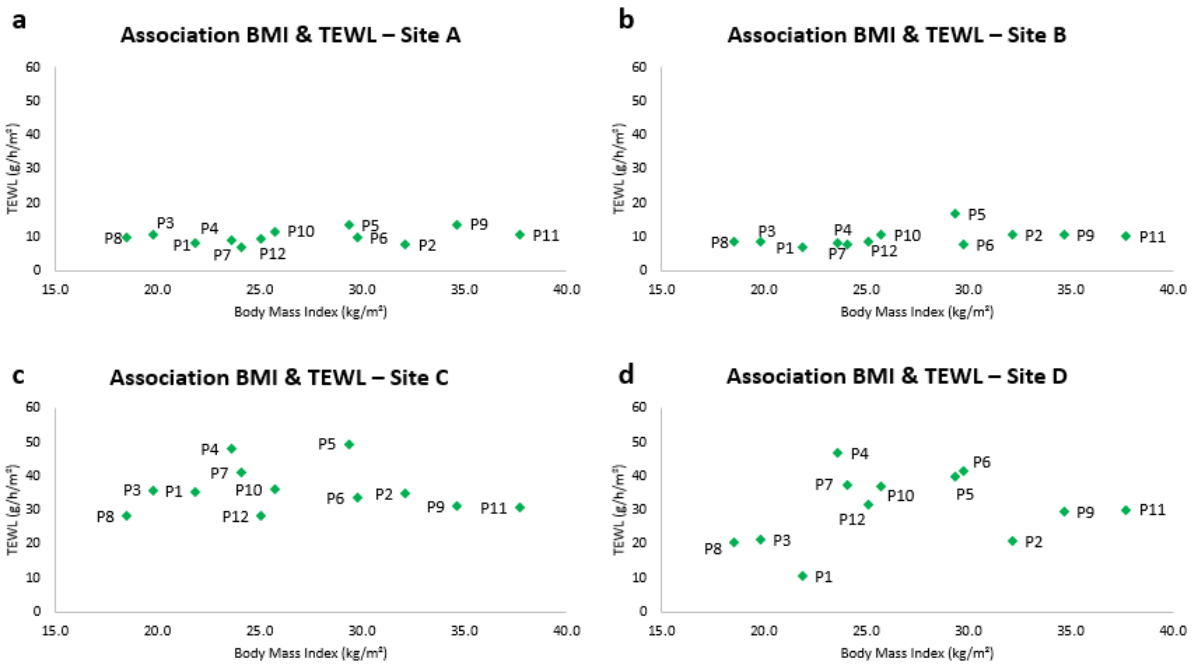


Figure 5.10. Relationship between absolute TEWL value and individual body mass index (BMI) at time point 60 minutes at four anatomical sites of assessment when exposed to dry sample pads (A and B) and saturated sample pads (C and D).

When both biophysical parameters, namely SC hydration and TEWL, were examined with respect to the age of the participant, no remarkable and/or consistent trends were evident for any of the test conditions.

Nonetheless, close examination of the data related to TEWL did reveal some differences at sites C and D, as illustrated at 60 and 120 minutes in Figure 5.11. For example, at site C, similar TEWL responses were detected in both young and older individuals following the exposure to moisture (60 minutes), with those presenting with elevated responses e.g. P4 generally maintaining their values at 120 minutes following the application of pressure. By contrast, at site D, the responses to moisture were variable. In particular, whereas some participants i.e. P1, P2 and P3 demonstrated relatively low values at 60 minutes, the responses for both the other three young participants and all the older participants were elevated following moisture exposure. By contrast, following the application of pressure, all younger participants displayed an elevated TEWL response, whereas two of the older participants i.e P7 and P11, displayed reduced values at 120 minutes.

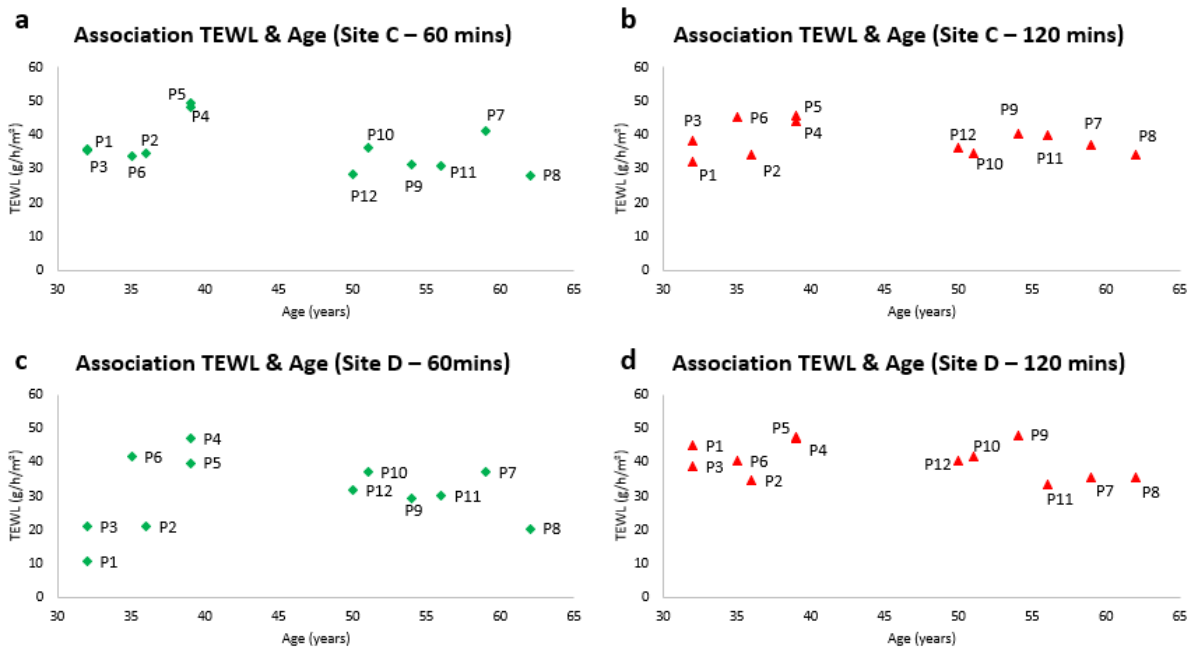


Figure 5.11. Relationship between absolute TEWL value and individual age at two different time points (60 and 120 minutes) when exposed to both saturated sample pads (i.e. sites C and D).

There were no remarkable associations between the SC hydration values and the age of participants, as illustrated in Figure 5.12.

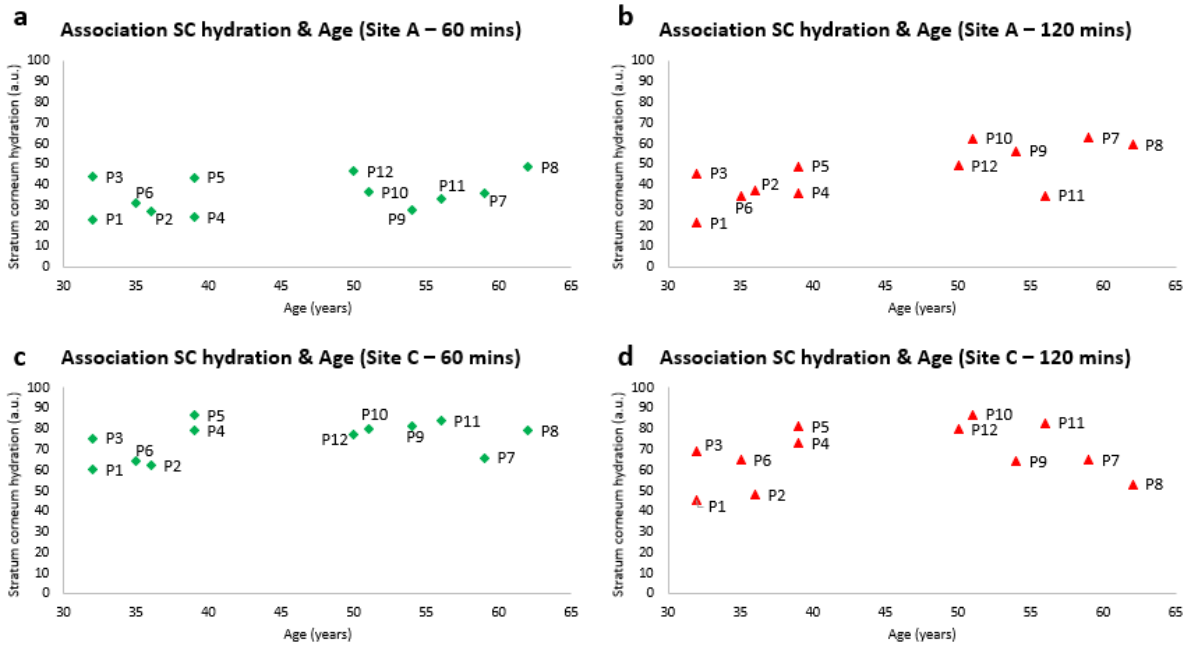


Figure 5.12. Relationship between SC hydration value and individual age at two different time points (60 and 120 minutes) when exposed to either dry sample pads (A) or saturated sample pads (C).

5.4.8 INTERFACE PRESSURE MONITORING

Data from the interface pressure monitoring system revealed distinct profiles for each participant, as exemplified by the pressure distribution of two randomly chosen participants, P3 (Figure 5.13A) and P8 (Figure 5.13B) who displayed one of the lowest and the highest peak pressure gradient, respectively.

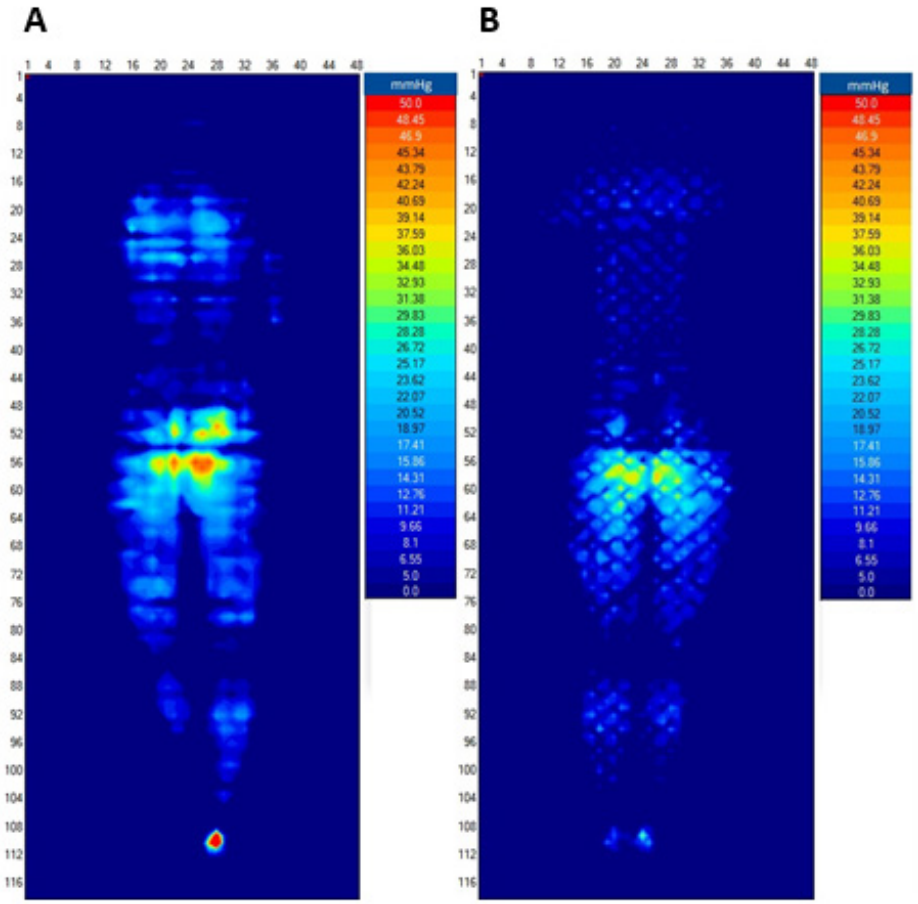


Figure 5.13. Temporal profiles of pressure distribution profile for two participants A) P3 and B) P8 over the 60-minute period in the high sitting posture. Elevated pressure could be noted at the sacral area.

Overall, these parameters ranged from 11.4 (P4) to 21.7 (P5) mmHg and from 20.7 (P4) to 55.9 (P8) mmHg/mm for peak pressure index and peak pressure gradient, respectively. The corresponding values for single peak pressure values were 41.4 (P8) and 64.8 (P9) mmHg, while the contact area ranged from 1182.4 – 2482.1 cm². A summary of these values for each participant is presented in Table 5.7.

Table 5.7. Median, range and interquartile range (IQR) of the four interface pressure parameters for each participant in the high-sitting posture

Participant ID	Peak pressure (mmHg)		Peak pressure index (mmHg)		Peak pressure gradient (mmHg/mm)		Contact area (cm^2)	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
P1	44.3	21.8	13.8	3.9	27.0	35.3	1529.5	323.6
P2	64.5	13.5	19.5	2.3	26.3	9.8	2040.2	305.9
P3	46.5	66.8	16.1	6.9	21.8	48.0	1400.6	611.8
P4	48.0	17.3	11.4	2.2	20.3	24.8	1190.7	311.0
P5	67.5	129.8	21.8	9.4	24.8	29.3	2045.2	715.5
P6	66.8	36.8	21.7	4.8	30.0	31.5	2065.5	318.5
P7	49.5	73.5	15.8	8.6	29.3	45.0	1481.5	912.6
P8	42.8	96.0	12.3	10.4	56.3	195.0	1271.6	988.5
P9	64.5	30.8	19.3	6.3	30.8	57.0	2490.2	351.4
P10	63.0	9.0	20.5	1.4	29.3	33.8	2113.5	106.2
P11	58.5	11.3	21.6	5.7	26.3	19.5	2341.0	654.8
P12	51.0	25.5	18.5	5.6	27.8	51.0	2027.5	219.9

5.4.8.1 INTERFACE PRESSURE ANALYSIS WITH RESPECT TO TEWL AND SC HYDRATION

Further analysis of the data derived from the pressure monitoring parameters was examined to determine possible associations with TEWL and SC hydration.

Interesting trends, although weak, were detected at the dry sites, namely A (r_s= 0.58, p= 0.05) and B (r_s= 0.72, p= 0.01), when TEWL parameter was associated with the peak pressure index. Indeed, data revealed increasing TEWL values with the increase in peak pressure index. By contrast, such trends were not detected at the moistened sites, C (r_s= 0.45, p= 0.14) and D (r_s= -0.01, p= 0.98), with individuals with high and low peak pressure index presenting similar TEWL responses (Figure 5.14). The association of TEWL with peak pressure gradient did not yield trends at any of the investigational sites.

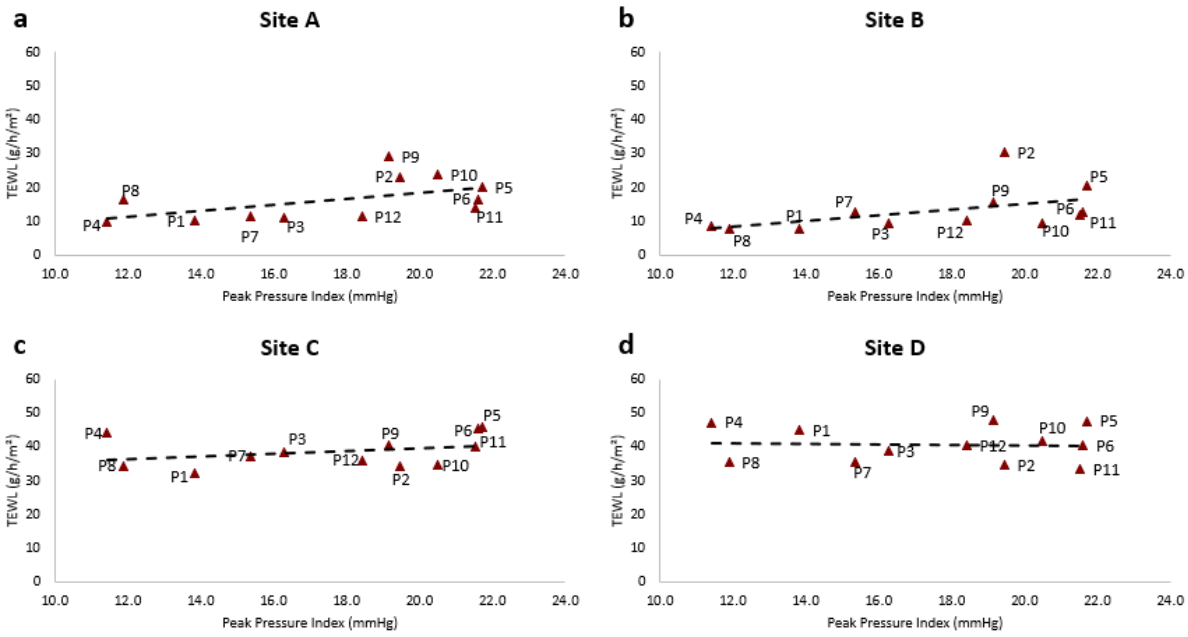


Figure 5.14. Association between Peak pressure index and TEWL at both dry (A and B) and moisture-insulted (C and D) sites. A weak trend detected between TEWL and peak pressure index

Analysis was performed to examine the association of SC hydration with the pressure monitoring parameters. While it was observed positive trends towards higher skin hydration responses with the increasing peak pressure index at the sites under moistened conditions, C (r_s = 0.42, p= 0 .17) and D (r_s = 0.39, p= 0.21), such trends were not evident at the sites challenged with dry incontinence products (A and B) (Figure 5.15). No findings worth mentioning were observed in the association between peak pressure gradient and skin hydration.

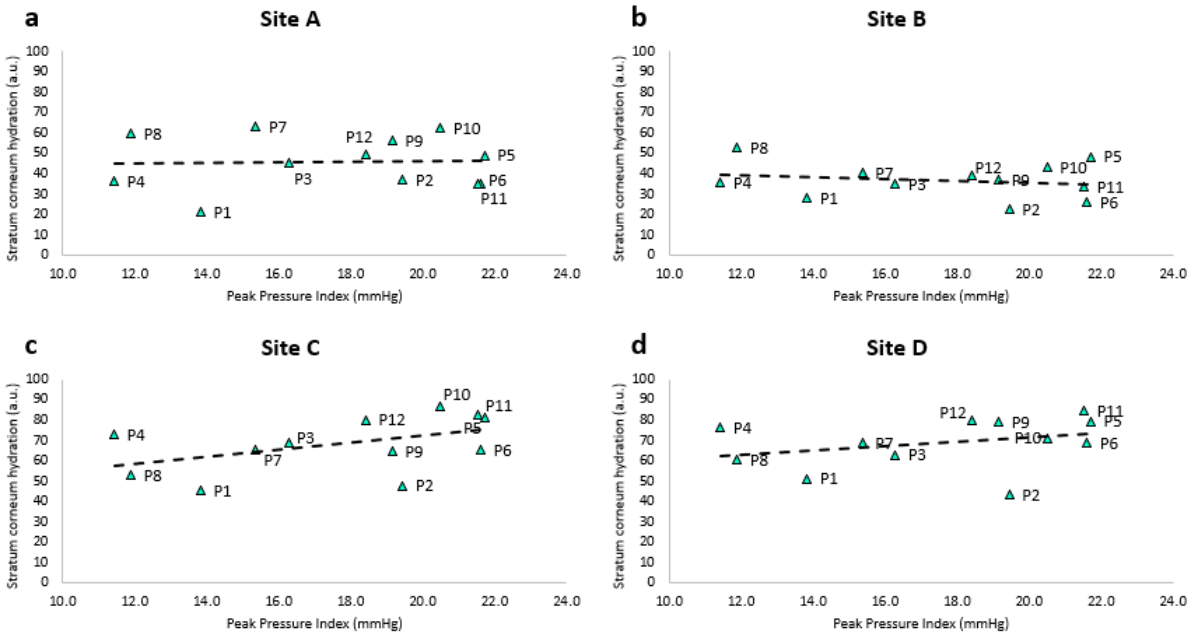


Figure 5.15. Association between Sc hydration parameter and peak pressure index. Weak associations were detected at the sites under moistened conditions, namely site C and site D.

5.5 DISCUSSION

This study was designed to examine changes in specific parameters reflecting skin health following the application of continence pads, either dry or saturated with synthetic urine (SU), to a cohort of healthy participants from two distinct age ranges. Data revealed significant increases in TEWL and SC hydration values at the sites exposed to saturated pads (60 minutes), which were maintained or exacerbated following the application of mechanical load corresponding to a high sitting posture. (Figures 5.5 and 5.6). These findings could be attributed to the combination of prolonged skin interaction with moisture in the presence of occlusion. In all cases after a 30-minute recovery period free of the sample pads, TEWL and hydration values reduced partially towards the baseline, with no evidence of prolonged skin changes. Other biophysical (erythema and pH) parameters of skin health did not reveal any consistent and significant trends across the cohort, although sub-groups of participants demonstrated elevated responses beyond the normal variation in the data.

Several studies have reported the detrimental implications of occlusion in terms of the overhydration of the stratum corneum, increase in the friction coefficient, and increase in skin susceptibility to damage (Phipps et al. 2019; Gerhardt et al. 2008). The present findings are consistent with previous studies, which examined the impact of exposure to moisture (water or sodium lauryl sulphate solution) and mechanical loads at a range of skin sites e.g. sacrum, and heel, which demonstrated disruption to skin barrier function (Firooz et al. 2015; Tomova-Simitchieva et al. 2018; Bostan et al. 2019). The data showed no remarkable differences in TEWL and skin

hydration values at the dry-occluded pad sites, although a few participants displayed small increases in TEWL following exposure to mechanical loads resulting from the high sitting posture (Figure 5.5). Based on the data, it is tempting to assert that the use of these engineered incontinence products limited the implication of occlusion on skin parameters. The skin hydration values, however, were influenced when the pads were saturated with SU (Figure 5.6). This is not surprising as the SC hydration method estimates the water gradient and as such, the superimposition of mechanical loads on saturated pads could play a role in enhancing the infiltration of water into the skin, thus altering the flow gradient (Jansen van Rensburg et al. 2019). In addition, both the TEWL and skin hydration values tended to return partially towards baseline values following the 30-minute recovery period, demonstrating that the changes at the insulted sites represent transient disruptions in skin barrier function and hydration.

No consistent changes were observed in the parameter reflecting erythema during the time course of the test session. Interestingly, some individuals demonstrated a decrease in erythema below basal levels following exposure to SU, with values generally returning to baseline after the recovery period. These findings are consistent with those from other studies which demonstrated no considerable changes in skin redness post exposure to moisture and/or mechanical loading (Jayabal et al. 2021; Denzinger et al. 2020). In addition, the unremarkable findings could be attributed to the lack of sensitivity of this parameter, as measured using the Mexameter, where outputs have been previously reported to be highly influenced by skin colour, anatomical location (see chapter 7, section 7.4.3) and various environmental factors including temperature and humidity (Denzinger et al. 2020). Nevertheless, in this particular study, it could be hypothesised that water penetration and over-hydration of the skin might have influence Mexameter outputs. Indeed, the device quantifies skin colour base on the concept of absorbance and reflectance. Water penetration and over-hydration can affect skin colour by altering the amount of water in the skin, which can affect light absorption and scattering properties. Over-hydration can cause swelling of the skin cells, which can increase the scattering of light and cause the skin to appear lighter or brighter (Chon et al., 2018).

With respect to pH, the study demonstrated that the skin pH of all participants remained in the acidic range when exposed to both dry and saturated pads with SU (pH of 8.0), irrespective of the incontinence product. These findings contrast with other studies where an increase in pH was reported (Koudounas et al. 2021; Koudounas et al. 2020). This difference could be a result of the different pad designs and compositions used across the studies. The analysis of the pad pH before and after it was in-situ revealed a transition of the products' surface pH from neutral or alkaline to an acidic pH and maintenance of the acidic core pH throughout the test session. The behaviour of the pads might have contributed to maintaining the acidic mantle of the skin, which was observed for both test products M and T. The ability of both non-woven and curly fibre pads to support the

skin in maintaining its barrier function following episodes of incontinence has been previously reported (Rippon et al. 2016; Ajmeri et al. 2011).

There were no consistent correlations between biophysical TEWL and SC hydration parameters with BMI and age of the participants. The results of this study are consistent with previous reports where no associations were found between TEWL and skin hydration with BMI (Iizaka 2018) and age (Jayabal et al. 2021). Nevertheless, these findings contrast with other studies where a significant influence of age on skin candidate biophysical parameters was detected (Cho et al. 2019; Firooz et al. 2012). The differences in the results reported between the studies could be due to the adoption of different testing protocols. Indeed, in these studies, TEWL and skin hydration values were measured in the absence of skin insults. In addition, interesting weak correlations were detected between the biophysical and interface pressure parameters. Indeed, at the dry sites, a significant positive correlation of peak pressure index with TEWL was observed. The mechanism underpinning this association is not fully understood, however, it could be a consequence of further skin barrier impairment due to the elevated pressure at the skin interface. More research is needed to elucidate this concept.

The study is limited by its small sample size, involving a lack of ethnic background diversity, which limits the generalisability of its findings. In addition, the two age groups in the cohort were separated by only 10 years. Also, the study did not recruit individuals over the age of 70 years, who are more representative of users of incontinence products and have changes in local skin structure and function which would increase their risk of skin damage following exposure to urine and mechanical loads. The study is also limited by both the practicality of the test session and the time exposure for each test condition, with the pads removed following the 120 minutes of application. This may not reflect the situation in care settings, where pads can be left in-situ for prolonged periods, increasing the risk of skin damage (Bliss et al. 2011). Furthermore, the ambient condition might have influenced the outputs of the parameters. However, a pragmatic approach was used to minimise the influences of external factors on the outputs of the skin parameters. Indeed, all the assessments were conducted in a temperature and humidity-controlled laboratory environment. In addition, the test sections were performed at similar hours of the day to reduce the impact of circadian influences. Nonetheless, the trivial findings when the two cohorts of participants were compared might be a result of the different seasons of recruitment and skin assessment. In particular, the young cohort was recruited and assessed during winter while the old cohort was subjected to skin challenges and assessment during summer. The influence of seasonal variation on the outputs of biophysical parameters has been previously reported (Green et al. 2022)

One of the major functions of the skin is to protect the body from the external environment by the provision of a physical barrier, achieved by maintaining the integrity of the epithelium (Graham et al. 2019). Absorbent products are used to contain body waste products in individuals with incontinence, however, this study has demonstrated marked changes in skin barrier function and hydration even after relatively short exposure periods. Thus, regular changing of these pads to reduce the time of interaction is essential to maintain skin health. Indeed, in the absence of moisture, minimal variations in the barrier properties were detected even in the presence of mechanical loads. Accordingly, caregivers must implement an efficient continence management regimen, as well as regular checks to enable skin off-loading, particularly in elderly immobile individuals who might present with impaired tolerance to moisture and mechanical loads.

5.6 CONCLUSION

This study employed an array of biophysical and biomechanical parameters to characterise skin health following periods of exposure to incontinence pads with and without synthetic urine. The study highlighted the potential beneficial effects of incontinence products when employed in a dry state. However, the presence of moisture alone or in combination with mechanical loads caused changes in skin barrier function and hydration, which were restored following 30 minutes of recovery period. There is therefore a compelling need to ensure that individuals presenting with incontinence have regular pad changes, as well as periodic intervals of skin off-loading.

Chapter 6 A CROSS-SECTIONAL SURVEY OF ADVERSE SKIN REACTIONS TO PROLONGED USE OF PERSONAL PROTECTIVE EQUIPMENT (PPE)

The results of this study were published in 2021:

Abiakam, N, *Worsley, P, Jayabal, H, Mitchell, k, Jones, M, Fletcher, J, Spratt, F and Bader, D. Personal protective equipment related skin reactions in healthcare professionals during COVID-19. Int Wound J. 2021; 18: 312– 322. https://doi.org/10.1111/iwj.13534*

&

Chopra J, ***Abiakam N****, Kim H, Metcalf, C, Worsley, P and Cheong, Y. The influence of gender and ethnicity on facemasks and respiratory protective equipment fit: a systematic review and meta-analysis. BMJ Global Health 2021;6: e005537.*

6.1 INTRODUCTION

The outbreak of COVID-19 has resulted in clinical staff worldwide employing protective measures whilst providing care to vulnerable patients. The use of Personal Protective Equipment (PPE) has given healthcare workers, who are predominantly female, a high level of safety by limiting direct contact between clinical staff and patients (Montero-Vilchez et al. 2021). However, the prolonged application of PPE during clinical shifts can affect skin health. Indeed, to provide protection against airborne particle transmission, devices such as respiratory protective equipment (RPE) are tightly fastened to the face to create an airtight seal. However, these masks have been traditionally designed for a white male workforce, providing a limited range of size and geometry (Trades Union Congress 2017). This can lead to overtightening to compensate for a poor fit of smaller and different-shaped faces associated with females and non-Caucasian ethnicities, which can result in soft tissue damage, as well as an increased risk of infection (Sari et al. 2022). Staff are required to accommodate critical clinical duties under this challenging condition, with a limited number of periods to permit skin recovery. Furthermore, staff are called to employ these single use devices for prolonged periods in order to mitigate PPE shortage issues (Ranney et al. 2020).

When the RPE devices are applied they can create pressure, shear, and friction at the skin interface which is potentially sustained over an entire working shift of 10-12 hours. In addition, due to the mental and physical stress on individual staff members who are managing COVID-19 patients, the skin can be further compromised by moisture, originating from excess sweating. Indeed, the exposure to moisture leads to the reduction of the strength and stiffness of the outermost skin

layer, the stratum corneum (SC), thereby reducing the overall tolerance to mechanical loading (Kottner et al. 2018). Particular anatomical regions at risk are, for example, the bridge of nose, cheeks and forehead representing vulnerable areas on the face (Etgu et al. 2021). Indeed, research in the host lab has revealed high interface pressures at the bridge of the nose during respiratory mask and RPE application (Caggiari et al. 2022; Worsley et al. 2016). The combination of altered microclimate at the skin-RPE device interface and prolonged exposure to pressure and shear forces can result in device-related pressure damage to the skin and underlying tissues (Worsley et al. 2020).

Although there are reports of skin damage from using PPE, there is a paucity of empirical evidence, particularly with respect to healthcare workers (HCWs). This has prompted some recent studies to examine the relationship between the long-term use of PPE and adverse skin reaction of HCWs (Akl et al. 2021). Studies reported that indentation from respirators was evident over the nasal bridge in cohorts of HCWs. In addition, HCWs also reported dry skin, acneiform eruptions, itchiness and skin rashes as a result of prolonged PPE usage (Manookian et al. 2022). Despite this relatively high incidence of adverse skin reactions, there are no definite recommendations in terms of length and frequency of PPE usage by HCWs. In addition, there is a paucity of evidence pertaining to the types of devices (manufacturer and model) which commonly cause skin reactions, with a range of PPE devices being used both within and between different healthcare institutions. Furthermore, there is a scarsity of empirical evidence pertaining to the time dependent skin behaviour following the reports of adverse reactions.

6.1.1 RESEARCH QUESTIONS

1. What is the point prevalence of skin reactions to personal protective equipment in a hospital setting?
2. What proportion of healthcare workers report skin reactions from wearing PPE in hospitals?
3. Which anatomical sites are more vulnerable to adverse skin reactions following PPE usage?
4. Does the nature and frequency of skin reactions change over time?
5. Do intrinsic and extrinsic factors play an important role in the severity of skin reaction following PPE application?
6. What is the prevalence of skin reaction following a single use of PPE against continuous and consecutive days of usage?

6.2 AIM OF THE STUDY

The present study aims to perform a cross-sectional survey of HCWs to evaluate the nature and frequency of adverse skin reactions to PPE. Secondary analysis will address factors which are implicated in compromising skin health, as well as report the status of skin health after a prescribed period of follow- up.

6.2.1 OBJECTIVES

1. Recruit a cohort of HCWs at high risk of manifesting skin adverse reactions due to the use of PPE
2. Disseminate survey questionnaires to the recruited cohort in order to acquire their experience following PPE usage.
3. Identify associations between skin adverse reactions with extrinsic and intrinsic factors
4. Monitor the skin health of HCWs over a defined period of time to identify improvement or worsening of reported adverse reactions

6.3 METHODOLOGY

6.3.1 STUDY DESIGN

This study, involving a number of UK NHS centres, was divided into two distinct phases using a standardised survey questionnaire. The first phase involved a 24-hour prevalence study of skin reactions from all HCWs using PPE and was conducted in high-risk departments, namely, the general intensive care unit (GICU), neurological intensive care unit (NICU), surgical high dependency unit (SHDU) of a single large university hospital trust. This was complemented with a second study designed to capture the impact of respirator protective equipment (RPE) on skin health, using a convenience sample of hospital staff from three hospitals during the COVID-19 pandemic.

Two distinct questionnaires were designed to meet the objectives of the studies (see Appendix A). The questionnaires consisted of some closed-ended questions, including multiple-choice answers, binary yes and no choices, Likert rating scales, as well as some open-ended questions. The questions were based on previous literature, relevant guidelines and media reports (Foo et al. 2006; Donovan et al. 2007; Yan et al. 2020). The questionnaire for the 24-hour prevalence sub-study consisted of only 12 questions while the multicentre study survey presented 26 questions. Indeed, the short version survey was purposely composed in order to meet the requirement of HCWs, who raised concerns about completing long questionnaires after a 12 – 13 hours shift. The short survey included details of each participant's demographics, type and make of PPE employed, average time

spent in PPE and the frequency of device removal. In addition, each participant was asked about the comfort and pain levels associated with wearing their protective equipment, any preventative measures which were adopted, and the nature and location of any skin adverse reactions. The multicentre survey questionnaire was divided into 4 sections namely:-

- Participant information – this section acquired information related to participants' demographics (age, gender, ethnicity), occupation (current clinical role, clinical department), and working pattern.
- PPE for eyes and face protection – the section investigated the different types of PPE and face protectors used by HCWs, length of PPE usage, whether fit testing was performed prior to use, daily hours of use and doffing timetable, and whether PPE was used on consecutive occasions.
- Skin care – this third section enquired on the various strategies employed by HCWs to protect skin health and the main anatomical locations affected by PPE usage
- Comfort – the fourth and last section examined the comfort and pain perception during the use of these protective devices.

The questionnaires were reviewed by a panel of experts consisting of clinical leads, nursing managers and experienced skin health researchers from the University of Southampton and University Hospital Southampton, who confirmed the content validity. Prior to being disseminated, a pilot study was performed to evaluate the feasibility of completing the questionnaires by HCWs, who found them comprehensible and of ease completion. To minimise the risk of transmission of coronavirus during the process, the short surveys were emailed to clinical managers to disseminate among their staff while the multicentre sub-study questionnaires were distributed electronically to all the staff using a Google Docs platform, with all results held on a secure server.

Inclusion criteria consisted of hospital staff working in areas deemed necessary for PPE usage, namely, staff caring for suspected and/or infected COVID-19 patients and staff at the frontline of hospitality services i.e. receptionists, ushers, who were required to wear protective equipment (at least, a surgical mask with or without eye visor). Participants were excluded if they were below 18 years of age and were not required to use PPE during their working shifts. Participation was purely voluntary and informed consent was implied through the completion of the questionnaire. The project was approved by the University of Southampton ethics committee (ERGO-FOHS-56430) (Appendix A.1).

6.3.2 DATA DISSEMINATION AND COLLECTION

The 24-hour prevalence sub-study data collection was carried out in June 2020. Questionnaires were distributed as hard copies to staff after their shift and they were kindly asked to detail their experience by answering the survey questions.

The data collection for the cross-sectional study was conducted from May 2020 to June 2020. Subsequently, a follow-up data collection was performed 3 weeks after the beginning of the study, from June to July 2020. The latter would enable a better understanding of temporal changes in the reported adverse skin reactions in terms of progression, no change, or regression. The questionnaire, which included more detailed questions than the prevalence survey, was disseminated to staff via emails, in the form of a link. Gatekeepers at each UK NHS trust were recruited to disseminate the survey, which was completed on a voluntary basis. In addition, dissemination was facilitated through the partnership with NHS England and NHS Improvement. Each participant had the option to leave any question unanswered and, as such, the result for each question was calculated based on the total number of respondents.

6.3.3 STATISTICAL ANALYSIS

The data from both studies were imported into Microsoft Excel (Microsoft 365, USA). Descriptive statistics (mean ± standard deviation (SD)) were used to represent continuous variables, while categorical data were presented as frequencies (percentages). Pivot tables were used to analyse the relationship between categories, and to examine trends within data. To examine the associations between dichotomous variables, Point-Biserial Correlation and Chi-Square Test of Independence were performed. Associations were considered to be statistically significant at the 5% level ($p < 0.05$).

6.4 RESULTS

6.4.1 24-HOUR PREVALENCE STUDY

A total of 108 questionnaires were completed over a 24-hour period. The majority of participants were female (81%), who were equipped with full protection, including RPE (FFP3) and eye protection, gloves, and gowns. The cohort included 75% nurses, 9% doctors, and 16% healthcare assistants (HCAs) or other health-related professions (Table 6.1). Because of infection and prevention policies, staff were required to adopt new personal protective equipment, if available, after each discontinuation of use. Accordingly, staff often employed different mask designs within

the same shift. A total of 119 RPE devices were used, with 68% of participants using 3M half respirators and the remaining using one or more designs from Alpha Solway, Medline Cardinal, EasyFit 300, and Valmy. The details of participants' demographic and PPE usage are illustrated in Table 6.1.

Table 6.1. Summary of demographics of participants and usage of PPE from the prevalence sub-study survey

Characteristics of participants					
Gender					
		Male	*Female*		*Total*
		21 (19%)	87 (81%)		108
Profession					
	Doctors	*Nurses*	**Others*		
	10 (9%)	81 (75%)	17 (16%)		108
Use of Personal Protective Equipment (PPE)					
Average time of RPE usage					
	0-7 hrs	*8-9.5 hrs*	*10-13.5 hrs*		
	17 (16%)	18 (17%)	73 (68%)		108
Types of respirator devices					
	3M	*Alpha Solway*	*Valmy*	***Others*	
	81 (68%)	20 (17%)	15 (13%)	3 (2%)	119
Number of breaks taken during shift					
No breaks	*1*	*2*	*3*	*4*	
6 (6%)	2 (2%)	30 (28%)	67 (63%)	2 (2%)	107
Average time per break					
	0 minutes	*25 minutes*	*30 minutes*	*45 minutes*	
	6 (6%)	2 (2%)	87 (81%)	12 (11%)	107
Types of skin adverse reactions					
Redness blanching	*Pressure damage*	*Itchiness*	*Spots*	*Rash*	*†Others*
49 (35%)	27 (19%)	30 (21%)	6 (4%)	16 (11%)	12 (9%)	140

**Healthcare assistants, technicians; **Cardinal, Easyfit; †acne, allergic reaction, burning sensation*

All the participants underwent either qualitative or quantitative fit testing prior to employing face protection, according to Fit2Fit accredited methodologies (Figure 6.1) (HSE 2021). The mean time spent in a daily shift using PPE was 9.2±2.6 hours. The average time in which PPE was removed (doffed) during a shift was 0.53±0.08 hours, although 64% of participants reported wearing PPE for more than two hours without relief. Of the total respondents, 66% reported changes in their skin health after their shift using PPE changes (140 adverse reactions). Redness blanching (35%), pressure damage (19%), and itchiness (21%) represented the most common skin adverse reactions. Other adverse reactions included rashes, spots, dry skin, acne, blisters, allergic reactions, and burning sensation. The anatomical sites most commonly affected were the bridge of the nose (37%), ears (16%), cheeks (12%), and chin (11%). Other affected locations included the forehead, scalp, neck, wrist, hands, and underneath the eyes.

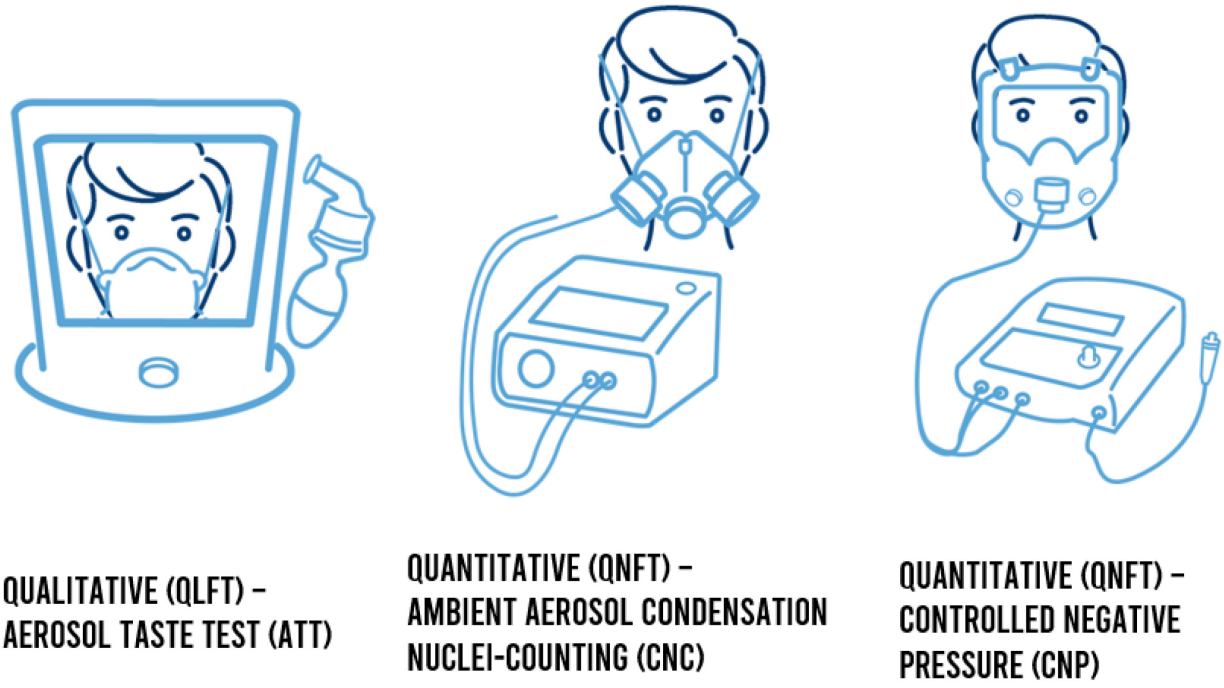


Figure 6.1. Qualitative and quantitative fit testing for the establishment of the protective capacity of a respirator.

The relationship between the time of PPE usage, classified into four groups, and the adverse skin reactions are summarised in Table 6.2. The results highlighted an increase in the participants reporting adverse reactions, particularly redness blanching and pressure damage, with an hourly increase in PPE usage. By contrast, there was a corresponding decrease in participants presenting with no adverse skin reactions with prolonged time of PPE usage. The prevalence of adverse skin reactions following a given shift was 66%.

Table 6.2. The distribution of adverse skin adverse reactions across the time period of PPE usage by staff during a single working pattern at any given anatomical location. The corresponding percentages at each time period are calculated based on the total number of participants.

Hours of PPE usage	***Redness blanching (%)***	***Pressure damage (%)***	***Itch. (%)***	***Rash (%)***	***Dry skin (%)***	***Spots (%)***	****Other (%)***	***No reaction (%)***	***Total (n)***
≤8 hours	2 (11)	2 (11)	4 (21)	2 (11)	0 (0)	0 (0)	0 (0)	13 (68)	19
9 hours	11 (69)	5 (31)	8 (50)	5 (31)	1 (6)	2 (13)	2 (13)	3 (19)	16
10 hours	20 (40)	11 (22)	10 (20)	5 (10)	1 (2)	3 (6)	6 (12)	16 (32)	50
≥11 hours	16 (70)	9 (39)	9 (39)	4 (17)	2 (9)	1 (4)	0 (0)	5 (22)	23

**Others include acne, blisters, allergic reactions and burning sensation.*

6.4.2 MULTI-CENTRE SURVEY

A total of 307 participants from three different UK NHS acute centres were recruited into the multicentre survey. Table 6.3 summaries their demographic details, their use of PPE, in terms of average daily hourly usage and consecutive day usage, as well as the pain perception while adopting the protective equipment. It is clear that the majority of respondents were females, with nurses representing the largest professional group.

Table 6.3. Summary of demographics of participants and usage of PPE from the cross-sectional survey

Characteristics of participants					
Gender					
		Male	*Female*		*Total*
		38 (12%)	269 (88%)		307
Profession					
	Doctors	*Nurses*	**Others*		
	51 (17%)	209 (68%)	47 (15%)		307
Ethnicity					
	White (Caucasian)	*Black/African/ Caribbean*	*Asian/Asian British*	*Mixed/Multiple backgrounds*	
	212 (79%)	28 (10%)	24 (9%)	6 (2%)	270
Age Range					
20-29 (yrs)	*30-39 (yrs)*	*40-49 (yrs)*	*50-59 (yrs)*	*60-69 (yrs)*	
43 (17%)	72 (29%)	74 (30%)	55 (22%)	6 (2%)	250
Average working days per week					
≤ 2 Days	3 Days	4 Days	5 Days	≥ 6 Days	
28 (9%)	100 (33%)	113 (37%)	62 (20%)	2 (1%)	305
Use of Personal Protective Equipment (PPE)					
Number of consecutive days of usage					
2 Days	*3 Days*	*4 Days*	*5 Days*	*≥ 6 Days*	
63 (29%)	83 (39%)	34 (16%)	23 (11%)	12 (5%)	215
Average daily hours of usage					
< 6 hrs	*6-8 hrs*	*8-10 hrs*	*10-12 hrs*	*12-13 hrs*	
65 (21%)	94 (31%)	39 (13%)	80 (26%)	27 (9%)	305
Frequency of skin relief from PPE during a shift					
Every 1 hr	Every 2 hrs	Every 3 hrs	Every 4 hrs	> Every 4 hrs	
46 (18%)	57 (22%)	60 (23%)	46 (18%)	47 (18%)	256
Pain perception scale while using PPE (0= no pain, 10= worst pain imaginable)					
	No pain (0)	*Mild (1-3)*	*Moderate (4-6)*	*Severe (7-10)*	
	42 (16%)	124 (48%)	59 (23%)	35 (13%)	260

**Others include healthcare assistants, biomedical scientists and technicians.*

Closer inspection between recruitment centres revealed clear differences in terms of ethnic background. Indeed, 91% of the participants from one acute centre were from the Black, Asian and

Minority Ethnic (BAME) population, compared with a mean value of 12% recruited from the other two centres. This corresponded to the demographics of the local hospital population.

6.4.2.1 SKIN ADVERSE REACTIONS DUE TO RESPIRATOR PROTECTIVE EQUIPMENT USAGE

Given that the majority of HCWs complained that most of the skin damage acquired was on the facial anatomical region, as a consequence of prolonged use of respirator protective equipment (RPE), the multicentre study questionnaire focused on the implications of the use of RPE on skin health.

Of the 307 survey responses, 269 participants (88% of the total) identified a total of 1257 adverse skin reactions from RPE use, occurring specifically at five locations of the face, namely, the forehead, the bridge of the nose, cheeks, chin and ears. There was a clear change in the perceived health of the skin assessed on a 0-10 scale, with 73% of the responders recording a relative decline in skin health following continuous/intermittent RPE usage.

Six types of adverse skin reactions were reported which included redness blanching, pressure damage, itchiness, rash, dry skin and spots. These were reported at various sites on the face, as summarised in Table 6.4. There were many cases of multiple skin reactions at one of the anatomical locations, with the highest proportion of ~28% occurring at the bridge of the nose and the cheeks. It is worthy of note that 129 adverse reactions occurred at the forehead due to the direct use of 359 eye protective equipment, 75% of which involved face shields and 41% general safety glasses.

Table 6.4. The distribution of adverse skin adverse reactions at different anatomical sites on facial skin. The corresponding percentages at each site are calculated based on the total number of reactions.

Anatomical sites	***Redness blanching (%)***	***Pressure damage (%)***	***Itch. (%)***	***Rash (%)***	***Dry skin (%)***	***Spots (%)***	***Total (%)***
Forehead	47 (36)	11 (9)	25 (19)	8 (6)	24 (19)	14 (11)	129 (10)
Nose bridge	135 (40)	55 (16)	67 (20)	22 (7)	37 (11)	19 (6)	335 (27)
Cheeks	114 (32)	25 (7)	84 (23)	36 (10)	56 (16)	45 (13)	360 (29)
Chin	41 (18)	5 (2)	54 (23)	35 (15)	33 (14)	64 (28)	232 (18)
Ears	73 (36)	55 (27)	51 (25)	7 (3)	14 (7)	1 (0)	201 (16)
Total	410 (35)	151 (12)	281 (22)	108 (9)	164 (13)	145 (11)	1257 (100)

Close examination of the data revealed some differences between the three acute-care centres. As an example, the centre with staff mostly from the Black, Asian and mix race ethnicity, revealed a

considerably higher incidence of adverse skin reactions at the ear, with percentage values of redness blanching (27%), pressure damage (24%) and itchiness (21%). By contrast, the corresponding combined values of the other centres that were predominantly White ethnicity were 20%, 16% and 16%, respectively.

6.4.2.2 ASSOCIATIONS BETWEEN SKIN ADVERSE REACTIONS WITH INTRINSIC AND EXTRINSIC FACTORS

The association between the various skin adverse reactions was examined with respect to one intrinsic factor, namely age, and a number of extrinsic factors associated with PPE usage. In addition, the performance of different designs of face protective equipment was analysed.

Figure 6.2 indicates that there were few differences in skin reaction with respect to respondents' ages conveniently categorised into decades from 20 to 69 years. However, there were some potential, but not significant ($p>0.05$), trends associated with a monotonic decrease across the age groups in participants presenting with redness blanching from 47% to 30% at the bridge of the nose (Figure 6.2A) and the cheeks (Figure 6.2B). A similar decrease was noted for spots present at the cheeks and chin (Figure 6.2C). By contrast, there was a gradual increase with age in the percentage of respondents presenting with no adverse reaction at both the ears (Figure 6.2D) and cheeks (Figure 6.2B), both of which were found to be statistically significant ($p<0.05$).

Figure 6.3 reveals a limited number of non-significant associations between the adverse skin reactions and the number of consecutive days of PPE usage, which varied from 1 to $\geq$6 days. However, it is evident that redness blanching was the most reported reaction at the bridge of the nose with, for example, an incidence of 50% for those wearing RPE for 3 consecutive days (Figure 6.3A). The corresponding values were ~40% at the cheeks (Figure 4.2B) and <20% at both the chin (Figure 6.3C) and ears (Figure 6.3D).

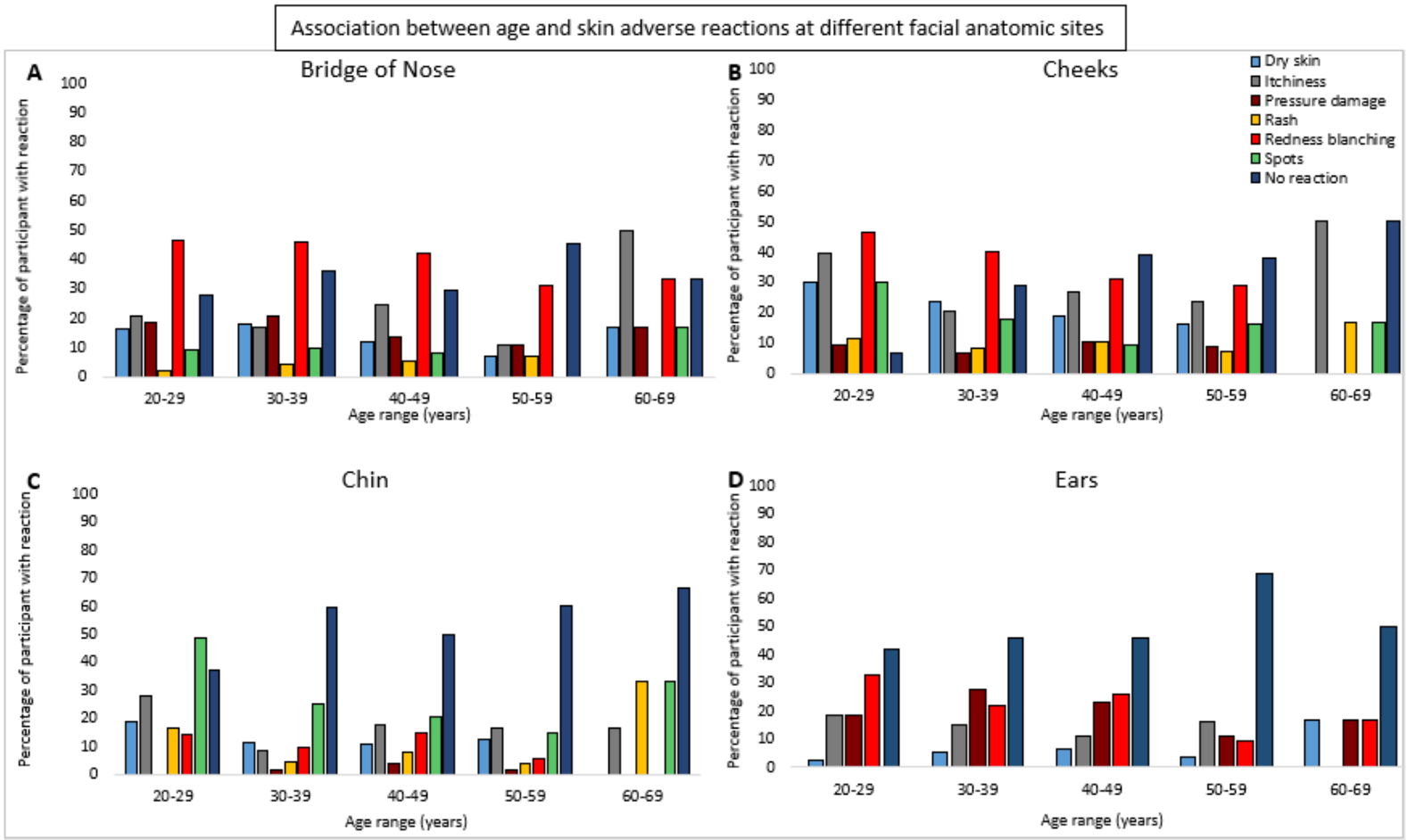


Figure 6.2. Correlation between age decade and seven distinct skin reactions at different facial skin locations, namely, the nasal bridge (A), cheeks (B), chin (C) and ears (D). While a decrease in redness blanching was observed with a decrease in age at the nose bridge and cheeks, an increase in no reaction was evident with an increase in age at the ear

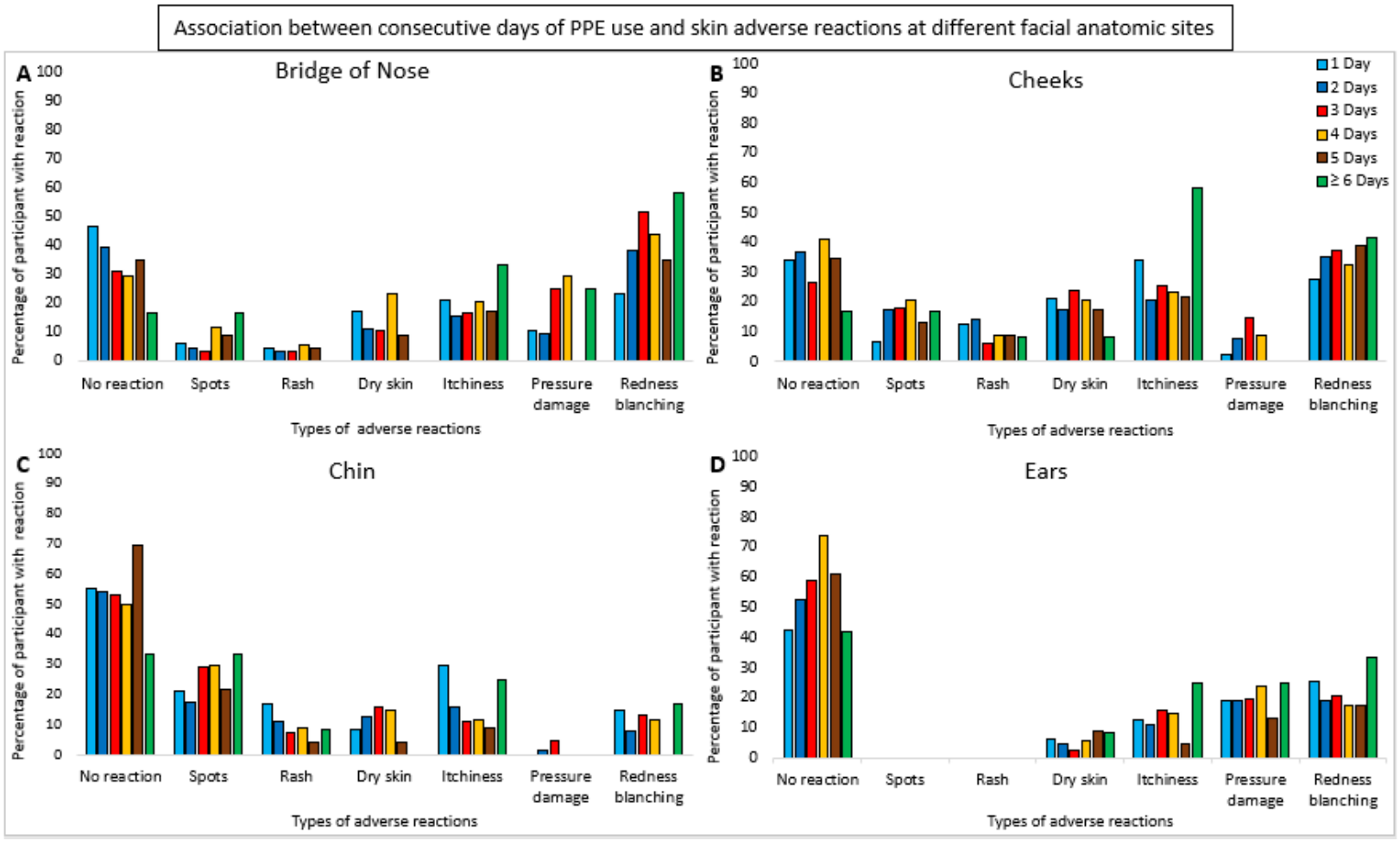


Figure 6.3. Association between the number of consecutive days of PPE usage and seven distinct skin reactions at different facial skin locations, namely, the nasal bridge (A), cheeks (B), chin (C) and ears (D). Peaks of adverse reaction observed following 3 days of consecutive PPE application at each facial sites

With respect to the average daily hours spent using PPE, the cohort of 305 responders was most conveniently classified into 5 separate groups ranging from less than 6 hours to greater than 12 hours. The results, as illustrated in Figure 6.4, generally reveal similar trends at each site, with a higher proportion of participants reporting skin adverse reactions with longer PPE usage. The corresponding participants reporting no adverse skin reaction decreased with prolonged usage at each site. However, there were distinct differences at the facial locations with, for example, a higher proportion of response in the form of redness and pressure damage at the nose (Figure 6.4A) and the ears (Figure 6.4D). By contrast, the chin was associated with spots with a 41% incidence in respondents using the PEE for 12-13 hours, with little evidence of pressure damage at this site (Figure 6.4C).

On closer examination, there was a continuous increase in the percentage of respondents presenting with redness blanching at the nose with extended hourly usage, ranging from 35% to 56% (Figure 6.4A). An increasing temporal trend was also noted with dry skin and itchiness, reaching maximum values of 26% and 30%, respectively. Similar changes were observed at the cheeks, with an increase in redness, itchiness, dryness and pressure damage associated with hourly usage of PPE (Figure 6.4B). The ears (Figure 6.4D) were associated with an increasing trend with time using PPE for itchiness, pressure damage and redness blanching, with the highest rates of reported pressure damage (41%) corresponding to 12-13 hours usage.

The findings highlighted statistically significant associations ($p<0.05$) between the average daily time of PPE usage and the manifestation of skin adverse reactions at the bridge of the nose, cheeks and ears (Figures 6.4A, 6.4C and 6.4D).

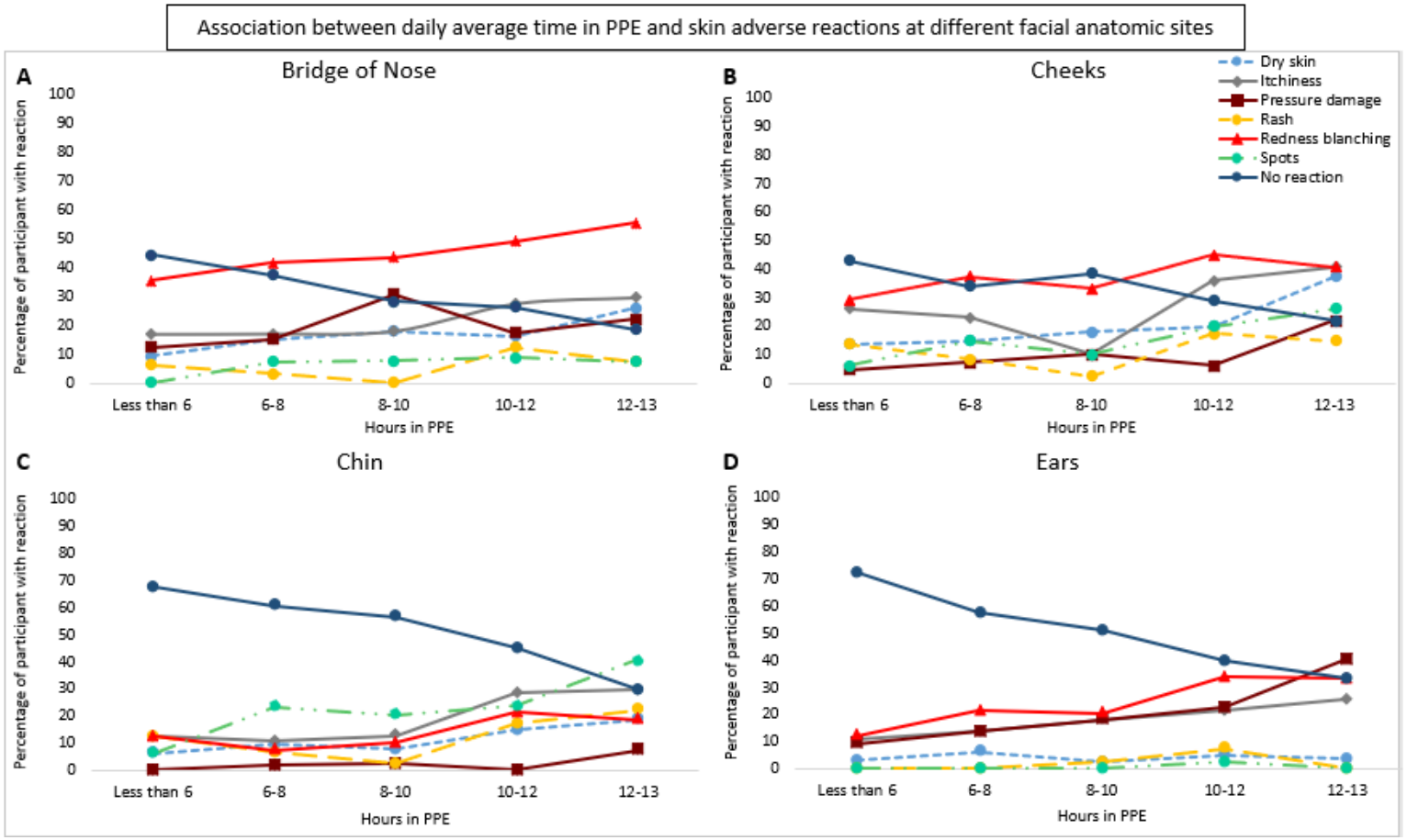


Figure 6.4. Correlation between the average daily time spent using PPE and seven distinct skin reactions at different facial skin locations, namely, the nasal bridge (A), cheeks (B), chin (C) and ears (D). Hours spent in PPE is plotted against the percentage of respondents presenting with skin reactions.

With respect to the frequency associated with skin relief, effectively doffing the PPE, the results from 256 participants were categorised into five time periods ranging from every 1 hour to in excess of 4 hours within a daily shift. The results at the four facial sites, as illustrated in Figure 6.5, indicate that 50% of the respondents acquiring redness blanching on the nasal bridge corresponded to continuous PPE usage in excess of three hours without relief (Figure 6.5A). Similar trends were also observed at the cheeks (Figure 6.5B) and the ears (Figure 6.5D) with maximum reported rates of 40% and 35%, respectively. It is also noteworthy that pressure damage was reported on both the bridge of the nose and the ears in approximately 30% of participants when PPE was worn continuously for three or more hours. For the bridge of the nose, cheeks and ears, the percentage of participants reporting no adverse reactions generally decreased with extended periods of PPE use without relief.

Statistical significance ($p<0.05$) was observed at the bridge of the nose (Figure 6.5A) and the ears (Figure 6.5D) when the adverse skin reactions were correlated with the time period for which the PPE was doffed from the skin. This was particularly evident at the cheeks for redness blanching (Figure 6.5B).

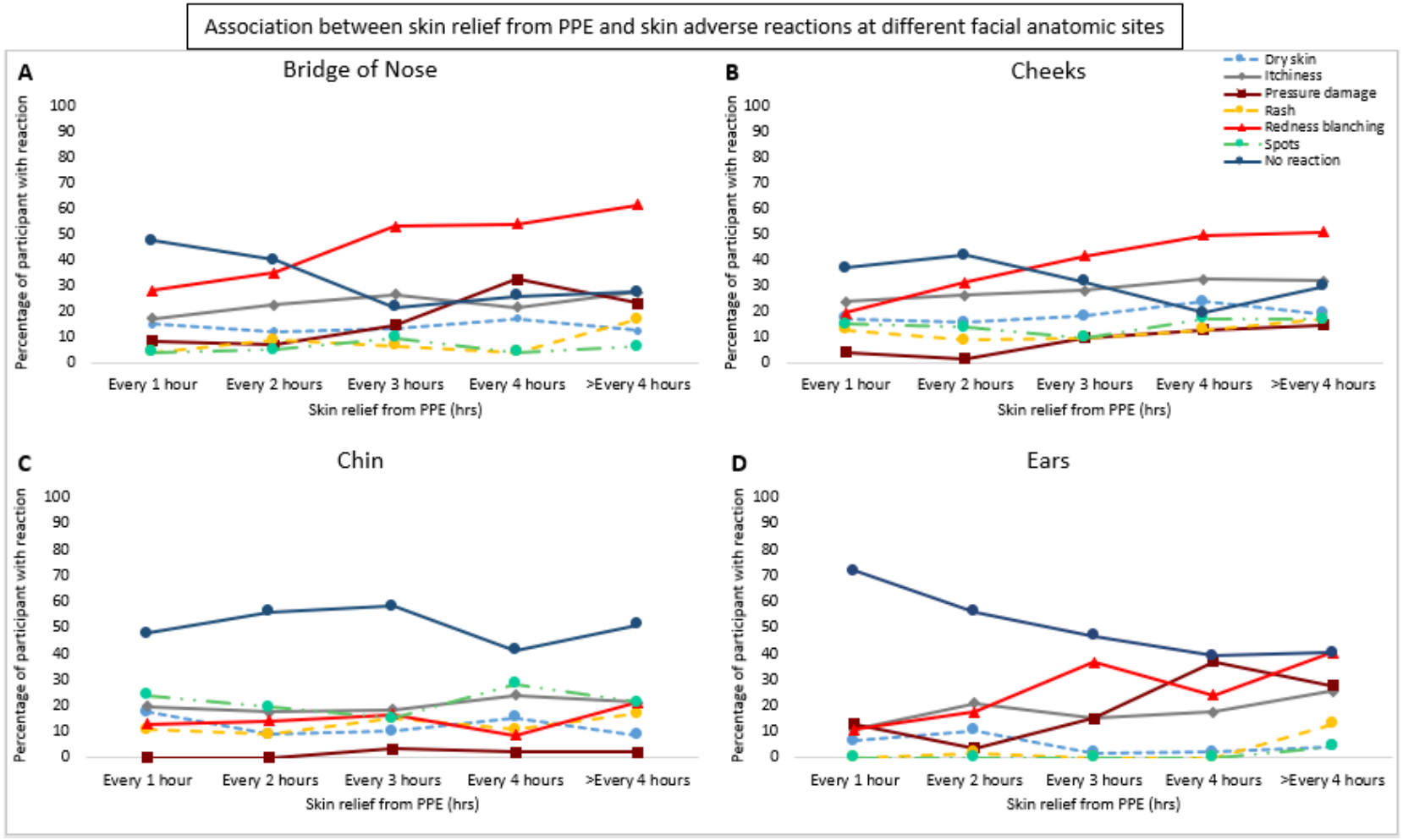
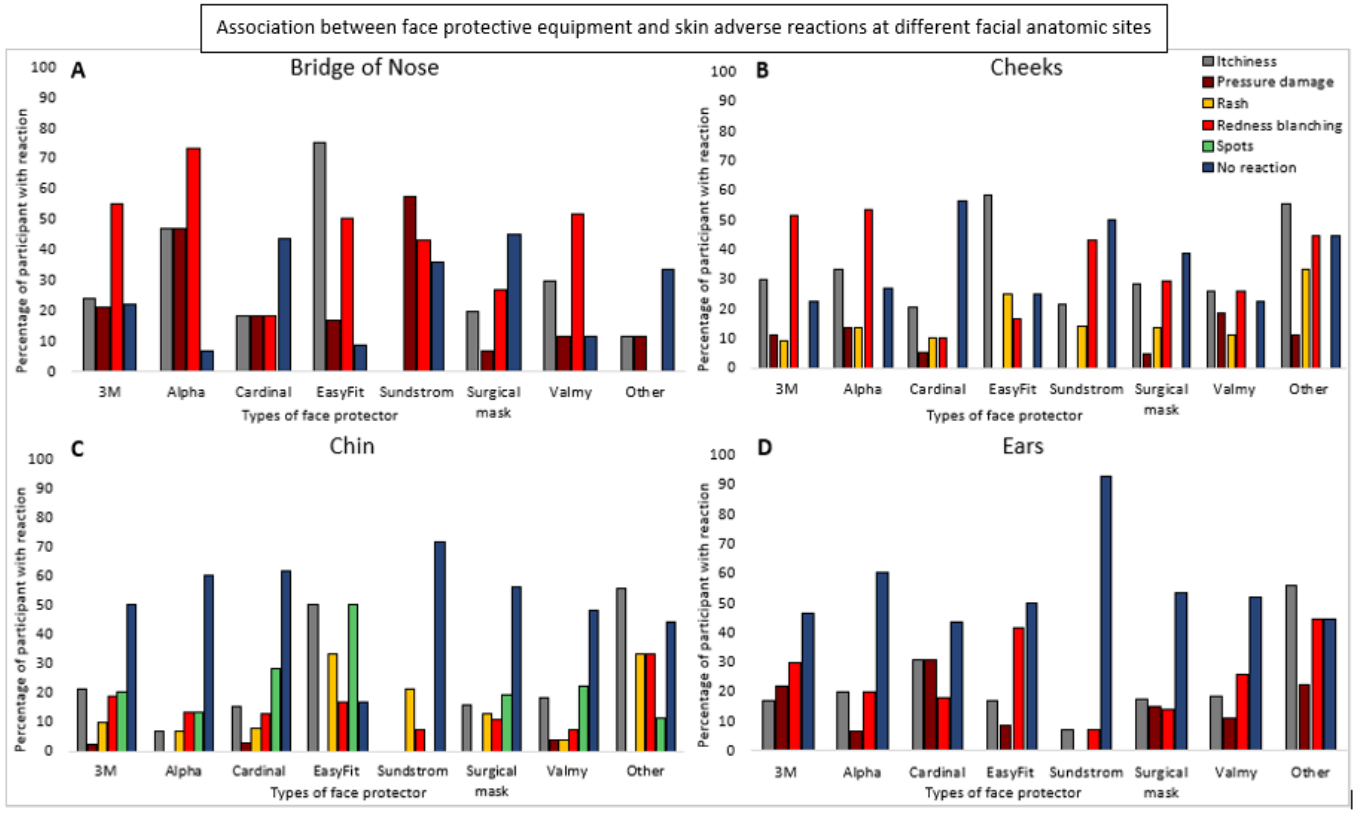


Figure 6.5. Correlation between the frequency of skin relief from PPE and seven distinct adverse reactions at different facial skin locations, namely the nasal bridge (A), cheeks (B), chin (C) and ears (D). Skin relief from PPE is plotted against the percentage of respondents presenting with skin reactions.

Different types of RPE devices were reported across the cohort, including FFP3 respirators, FFP1 models and other varieties of face protective equipment. The results at the four facial sites, as illustrated in Figure 6.6, revealed no statistical differences attributed to the design of face protective equipment. Nonetheless, close examination revealed some trends, namely,

- With most device types, redness was the most prominent skin adverse reaction, particularly over the bridge of the nose and the cheeks (Figures 6.6 A and B)
- Redness blanching was evident for more than 50% on the bridge of the nose and cheeks for 3M, which was used by 46% of the cohort
- Pressure damage at the nose was relatively high for the 10% of the cohort, who used the Alpha and Sundstrom designs of face protectors.
- Itchiness at all anatomical sites was relatively high for the 7% of the cohort who used the Easy-Fit and other designs of face protectors.
- Rashes were more prominent at the cheeks and chin for all mask designs, with absolute percentage occasionally exceeding 30%
- Spot was elevated on the chin with a value of 50% reported by Easy Fit users
- The surgical mask used by 35% of participants produced no adverse reactions which were greater than 30% at any of the skin sites.
- No adverse reaction was reported in >40% of the cohort at the chin and ears and for many designs at the cheeks.



Types of face protection	*3M*	*Alpha*	*Cardinal*	*Easy Fit*	*Sundström*	*Surgical mask*	*Valmy*	*Other*	*Total RPE*
Nº of Respondent	142	15	39	12	14	109	27	9	367
Frequency (%)	46	5	13	4	5	36	9	3	

Figure 6.6. Correlation between various designs of face protective equipment and percentage incidence of adverse skin reactions at different facial sites, namely, the bridge of the nose (A), cheeks (B), chin (C) and ears (D). The number of respondents and their distribution for each design of face protection equipment, with relative frequencies, calculated based on the total number of participants, is indicated at the bottom of the figure.

The responses to the other closed questions related to fit testing, skincare while using PPE, comfort and safety, and skin health are summarised in Table 6.5. In contrast to the prevalence study, it was evident that some respondents i.e. 24% did not undergo fit testing before using RPE. However, this was not consistent between centres as one acute centre revealed that 72% of their 33 respondents, mostly from the minority ethnic community, did not attend fit testing prior to using RPE. Although 50% of the overall cohort used cosmetics, a smaller proportion of participants employed skin protective measures, including moisturizers and/or preventive dressings. In addition, while more than 60% of participants reported discomfort and breathing difficulties, the protective equipment was recognised as ensuring safety by more than 60% of HCWs. It was also of note that although pressure damage was often reported, only 15% of HCWs presented with broken skin.

Table 6.5. Summary of the response related to skincare, comfort and safety, and facial skin health while using PPE.

Questions (?)	***Yes (%)***	***No (%)***	***Total respondents***
	USE OF PPE		
Fit tested	235 (76)	72 (24)	307
	SKIN CARE WHILE USING PPE		
Use of cosmetics	124 (52)	116 (48)	240
Use of moisturizer/cream	80 (30)	185 (70)	265
Use of preventive dressing material	31 (12)	234 (88)	265
Regular break taken from PPE	214 (71)	88 (29)	302
	COMFORT AND SAFETY WHILE USING PPE		
Comfortable wearing PPE	69 (26)	194 (74)	263
Breathe easily wearing PPE	98 (37)	165 (63)	263
Feel safe & in control using PPE	161 (61)	102 (39)	263
	FACE SKIN HEALTH		
Presence of indentation marks	165 (55)	138 (45)	303
Presence of broken skin	46 (15)	259 (85)	305

6.4.3 MULTI-CENTRE SURVEY FOLLOW-UP

A total of 144 participants were followed up from the original cohort after 3 weeks from the dissemination of the original survey. The time gap (3 weeks) was deemed as an adequate period necessary to enable staff to get a better understanding of how their skin reacts to PPE, as well as educate themselves on strategies to prevent further skin adverse reactions. Findings indicated that 85% of respondents remained with the same protective equipment, while 15% reported a change in mask design or type. The data revealed some differences when compared to the original cross-sectional survey data (Table 6.3 vs 6.6). As an example, there was a slight decrease in the percentage of participants using PPE in excess of 4 consecutive days during the time of follow-up, i.e. 30% vs 34%. A similar reduction was also reported in terms of the average daily time spent in PPE, with 29% of participants adopting the equipment for >10 hrs, against 35% of participants in the original data set. Although there was an overall decrease in the level of perceived pain, nonetheless, almost 11% of HCWs reported a feeling of low morale and struggling with their daily life activities as a result of PPE-related pain. The percentage of staff who adopted protective measures, in terms of creams and/or moisturizers, increased by 4% compared to the original data i.e. 30% to 34%. However, there was a slight decrease (~3%) in the number of participants using prophylactic dressings.

Table 6.6. Summary of the working pattern of participants and usage of PPE from the follow-up survey

Experience and PPE Usage – Follow-up questionnaire					
Average working days per week					
≤ 2 Day	3 Days	4 Days	5 Days	≥ 6 Days	**Total**
26 (19%)	40 (29%)	33 (24%)	31 (22%)	8 (6%)	138
Number of consecutive days of PPE usage					
2 Days	*3 Days*	*4 Days*	*5 Days*	*≥ 6 Days*	
50 (49%)	23 (22%)	14 (14%)	13 (13%)	3 (3%)	103
Average daily hours of usage					
< 6 hrs	*6-8 hrs*	*8-10 hrs*	*10-12 hrs*	*12-13 hrs*	
32 (22%)	46 (32%)	24 (17%)	25 (17%)	16 (11%)	143
Pain perception scale while using PPE (0= no pain, 10= worst pain imaginable)					
	No pain (0)	*Mild (1-3)*	*Moderate (4-6)*	*Severe (7-10)*	
	61 (43%)	53(37%)	24 (17%)	5 (3%)	143

A total of 380 adverse skin reactions were reported at the time of follow-up. The types and distribution of these reactions across the 5 anatomic sites of the face are summarised in Table 6.7.

Compared to the original data (Table 6.4), there was an overall 15% increase in redness blanching and a 3-fold decrease in dry skin. There was also an overall slight decrease (<4%) in terms of pressure damage, itchiness and spots. There were no significant differences in the total skin reactions reported at the different anatomical sites, with the exception of at the forehead and chin, where there was a decrease of 4% and an increase of 6%, respectively.

Table 6.7. Distribution and types of adverse skin adverse reactions, with relative frequencies, at the different anatomical sites of the facial skin at the date of follow-up. The corresponding frequencies (percentages) of adverse reactions at each site are calculated based on the total number of reactions.

Anatomic sites	***Redness blanching – response (%)***	***Pressure damage – response (%)***	***Itchiness – response (%)***	***Rash – response (%)***	***Dry skin – response (%)***	***Spots – response (%)***	***Total reaction – response (%)***
Forehead	10 (43)	0 (0)	8 (35)	3 (13)	1 (4)	1 (4)	23 (6)
Nose bridge	57 (57)	9 (9)	17 (17)	6 (6)	4 (4)	7 (7)	100 (26)
Cheeks	44 (42)	6 (6)	24 (23)	14 (13)	6 (6)	10 (10)	104 (27)
Chin	27 (30)	11 (12)	21 (23)	18 (20)	3 (3)	10 (11)	90 (24)
Ears	43 (68)	8 (13)	10 (16)	1 (2)	1 (2)	0 (0)	63 (17)
Total	181 (48)	34 (9)	80 (21)	42 (11)	15 (4)	28 (7)	380 (100)

6.5 DISCUSSION

The worldwide spread of COVID-19 has imposed a considerable strain on both healthcare systems and healthcare professionals, who are required to adopt strict protective measures in order to ensure safety while managing affected patients. The present study included a single-site prevalence study and a multicentre survey of 307 HCWs with associated follow-up, from three acute UK hospital trusts to evaluate skin reactions directly from the use of PPE. The comprehensive survey identified the nature of the adverse skin reactions and their associations with time spent in PPE and the frequency of skin relief resulting from doffing the device. In addition, it examined the distinct patterns of reactions in different facial locations and any associations attributed to mask designs.

The multicentre survey data on a convenience sample of HCWs from three hospital trusts revealed that more than 87% of the participants reported changes to skin health as a direct consequence of PPE usage. These findings differ from what has been reported in recent studies (Yuan et al. 2021; Nguyen et al. 2022), where it was reported that the general prevalence of skin reaction in healthcare workers wearing PPE ranged between 54% and 77%. However, while these studies investigated different anatomical locations across the body, the present study only focused on the facial skin health of clinical staff. It revealed that the most affected sites were the cheeks and the nasal bridge (Table 6.4), which is in accordance with other findings (Skiveren et al. 2022).

There are some evident trends in the nature of the adverse reactions across the five age groups (Figure 6.2). In particular, there was an observed decrease in the prevalence of redness blanching, dry skin and spots with an increase in age at different facial sites. This trend contrasts with that which may be predicted, based on the established loss of skin water content and elasticity with age (Krutmann et al. 2017). However, it must be accepted that the HCWs were exposed to exceptional circumstances involving many uncontrollable variables, such as temperature, humidity, hormonal variations and psychological stresses, all of which could affect the tolerance of skin to PPE usage (Coleman et al. 2006; Gosain et al. 2005). Consecutive days usage of PPE did not highlight specific differences, although peaks of adverse reactions were observed between days 3 and 4 (Figure 6.3). This could be associated with a cumulative effect of repetitive insults, thereby decreasing the skin tolerance to loading at vulnerable sites.

For both the prevalence study and multicentre survey, independent of the facial anatomical site, there was clear evidence that participants who spent fewer hours in PPE presented with a higher probability of no adverse reactions (Table 6.2 and Figure 6.4). By contrast, there is an increase in skin reactions as a result of an increased time of PPE usage. From this finding, it is tempting to suggest that the time limit for daily use of PPE should range from 8-10 hours. These results are in accord with other recent studies on PPE usage with COVID-19 (Liu et al. 2022; Sari et al. 2022). This is not surprising as it could be predicted that the longer the time of PPE usage, there is enhanced probability of incurring increased perspiration, with exposure to moisture being associated with a reduction in the strength and stiffness of the skin, particularly in its outermost layer (Kottner et al. 2018). The present study has also revealed compelling evidence that the frequencies of skin relief from PPE are associated with the occurrence of reactions. Figure 6.5 showed that, aligned with other studies where load exposure and frequency of relief are critical to skin health (Bouten et al. 2003), after 3 hours of continuous usage of protective equipment, up to 40% of respondents reported pressure damage at different anatomical locations of the face. These findings are of fundamental importance as current guidelines lack specific information regarding the duration of PPE application and the frequency of relief required to protect skin health (NHS Improvement

2020). Indeed, the findings from this study enabled NHS England and NHS Improvement to action an immediate update on the policies surrounding the use of PPE.

Implications of the manufacturer and design of RPE were also examined, in the light of previous studies which focused on the effects of N95 respirators (Lam et al. 2020; Hua et al. 2020), which are classified as FFP2 devices and deemed inadequate in high-risk settings. To the best of our knowledge, this was the first study where the effects of appropriate protection in COVID-19 settings i.e. FFP3 face masks were investigated in terms of adverse skin reactions to healthcare workers. Figure 6.6 clearly indicates that with prolonged use there was no specific mask design that maintained skin health over the face. Indeed, the bridge of the nose seemed to be particularly vulnerable to adverse reactions, in the form of redness blanching, pressure damage and itchiness, when exposed to all designs. This has been emphasised in subsequent studies where the use of FFP face protectors for an excess of 4 hours has resulted in detrimental implications to skin healthy (Skiveren et al. 2022; Westermann et al. 2022). It is worthy of note that most protective equipment for the face are predominantly designed for male face shapes (Merson 2020), incorporate components of stiff materials (Bader et al. 2019), and are produced based on the “one-size-fits all” principle. It is apparent from the present findings that ideal face protective equipment should incorporate a combination of soft material interfaces and include size ranges to accommodate face shapes of different genders and ethnicities. Indeed, a recent systematic review and meta-analysis on the implications of gender and ethnicity on facemasks and respiratory protective equipment fit, in which the researcher was one of the co-authors, showed a significant gender- and ethnicity-based variance across studies in facial anthropometric measurements, with females generally presenting with a smaller size of head and face, as well as smaller nose width, lip thickness and chin width compared to their male counterparts (Chopra et al. 2021). In addition, it was also noteworthy that there was a higher failure rate for RPE fit tests and/or lower fit factor scores amongst females (Manganyi et al. 2017). Significant differences in all facial measurements were also reported, with black males ascribed to have greater nasal root breadths compared to their white counterparts (Manganyi et al. 2017). A similar comparison highlighted Korean individuals presenting with wider face width and nose breadth, narrower nasal root breadth and lip width (Yu et al. 2014).

Although all manufacturers recommend fit testing prior to the use of FFP3 masks, it is disappointing to note that this was not consistently followed particularly at one test centre. Close examination revealed that this corresponded disproportionately with HCWs from the ethnic minority population. Indeed, the higher skin reactions reported particularly at the ears might be attributed to the lack of an adequate fitting process. Studies have reported the highest failure rates associated with cohorts involving the majority of BAME participants (Gilroy 2020). This issue has been recently examined in a computational-based study by the host authors, who highlighted the challenge of

fitting face masks, particularly for BAME individuals, to minimise the risk of both gapping and indentation at vulnerable sites including the bridge of the nose, cheeks and chin (Verberne et al. 2020). It is of critical importance that all HCWs undergo a comprehensive fitting process to minimise both skin damage and gapping at the mask-skin interface (Caggiari et al. 2022), the latter of which must be avoided to minimise the risk of viral infection. Further investigation is needed to ensure that good practice is implemented within care settings across the world.

In addition, it was observed that only a small proportion of participants (36%) adopted protective measures, in the form of moisturizers and/or preventive dressings, to ensure skin health (Table 6.5). This might be attributed to a lack of staff education on skin prevention and/or the paucity of information on appropriate prophylactic dressings able to fit under the face protection without compromising their overall function. Indeed, national and international guidelines have been cautious to advocate dressings, as these may affect the seal and therefore the functionality of respirators (Padula et al. 2021). The follow-up survey revealed a slight reduction in the proportion of participants with adverse skin reactions. This might be due to the contribution of many factors, such as an improved understanding of the risks of PPE usage, the redeployment of staff to non-COVID areas, and the reduction of the stress level to participants as fewer patients with COVID-19 were admitted.

The study is clearly limited by gender diversity and ethical background. Indeed, the vast majority of participants were females of White (Caucasian) ethnicity. The sample size in the multicentre study may not be reflective of the overall effective UK NHS workforce. In addition, there might be a self-selection bias, as staff with skin reactions might have been more prone to engage in the survey. However, these limitations were, in part, mitigated with the prevalence study, which confirmed a clear association between the manifestation of skin adverse reactions with the time of PPE usage (Table 6.2). Furthermore, the majority of participants in both prevalence and multicentre studies used 3M mask designs, limiting adequate comparisons between different designs of FFP3 face protective equipment. In addition, given that there was no clear explanation to the study participants on how to distinguish between the different types of skin adverse reactions (i.e. spots vs rash), the likelihood of addressing an adverse reaction with the incorrect terminology is very high and this might have influenced the findings of this study.

Based on the findings of this study, it was recommendable that medical staff implement frequent relief from PPE usage to ensure adequate skin recovery from mechanical and environmental loading. Additionally, it was highly recommended a daily average use of PPE not exceeding 10 hours. Although it was not statistically significant, the findings suggest that the highest number of adverse reactions corresponded with 3-4 days of PPE consecutive usage. As such, staff should avoid, where

possible, the use of PPE for more than 3 consecutive days. Prevention strategies to maintain skin health should be implemented within the UK healthcare system, which will benefit both staff and clinical services. Indeed, skin health management and prevention should be an essential component in clinical training and educational requirements.

6.6 CONCLUSION

A series of approaches were adopted to examine skin adverse reactions following periods of PPE usage. Increasing reports of adverse reactions were associated with the average daily time spent in PPE and the duration of PPE use without relief. Trends in skin adverse reactions were also associated with the number of consecutive days of PPE usage, as well as the type and model of RPE. There is a compelling need to improve the guidelines for PPE use and the design/materials of which the protective equipment are manufactured, in order to minimise the risk of skin damage to valuable healthcare workers.

Chapter 7 MONITORING THE FACIAL SKIN HEALTH OF HEALTHCARE PROFESSIONALS DURING COVID-19 PANDEMIC USING BIOPHYSICAL AND BIOCHEMICAL PARAMETERS

The results of this study have been published in Skin Research and Technology:

Abiakam, N, *Jayabal, H, Mitchell, K, Bader, D, Worsley, P. Biophysical and biochemical changes in skin health of healthcare professionals using respirators during COVID-19 pandemic. Skin Res Technol. 2022; 1- 11. https://doi.org/10.1111/srt.13239*

7.1 INTRODUCTION

The nature and the frequencies of adverse skin reactions following prolonged and continuous use of PPE, in context with the outbreak of coronavirus pandemic, have been extensively reported in the literature (Sari et al. 2022). A complete summary of the associated literature is provided in chapters 1, sections 1.2.4 and 4. The most prominent of these reactions include pressure damage, erythema, indentation marks and itchiness (Figure 7.1). Nonetheless, it is worth noting that these manuscripts have predominantly relied on survey questionnaires, as such, they captured participants' perception of their skin health, rather than measured changes in skin integrity. Indeed, it is most probable that staff presenting with skin adverse reactions were more likely to engage with the surveys, increasing the likelihood of reporting rates.

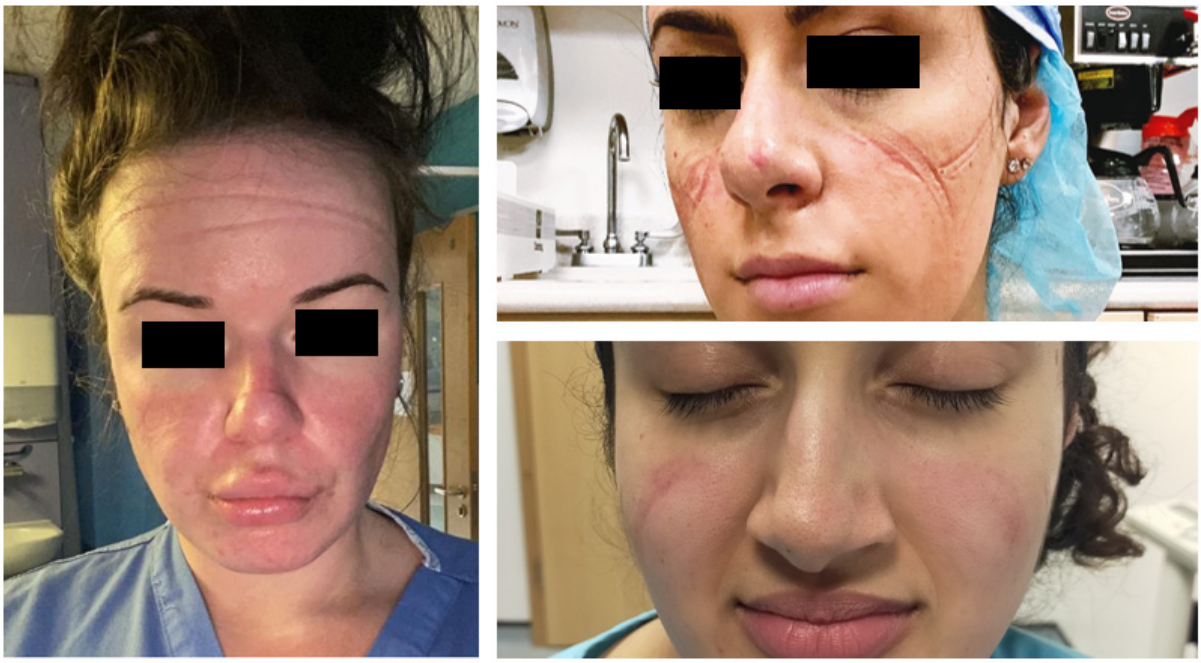


Figure 7.1. Image of an NHS clinical staff after various hours shift wearing respirator masks. The image highlights visible redness and indentation marks over the nasal bridge and on the cheeks due to prolonged use of the mask. The image is courtesy of NHS front-line workers (with permission).

Following the multicentre quantitative survey research project, detailed in chapter 6, which showed a significant correlation between the time of respirator protective equipment (RPE) usage and the doffing frequency with the manifestation of adverse skin reactions, there is a compelling need to examine these trends with objective parameters of skin health. The use of non-invasive biophysical technologies to monitor skin responses post challenges has been extensively described in chapter 2.

Indeed, several studies have been published in recent years exploring the biophysical response to RPE application. The vast majority of these studies have been limited by the time exposure to devices (2-4 hours), the use of a non-clinical cohort and types of respirators assessed (predominant FFP2 masks). Of the published studies, most have reported an increase in TEWL, SC hydration and skin surface pH following respirator application (Hua et al. 2020). For example, increased TEWL and erythema were detected at the areas under the influence of the RPE post 2 hours usage by HCWs, with values of the covered against the uncovered area being 22.8 vs 13.7 g/h/m^2 and 411.4 vs 335.5 AU for TEWL and erythema, respectively (Montero-Vilchez et al. 2021).

It is worth noting that none of these studies reported the implications of consecutive days of RPE usage, nor assessed correlation with extrinsic (different hours of usage, the number of breaks from equipment) and intrinsic (age, gender, BMI, ethnicity) factors. Montero-Vichelz and colleagues found a positive correlation between age and temperature increase but did not detect similar

trends in terms of gender (Montero-Vilchez et al. 2021). Although these studies represent a benchmark for future research investigating the impact of RPE on the skin health of HCWs, there is a need for more empirical data pertaining to skin behaviour before and after RPE application in clinically relevant shift patterns. This could be used to inform new designs of PPE, improved fitting protocols, and skincare regimens to protect healthcare workers from skin damage.

7.2 AIM OF THE STUDY

The present study aimed to investigate the biophysical changes in skin health following RPE application using a longitudinal design over repeated clinical shifts in HCWs.

7.2.1 RESEARCH QUESTIONS

1. Are there changes in the biophysical properties of skin under the RPE device interface?
2. What are the effects of consecutive days of RPE usage on skin health?
3. Are there correlations between intrinsic factors and skin health following extensive use of RPE?

7.2.2 OBJECTIVES

1. Recruit a group of hospital workers considered to be at risk of skin damage due to the prolonged use of a FFP2/3 respirator mask.
2. Collect data using a series of biophysical measurements collected from the participant cohort pre- and post-device application in relevant skin sites
3. Evaluate changes in skin parameters between a loaded under the RPE and unloaded control site.
4. Assess time dependent changes in biophysical parameters following sequential clinical shifts.
5. Correlate changes in skin integrity with respect to intrinsic (age, gender, health status) and extrinsic factors (type and duration of RPE use)

7.3 MATERIALS AND METHOD

7.3.1 PARTICIPANTS

Healthcare workers were recruited from COVID-19 high-risk departments of one UK University Hospital healthcare provider via poster advertisement and gatekeeper communication. Inclusion

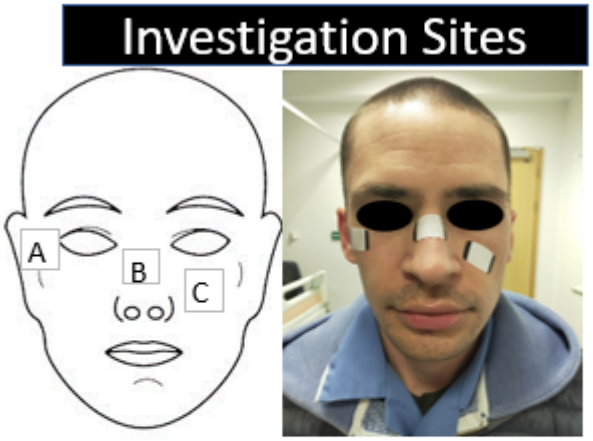
criteria consisted of individuals over 18 years of age, who employ FFP2/3 masks on a daily basis while attending to clinical commitments, who worked a minimum of three consecutive clinical shifts per week. Exclusion criteria included individuals with active skin conditions at the facial sites of investigation, allergies or sensitivity to adhesive tape and the inability to attend a minimum of two out of the three assessment sessions. The study was approved by the UK Health Research Authority committee (IRAS 285764) (see Appendix B) and written informed consent was obtained from participants prior to commencing the study.

7.3.2 STUDY PROTOCOL

The study was conducted during the second wave of the COVID-19 pandemic in the UK (December 2020 to March 2021). Three anatomical locations on the face, namely an area outside the perimeter of respirator application (negative control denoted A), the bridge of the nose (B) and the left cheek (C), were investigated (Figure 7.2a). Participants who agreed to take part in the study were tested on three different occasions based on a standardised protocol, as summarised in Figure 7.2b. Participants were requested to avoid the application of any moisturizer and/or cosmetics on the face for each of the assessment days. During the test session, participants acclimatized to an indoor environment and their face dried with paper towels (Tork®, Bedfordshire, UK) prior to commencing skin assessments. All test sessions were conducted in a temperature and humidity-controlled laboratory (room temperature of 22.5± 0.7^{o}C and relative humidity of 42± 6%) before and after the participant's working shift. Three distinct data collection sessions were used:

- Session 1: participant first day of mask usage following return to work after a period of absence (minimum of 24 hours)
- Session 2: second consecutive day of mask usage in a given working week
- Session 3: third consecutive day of mask usage in a given working week

a)



Site A: Area outside the perimeter of mask application (negative control)

Site B: Bridge of the nose

Site C: Left cheek

b)

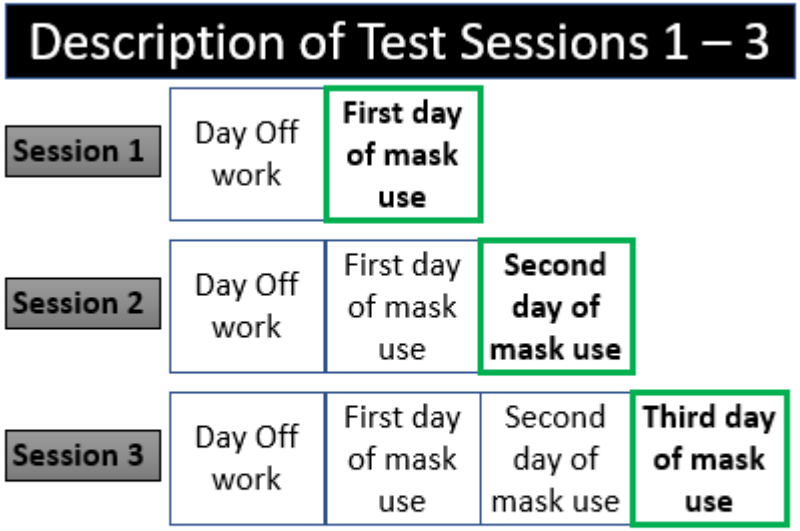


Figure 7.2. (A) Investigation sites associated with a control site 'A', and sites under the respirator mask, namely the bridge of the nose 'B' and left cheek 'C'. (B) Study protocol including each test session and the corresponding repeated days of respirator application.

7.3.3 SKIN BIOPHYSICAL AND BIOCHEMICAL ASSESSMENT

At the start of the first test session demographic and anthropometric data were collected from each participant. This including age, gender, ethnicity, height, weight, body mass index (BMI), working hours, type of respirator used, number and frequency of respirator removal, and pain perception while wearing the device.

The facial skin health of participants was assessed during each test session pre- and post-respirator application from their respective clinical shift, using three biophysical parameters. These included transepidermal water loss (TEWL), stratum corneum (SC) hydration and erythema. TEWL values were captured using a Tewameter (TM 300, Courage & Khazaka, Germany), which incorporates an open-chamber probe which was gently placed in contact with the skin for 1 minute during which equilibrium was achieved. SC hydration and erythema were assessed using the capacitance-based Corneometer (CM 825, Courage & Khazaka, Germany) and the narrow-band reflectance spectrophotometer Mexameter (MX18, Courage & Khazaka, Germany), respectively. Both devices were placed in contact with the test sites and the mean of 5 repeated measurements were recorded for each parameter. The outputs of the parameters were measured in $g/h/m^2$ for TEWL and arbitrary units (AUs) for both SC hydration and erythema. Participants' skin assessments were performed generally after 10 min following the removal of RPE, which corresponded to the time taken for the individual HCW to transfer from their various hospital departments to the controlled lab setting. All measurements were performed following recommended guidelines for their use (du Plessis et al. 2013).

Inflammatory skin biomarkers were evaluated non-invasively by collecting sebum from the skin surface of each participant, using commercial Sebutape^{TM} patches (32x19 mm) (CuDerm, Dallas, TX, USA). The sebutapes were attached to the skin (Figure 1a), by means of tweezer and gloved hands, and held in place for 2 minutes prior to removal. Subsequently, they were placed in appropriate labelled sterile containers and stored at -80^{o}C until biochemical analysis. Cells from the skin surface were also taken using commercial sellotapes at the end of each working shift. The biochemical markers and evaluation of corneocyte cells were conducted by collaborators on the project, the results of which will be discussed in the context of the biophysical results at the end of the thesis (Chapter 9).

7.3.4 DATA ANALYSIS

Raw data were imported into Excel (Microsoft office package 2019, USA) for analysis. Values from TEWL, SC hydration and erythema were normalised to the baseline for each test session (post/pre-respirator application ratio) in order to enable comparisons of relative variations across the experimental time frame and between the participants (Henshaw et al. 2020; Bostan et al. 2019). Shapiro Wilk test and D'Agostino-Pearson were used to assess the distribution of the data. Accordingly, a parametric two-way analysis of variance (ANOVA) with replication was employed to evaluate the effect of repeated respirator application derived from each test session and the difference between measurement sites for each parameter. Paired samples t-Test was used to compare measurements taken between specific test sessions. Pearson correlation coefficient was used to examine possible associations between skin health and other demographic and anthropometric data. Given the variable distribution nature of the data in some sessions, box and whiskers plots were used to describe the absolute values of the parameters. Differences were considered to be statistically significant at the 5% level ($p < 0.05$).

7.4 RESULTS

The study recruited 17 HCWs (15 females and 2 males), who use RPE (FFP2 or FFP3) on a regular basis during established clinical shift patterns (Table 7.1). One participant contracted COVID-19 during the study and was withdrawn for further assessment. The periods between consecutive test sessions varied for practical reasons, ranging between 1 to 8 weeks. The participants' age ranged between 22 and 61 years (mean age 33 ± 11 years), with a mean height and weight of 1.70 ± 0.1 m and 69.7 ± 17.1 kg, respectively. The mean corresponding body mass index (BMI) was 25.1 ± 5.4 kg/m². Participants included nurses (n=8), doctors (n=2) and other health-related professions (n=7). All participants were fit tested using a standardised procedure (HSE 2021) prior to employing FFP3 respirators, except for the two who used the N95 device (FFP2). Approximately one half of the participants (9/17) reported pain when using RPE during clinical duties. The approximate frequency of breaks recorded by participants, as summarised in Table 7.1, were similar for each session of data collection, as they followed an established working pattern.

Table 7.1. Demographic and anthropometric data of study participants with detail of respirator use and any associated adverse reactions to the skin.

ID	PROFESSION	GENDER	ETHNICITY	AGE (Years)	BMI (kg/m²)	MASK MAKE	WORKING HOURS	BREAKS FROM MASK	ADVERSE REACTIONS TO RPE
1	Nurse	Female	White	29	20.3	Aura 1863+	12	4	Spots, dry skin
2	Nurse	Female	White	28	22.5	Aura 1863+	12	2	Itchiness, excessive sweating
3	Doctor	Female	White	41	23.1	Aura 9330+	8	2	Spots, itchiness
4	Nurse	Female	White	61	24.6	Aura 1863+	8	2	None
5	Nurse	Female	White	33	39.4	Aura 1863+	12	3	Spots, lumps
6	Other	Female	White	40	34.5	Alpha Solway 3030v	10	3	None
7	Nurse	Female	White	28	22.3	Aura 9330+	12	3	Spots, itchiness
8	Other	Male	White	30	23.0	N95	10	4	Excessive sweating
9	Other	Female	White	22	25.1	Aura 1863+	7.5	1	Spots, dry skin
10	Other	Female	Asian	26	25.0	3M 8835+	8.5	1	Spots, dry skin, excessive sweating, headaches
11	Nurse	Female	White	28	20.3	Aura 9330+	12	2	Itchiness, spots, excessive sweating
12	Other	Female	White	30	19.8	N95	8	4	None
13	Other	Female	White	26	25.1	Aura 9330+	8	1	Spots
14	Nurse	Female	White	57	25.2	Aura 9330+	10	1	None
15	Other	Female	White	23	33.5	Aura 1863+	12	3	Dry skin, rashes, spots, itchiness
16	Doctor	Female	Asian	35	20.2	Aura 1863+	9	4	Dry skin, spots, itchiness, rashes, excessive sweating
17	Nurse	Male	White	31	22.5	Aura 9330+	12	3	Dry skin

**Other includes healthcare assistants, operations managers and clinical trials assistants who were redeployed to COVID departments*

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7.4.1 TRANSEPIDERMAL WATER LOSS

Variations in the skin barrier properties, as a function of the TEWL parameter, assessed across the test sessions are shown in Figure 7.3. Absolute TEWL values at the negative control site (site A) ranged from 7.1 to 17.5 g/h/m², from 8.5 to 30.1 g/h/m² and from 7.6 to 31.1 g/h/m² on sessions 1, 2 and 3 respectively. The corresponding values at the nasal bridge (site B) were between 3.1 – 45.4 g/h/m², 3.9 – 53.2 g/h/m² and 4.3 – 66.9 g/h/m², while the corresponding values at the cheek (site C) ranged from 8.2 – 45.1 g/h/m², 7.7 – 42.4 g/h/m² and from 6.8 – 40.9 g/h/m² (Figure 7.3). A group normalisation of the data revealed relatively small within-participant variability between time points at site A, with ratio values ranging between 0.4 – 2.2. By contrast, increased ratio TEWL values were evident at site B (Figure 7.4), with values post-respirator application exceeding those measured pre-application in the vast majority of cases i.e. ratio >1 (Table 7.2). Analysis of the ratio TEWL values revealed differences which were statistically significant between the three test sites ($p < 0.001$), but not significantly different between the three test sessions ($p > 0.05$), although, on closer examination site B revealed significant differences in TEWL ratio values between sessions 1 and 2 ($p < 0.05$).

There was a high degree of variation in the response at the nasal bridge (Site B), with a sub-group of participants (#5, #6, #10, #13, #15) presenting with consistently high TEWL ratios following respirator application in each of the three sessions. By contrast, some participants (#1, #2, #3, #4, #14, #16) demonstrated correspondingly lower TEWL ratios (≤ 2.2 fold). Data revealed Site C demonstrated more consistent TEWL values, only 5 participants (#5, #6, #7, #10, #11 and #13) demonstrating any increases in the TEWL ratios (Figure 7.4).

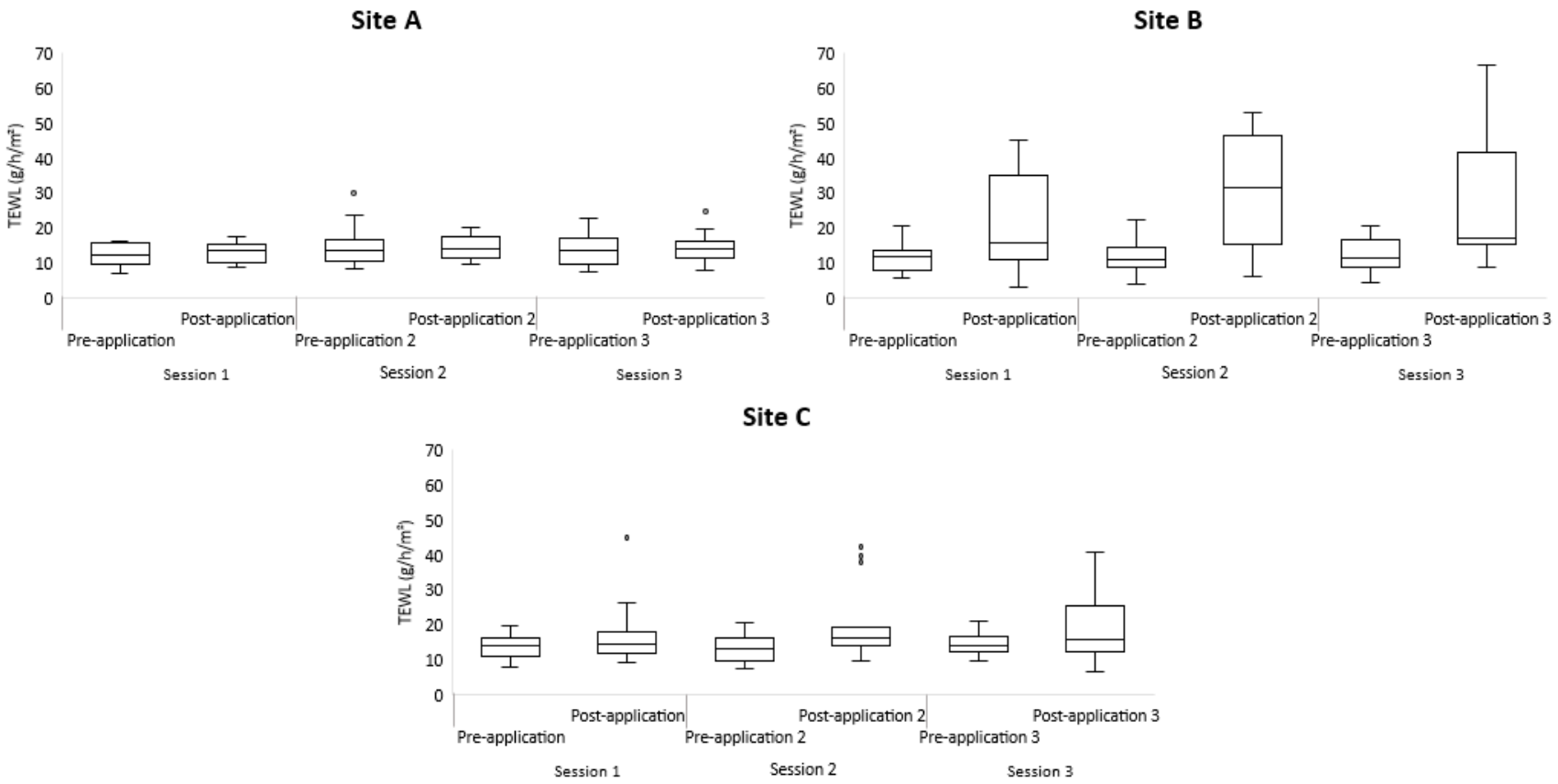

Figure 7.3. Box and whisker plot illustrating absolute TEWL values pre- and post-respirator application on the three sessions at sites A, B and C. Small variation observed at sites A and C, while a considerable increase in TEWL was exhibited at the nasal bridge (site B).

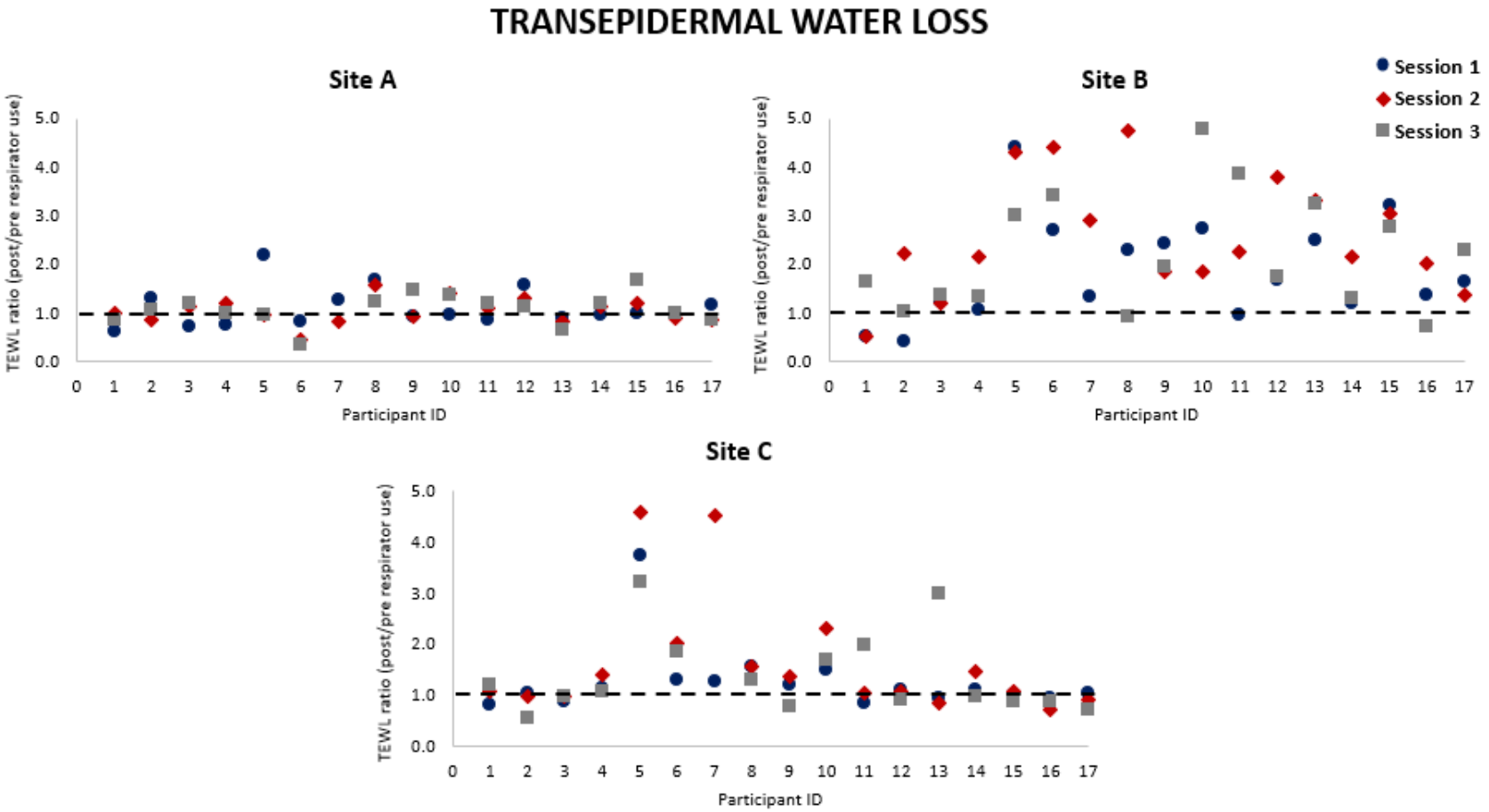

Figure 7.4. Ratio changes in TEWL values from pre- to post-respirator application for each participant on the three test sessions at sites A, B and C.

7.4.2 STRATUM CORNEUM HYDRATION

With respect to the SC hydration, a high degree of inter-participant variation was observed at sites A and C, with participants displaying absolute values at site A, which ranged between 30.4 and 97.1 AUs, between 37.6 and 95.1 AUs and from 33.2 to 99.5 AUs on sessions 1, 2 and 3, respectively. The corresponding values at site C were between 17.9 – 74.0 AUs, from 18.3 – 93.4 AUs and between 10.3 – 92.0 AUs (Figure 7.5). Similar considerable intersubject variation was also observed at site B, with absolute values ranging from 8.6 to 113.2 AUs across the test sessions (Figure 7.5). In addition, this site presented more elevated skin hydration values when compared to the other investigation sites. Differently from sites A and C which exhibited minimal variation in the pre-and post-respirator application ratio, some changes in SC hydration following respirator usage were observed for a sub-group of participants at site B (Figure 7.6). As an example, participant #6 presented with elevated skin hydration values (2-fold change), which remained consistent throughout the test sessions. In addition, a sub-cohort of participants (#1, #5, #9, #10, #11, #15 and #17) demonstrated increased SC hydration at session 2, equivalent to ≥ 1.8 ratio change. Increase in skin hydration at session 3, was below 2-fold, except for one participant (#15) who demonstrated a 4-fold increase. Nonetheless, all the differences associated with anatomical sites and test sessions were not found to be statistically significant ($p > 0.05$ in all cases)

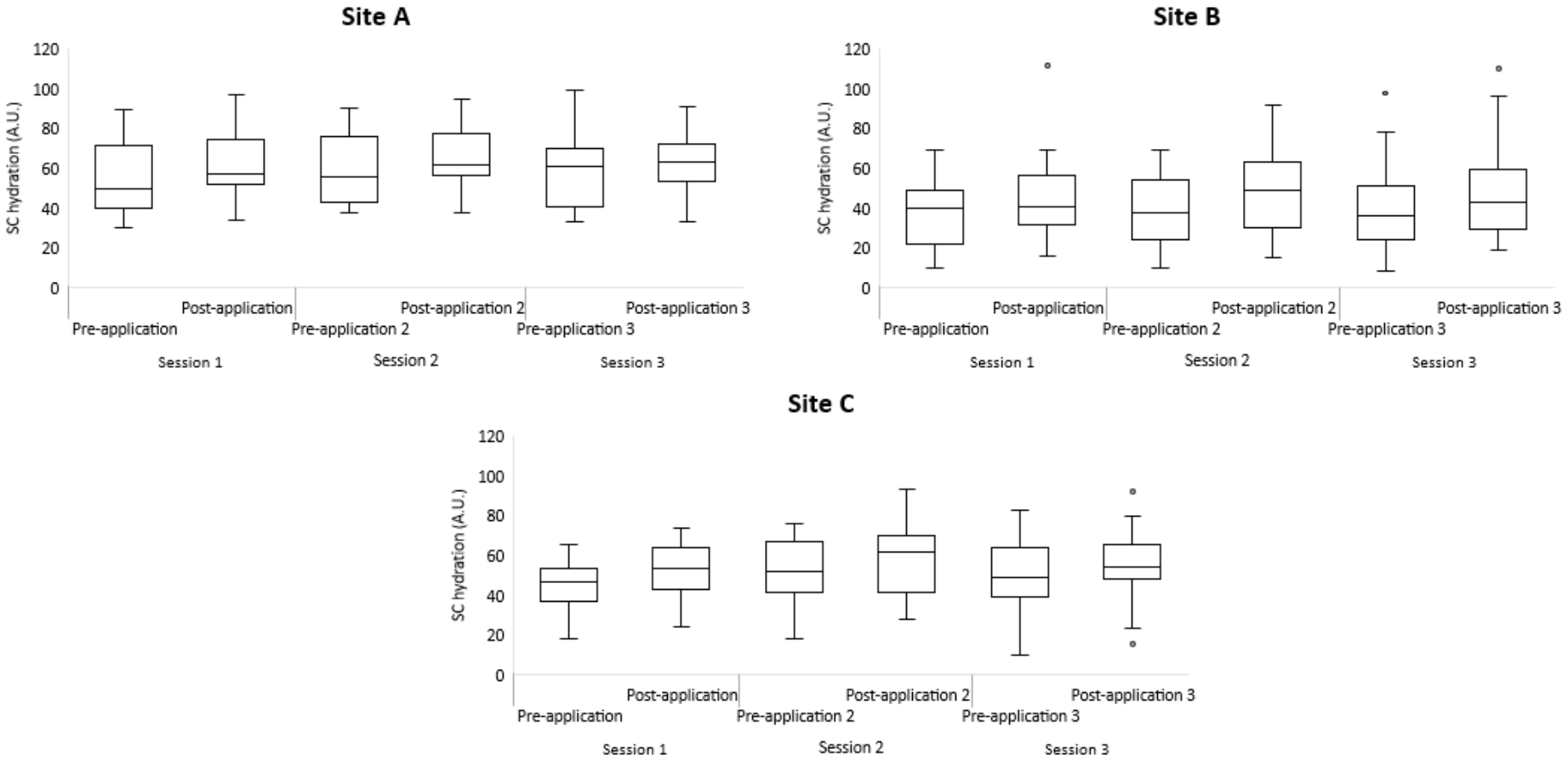

Figure 7.5. Box and whisker plot illustrating absolute SC hydration values pre- and post-respirator application on the three sessions at sites A, B and C.

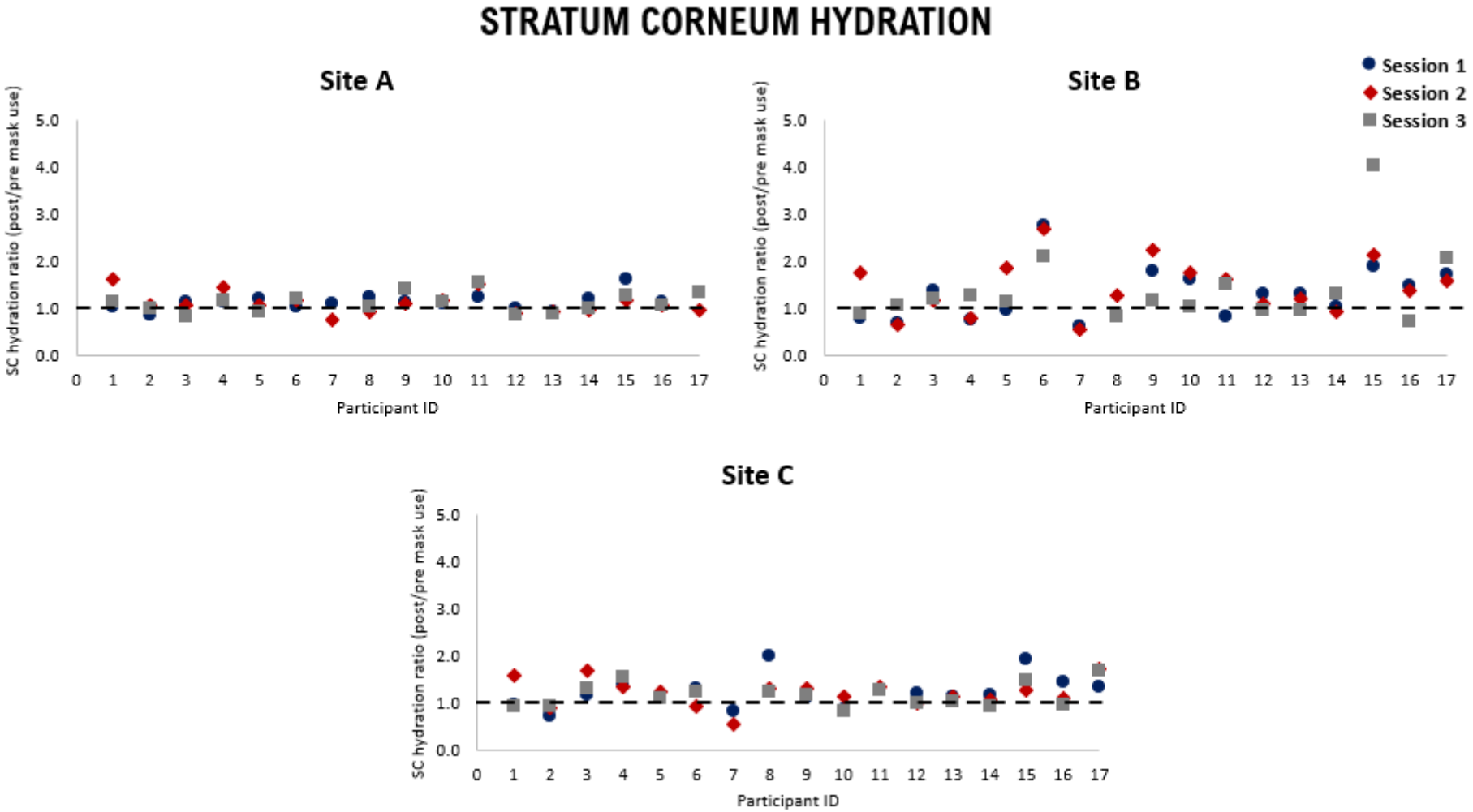

Figure 7.6. Fold changes in SC hydration values from pre- to post-respirator application for each participant on the three test sessions at sites A, B and C.

7.4.3 ERYTHEMA

There were few remarkable trends in relation to erythema, with participants demonstrating values across the test sessions, which ranged between 182.2 and 526.8 AUs, from 105.4 to 898.8 AUs and between 209 and 574.8 AUs at sites A, B and C, respectively (Figure 7.7). Indeed, for the vast majority of participants, erythema ratio changes were $\leq$1.5 at the anatomical sites for each of the test sessions (Figure 7.8). The one exception involved participant #6, who presented with a 2- and 1.5 ratio increase at site B on sessions 1 and 3, respectively.

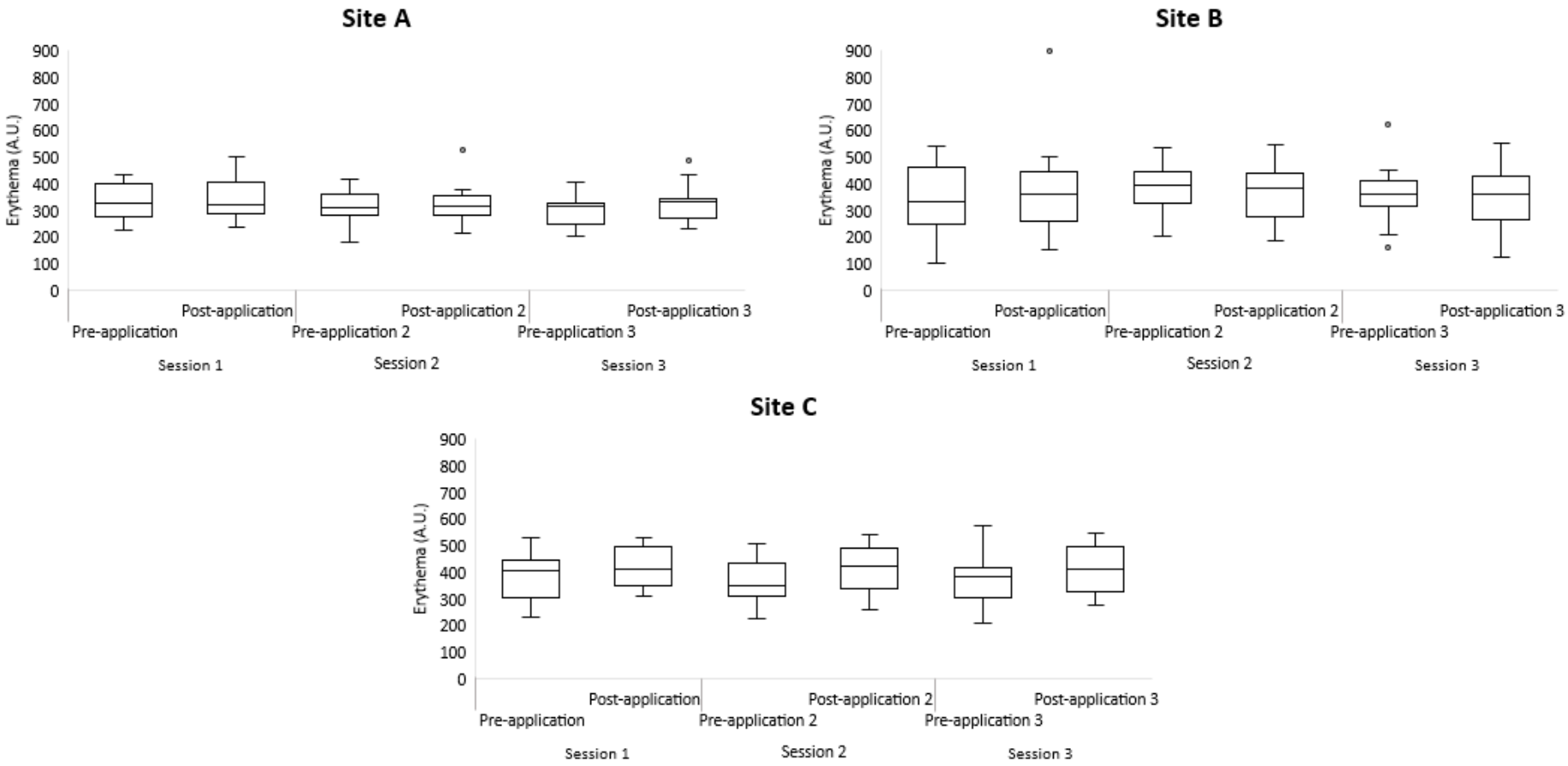

Figure 7.7. Box and whisker plot illustrating absolute erythema values pre- and post-respirator application on the three sessions at sites A, B and C

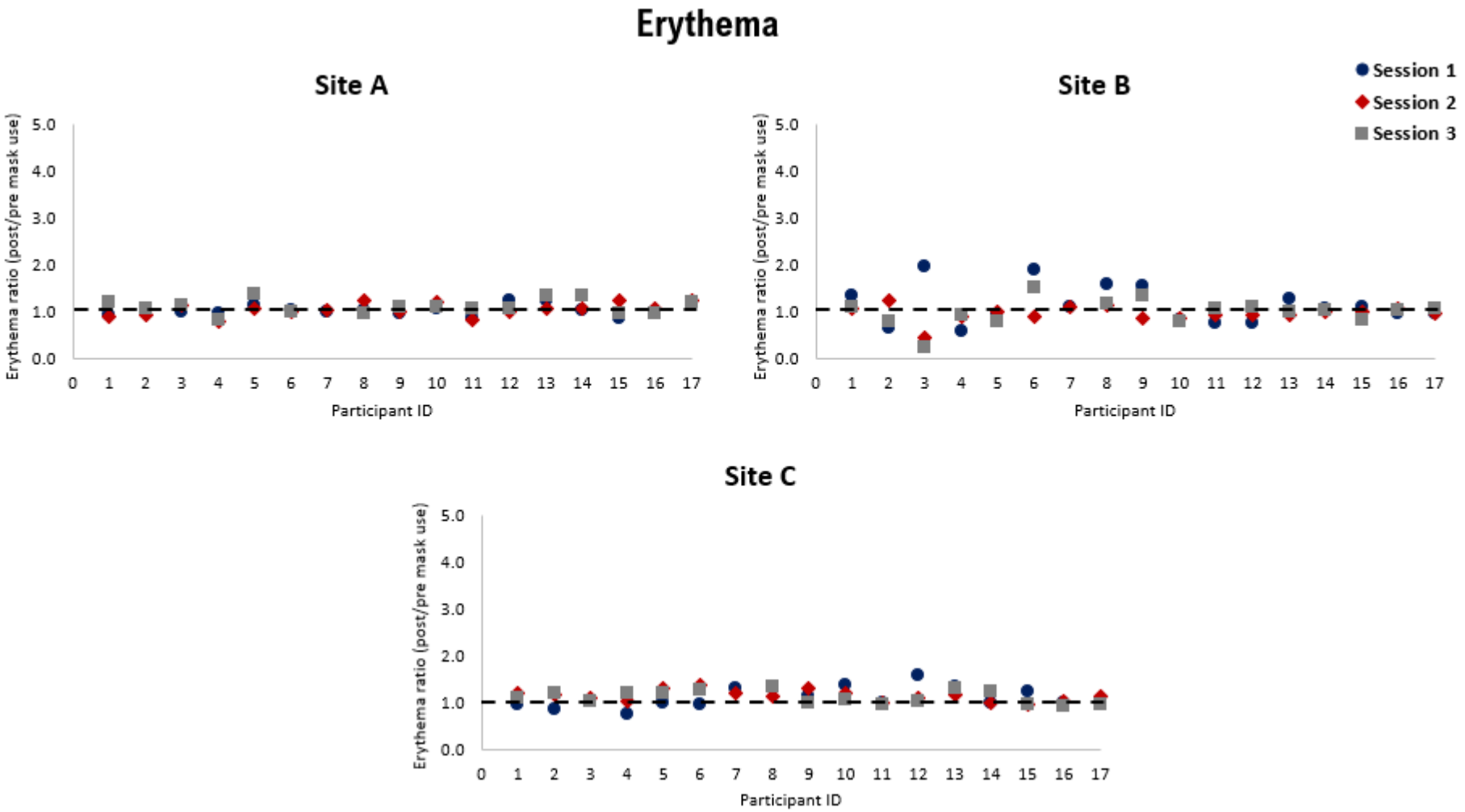

Figure 7.8. Fold changes in erythema values from pre- to post-respirator application for each participant on the three test sessions at sites A, B and C. Changes in erythema were <1.5 at all sites, with only participants 3 and 6 exceeding this threshold during the sessions.

7.4.4 SENSITIVITY ANALYSIS OF BIOPHYSICAL SKIN PARAMETERS

A sensitivity analysis was performed for each parameter associated with TEWL, SC hydration and erythema using arbitrary thresholds in the pre- to post-measurement ratios ranging from 1.0 to 3.0. Table 7.2 highlights the percentage of participants whose responses exceeded these thresholds at the different test sites. It confirms that the proportion of participants remained high with increasing thresholds for TEWL, particularly for the nasal bridge test site (B) for each of the test sessions. By contrast, with SC hydration and erythema, there was a marked reduction of these percentage values when the threshold was set above 1.0.

Table 7.2. Sensitivity analysis of the ratio changes in the three biophysical parameters at the three facial sites for each test session

PARAMETER	Threshold (ratio change)	Site A % of participants according to threshold			Site B % of participants according to the threshold			Site C % of participants according to the threshold		
		Session 1	Session 2	Session 3	Session 1	Session 2	Session 3	Session 1	Session 2	Session 3
TEWL	< 1.0	41	41	25	12	6	12	18	18	37
	≥ 1.0	59	59	75	88	94	88	82	82	63
	≥ 1.5	18	6	13	53	82	63	18	35	31
	≥ 2.0	6	0	0	41	71	44	6	24	19
	≥ 2.5	0	0	0	29	41	38	6	12	13
	≥ 3.0	0	0	0	12	35	31	6	12	13
SC Hydration	< 1.0	12	24	25	35	24	19	18	18	31
	≥ 1.0	88	76	75	65	76	81	82	82	69
	≥ 1.5	6	12	6	35	47	25	12	18	19
	≥ 2.0	0	0	0	6	18	19	6	0	0
	≥ 2.5	0	0	0	6	6	6	0	0	0
	≥ 3.0	0	0	0	0	0	6	0	0	0

PARAMETER	Threshold (ratio change)	Site A % of participants according to threshold			Site B % of participants according to the threshold			Site C % of participants according to the threshold		
		Session 1	Session 2	Session 3	Session 1	Session 2	Session 3	Session 1	Session 2	Session 3
Erythema	< 1.0	18	24	12	35	47	37	12	0	12
	≥ 1.0	82	76	88	65	53	63	88	100	88
	≥ 1.5	0	0	0	24	0	6	6	0	0
	≥ 2.0	0	0	0	6	0	0	0	0	0
	≥ 2.5	0	0	0	0	0	0	0	0	0
	≥ 3.0	0	0	0	0	0	0	0	0	0

7.4.5 CORRELATIONAL ANALYSIS WITH RESPECT TO INTRINSIC AND EXTRINSIC FACTORS

The role of intrinsic factors (BMI and Age) and extrinsic factors i.e., the nature of daily respirator usage, on skin health was also investigated. TEWL at site B was selected as this represented the most sensitive skin parameter to be influenced by respirator application. The linear models revealed that there were positive correlations between BMI and TEWL ratios at the nasal bridge, as illustrated in Figure 7.9, which were statistically significant at sessions 1 ($p<0.001$) and 2 ($p<0.05$). Individual data also revealed that three participants (#5, #6, #15) in the obese BMI range (>30 kg/m²) (WHO 2021) presented with high TEWL values, with ratio increases ranging between 2.7 and 4.4, which were sustained throughout the three test sessions. With respect to the age of participants, data revealed no interesting trends (Appendix C).

An equivalent analysis was performed with respect to daily hours and the number of breaks during respirator application (Figure 7.10). Data revealed considerable variability at each session, as exemplified by participants #6, #8, #10 across the three test sessions. Neither the working hours nor the number of breaks taken during shifts yielded a significant correlation with changes in TEWL ($p>0.05$). Indeed, some participants i.e. #2, #14 worked long shift periods (>10 hours) with limited breaks and demonstrated low TEWL ratio values. By contrast, other participants i.e. #9, #10, #13, who worked an 8 hour shift with a number of breaks demonstrated consistently higher TEWL ratio values.

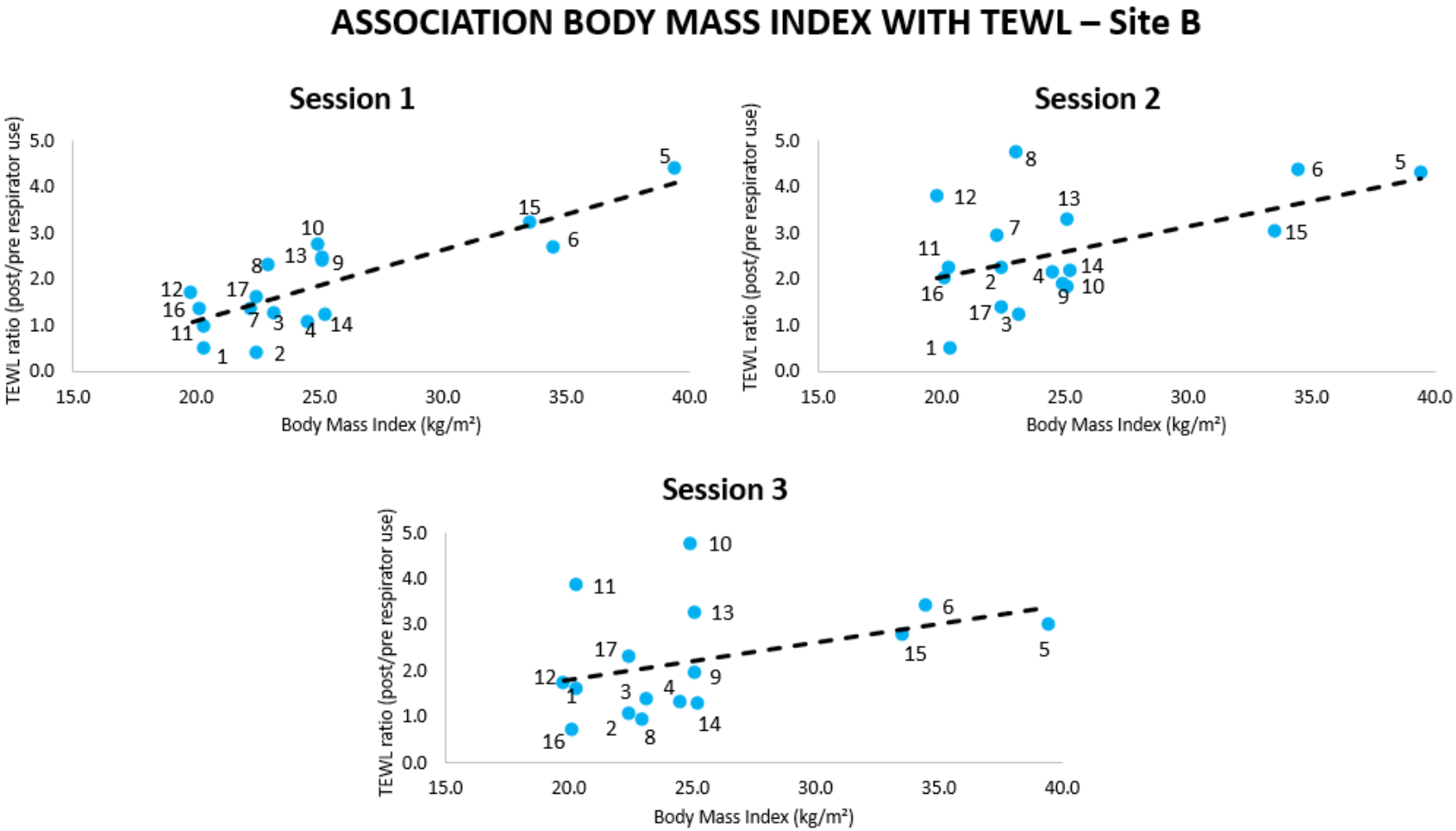

Figure 7.9. Correlations between BMI and TEWL ratio values at the nasal bridge site on the three test sessions, with participants in the obese BMI range expressing elevated TEWL values. The data labels on the coloured dots indicate the participant ID

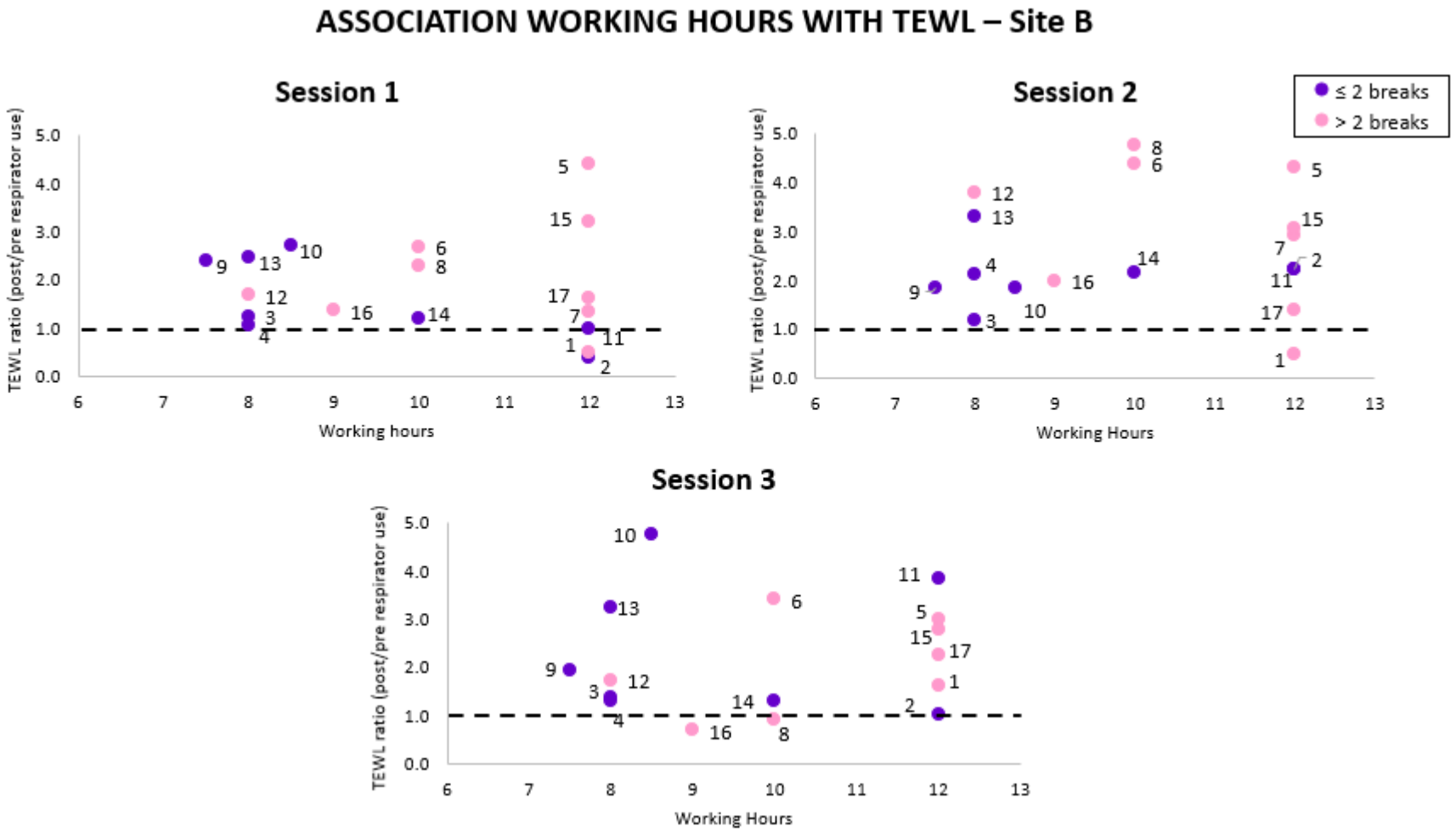

Figure 7.10. Scatter plot depicting working hours and TEWL ratio values at the nasal bridge site on the three test sessions. A reference line corresponding to a ratio change equal to 1 is depicted in the dashed line. Data showed no implication of working hours of TEWL expression

Correlations were also analysed between SC hydration and the daily working hours of the participants at the bridge of the nose, as illustrated in Figure 7.11. The following observations were made:

- There was an overall increase in skin hydration for subjects working 10 or more hours across the 3 sessions, which was remarked by participants #6 and #15
- Participants working for less than 10 hours presented with skin hydration values which were consistently below 1.5 folds increase, with the exception of #9 and #10, who occasionally exceeded this point.
- There seems to be no direct relationship between the number of breaks taken from RPE with skin hydration levels.

A similar approach was used to determine a possible correlation between skin hydration parameter with staff BMI on the bridge of the nose. The results showed no remarkable trends (data not shown).

A secondary sensitivity analysis was performed on the correlation between SC hydration and average daily working hours of staff to including threshold values from 1.0 to 2.5 folds, with participants conveniently categorised into two groups namely <10 hrs (n=7) and ≥10 hrs (n=10) (Table 7.3). Examination of the data revealed the importance of thresholds to assess changes in the skin status of participants on the basis of their working hours across the period of experiment, in particular when this was set at 1.5. As an example, it was noted that while only 30% (3/10) of the participants from the ≥10 hrs group exceeded this threshold on session 1, this was not the case in the following sessions, which presented with 60% (6/10) and 44% (4/9) of the subjects exceeding the threshold at sessions 2 and 3, respectively. The corresponding values for the group of participants working <10 hrs were 43% (3/7), 29% (2/7), and 0% (0/7). In addition, at least one or more participants of the ≥10 hrs group exceeded all the thresholds.

Table 7.3. Sensitivity analysis of the ratio changes in skin hydration parameters for participants working above and below 10 hours daily at the nasal bridge for each test session

Threshold	Session 1 No. of participants exceeding the threshold		Session 2 No. of participants exceeding the threshold		Session 3 No. of participants exceeding the threshold	
	<10 hrs	≥10 hrs	<10 hrs	≥10 hrs	<10 hrs	≥10 hrs
≥ 1.0	6	5	6	7	6	7
≥ 1.5	3	3	2	6	0	4
≥ 2.0	0	1	1	2	0	3
≥ 2.5	0	1	0	1	0	1

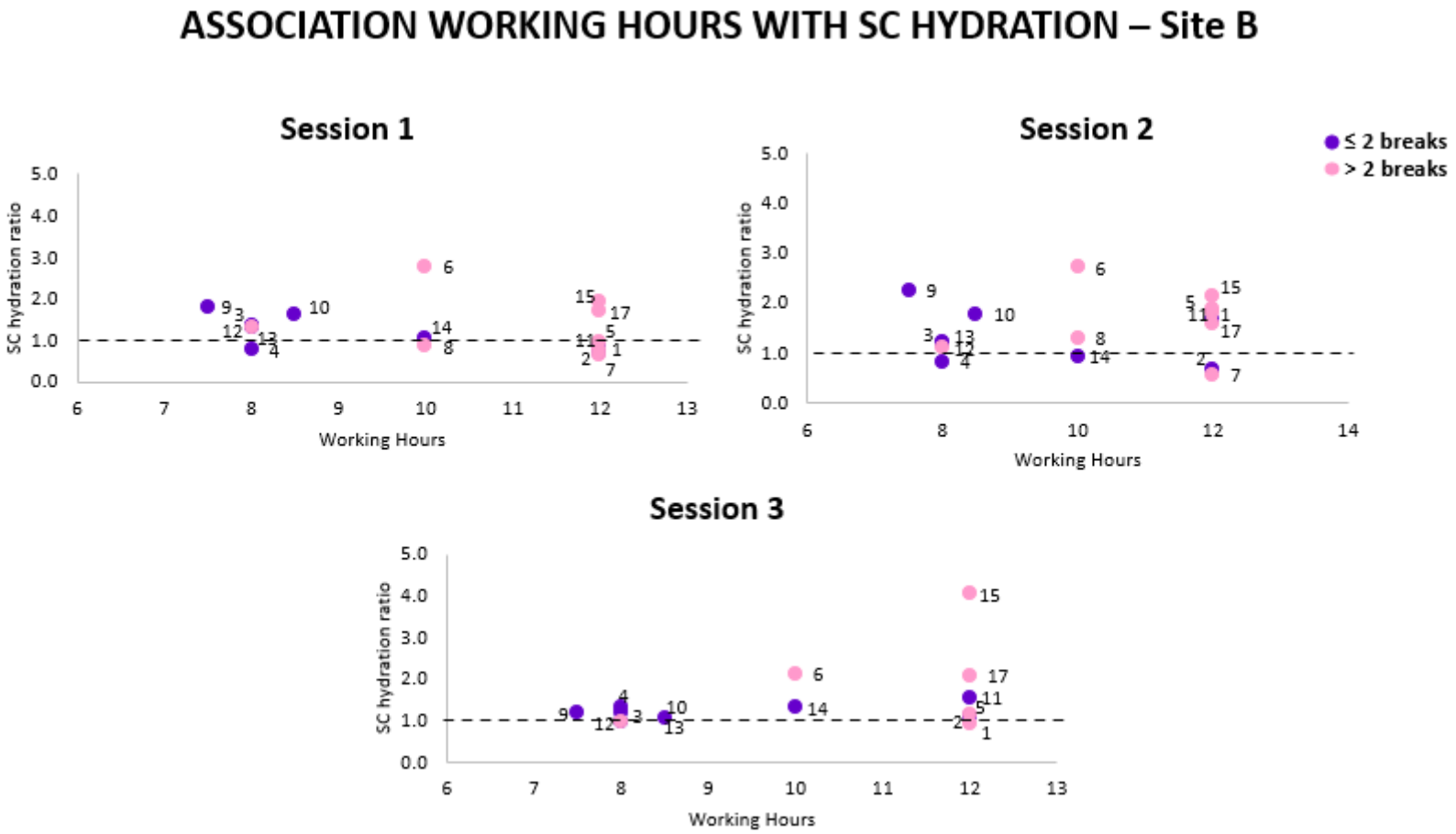

Figure 7.11. Correlations between the working hours and SC hydration ratio values at the nasal bridge site on the three test sessions. Figure shows an increase in SC hydration following >10 hours of shift with RPE.

7.5 DISCUSSION

One of the indirect consequences of the outbreak of the coronavirus pandemic is the adverse skin reactions as a direct result of the extensive use of personal protective equipment (PPE). Healthcare professionals are particularly affected, where the prolonged application has left them exposed to damaging mechanical and microclimate loads on the skin (Abiakam et al. 2021; Lan et al. 2020; Jiang et al. 2020). Although the nature and frequency of these adverse reactions have been extensively reported, there is little understanding of the effects of respiratory application on the integrity of skin sites of the face. In this context, the present study demonstrated how the temporal evaluation of biophysical parameters could enable a comprehensive analysis of changes in local skin physiology and barrier function. Significant changes in skin parameters have been observed within this cohort of 17 HCWs, which were particularly pronounced following two consecutive days of respirator use.

The study used an array of parameters to monitor changes in skin health in a cohort of HCWs based at one UK acute care provider. These included TEWL, SC hydration, and erythema, during three consecutive sessions of respirator use. Data revealed that participants responded differently to the use of RPE, as evidenced by the variable absolute values of the parameters during the sessions (Figures 7.3, 7.5 and 7.7). Nevertheless, the consistency in TEWL and SC hydration values observed at the control site (A) for each test session provides confidence that the absolute changes at the sites in direct contact with the respirator (B and C) represented changes in local skin properties. Indeed, the nasal bridge (site B) was most affected by the respirator application, as confirmed by the increased values in the biophysical parameters. For example, 35% of participants consistently demonstrated high TEWL values across the three test sessions (Table 7.2). These findings are consistent with previous studies, where a statistical increase in TEWL was reported at skin sites following 2 hours (Montero-Vilchez et al. 2021) and 8 hours (Han et al. 2021) of N95 respirator application. The mean values reported in these studies for the sites under the perimeter of mask application, i.e. nose bridge, were generally similar compared to those of the present study (22.8 vs 22.2 vs 21.3 g/h/m^2). Indeed, due to the bony prominence and cartilaginous substrate, the nasal bridge has been regularly identified as a vulnerable site for skin damage (Worsley et al. 2016). Indeed, at this site, TEWL and SC hydration values were generally higher on the second consecutive day of mask application (session 2) with more than 70% of the participants presenting in excess of a two-fold change from basal values in TEWL post-respirator usage (Table 7.2). Similar results have been reported in recent research where it was shown that the nasal bridge was the site subjected to the highest loading during mask-wearing (Caggiari et al. 2022).

By contrast, the cheek (site C) presented fewer events (~12%) where the TEWL value was increased and revealed a few interesting trends in terms of SC hydration. This could be explained by the fact that the middle cheek and the nasolabial areas present with poor hydration compared to other

facial sites (Voegeli et al. 2019). In addition, the cheek incorporates a higher proportion of soft tissues which can provide load-bearing capacity (Cyron et al. 2017). Although our findings contrast with a recent study which highlighted a significant SC hydration and TEWL increase at the cheek following the employment of respirators (Hua et al. 2020), these studies did not allocate breaks for respirator usage during their study design. This contrasts with the protocol adopted in the present study, where breaks were allowed (Table 7.1). The length of the breaks was different for each participant, ranging from 15 to 50 minutes and they were part of established clinical work patterns, which were uncontrolled in the study. These breaks from respirator application could have restored the TEWL and skin hydration values toward basal (unloaded) levels. In addition, these studies employed different mask designs different from the ones used in the current study

It is of note that there were no remarkable differences observed in skin erythema, evaluated using the Mexameter device. This was surprising given that skin redness was visibly evident in the loaded sites of some participants (Figure 7.12) and other studies have reported significant evidence of redness following respirator application (Han et al. 2021; Montero-Vilchez et al. 2021). This may be due to the lack of sensitivity in measurement system (Baquié et al. 2014; Kasraee 2017) and its dependence on skin pigmentation (Jayabal et al. 2021). The application of the Mexameter was also limited by the curved location of the nasal bridge, which could have introduced errors in the detection of erythema.

Figure 7.12. Session 3 data collection of a participant before (A) and after (B) 8.5 hours shift using a 3M FFP3 mask. The image details the inability of Mexameter to detect variations in skin redness as evidenced by the similarity in erythema scores: A) Baseline= 303.2 ± 57.6 A.U. and B) Post= 322.8 ± 18.8 A.U.

The present study has examined any correlations between intrinsic and extrinsic factors and the ratio changes in TEWL. As an example, the ratio changes in TEWL were significantly associated with participants' BMI at the bridge of the nose (Figure 7.9). Although the underlying mechanisms behind the correlation between BMI and TEWL are not fully elucidated, however, this association might be explained by the elevated perspiration generated within the occlusive micro-environment created by the devices, as a consequence of the combined effect of high body fat mass together with the adherence of HCWs to the demanding schedules in the COVID units. Indeed, exposure to elevated moisture at the skin interface can reduce the mechanical stiffness and strength of the stratum corneum thereby increasing its susceptibility to damage (Kottner et al. 2018). Changes in the biophysical parameters did not correlate to either the average daily working hours or the number of breaks taken by the participants during their clinical shifts (Figures 7.10 and 7.11). Indeed, the considerable variability in the responses across the cohort suggests that there are intrinsic factors coupled with other extrinsic factors which determine the skin tolerance to load-bearing. This contrast with findings from surveys, where HCWs subjectively reported skin reactions were associated with both the working shift time and the frequency of breaks (Abiakam et al. 2021). The inconsistent findings might be due to the subjective nature of the surveys, with participants probably overrating the perception of adverse reactions to the skin given the limited breaks and long hours of work.

The study cohort was limited by the relatively small HCW cohort from a single UK acute care provider. In addition, most of the participants were females of white (Caucasian) ethnicity (Table 7.1). There were also only small variations with respect to the age and BMI of the participants. Furthermore, the data collection was conducted over the course of three sessions and except for the testing days, participants were allowed to use skin protective measures during their shifts. In addition, due to practical reasons, data collection occurred over varying time periods (1-8 weeks), which might have impacted the nature of the skin response and in particular the inter-subject variability in the data. It is of note that the time between RPE removal and skin assessment (~10 minutes) might have influenced the biophysical parameters outputs. Influences of extrinsic factors such as temperature and humidity might have influenced the outputs of skin parameters. Nevertheless, these were minimised by performing the test session in a humidity and temperature-controlled environment. In addition, prior to assessing the skin of the participants, both at the baseline and following mask application, they were allowed to acclimatise to the room's ambient conditions. Furthermore, given that most of the participants worked long hours, the assessment of their skin pre- and post-RPE application was carried out at similar hours of the day.

Although it is essential to adopt RPE while working in COVID-19 high-risk units, strategies are required to protect skin health of heavily resourced healthcare workers. Indeed, what is initially

seen as skin erythema and indentation marks could easily lead to skin breakdown, which could provide an access site to coronavirus, as well as other hospital-acquired infections. Regardless of the successful fit test (HSE 2021), HCWs still continue to report adverse skin reactions (Table 7.1) and discomfort while employing these devices. Therefore, healthcare organisations worldwide must acknowledge these issues and create policies to protect skin health. Collaboration with industries is required to develop new respirator designs to provide comfortable and effective respirator devices (NHS England 2020).

7.6 CONCLUSION

The current study used a multi-array approach to assess changes in the skin health of healthcare workers before and after the use of respiratory protective equipment in routine clinical shifts. Participants varied in their responses, with the nasal bridge representing the anatomical site most affected by the devices. The study demonstrated that for a sub-group of healthcare workers, current respirators impair the barrier function of the skin which, if left untreated, could lead to changes in skin integrity. TEWL was the most sensitive parameter to change over the course of the longitudinal evaluation. Further studies are required to define relationships between mask designs, application periods and skin reactions.

Chapter 8 EVALUATION OF CHANGES IN SPATIAL AND TEMPORAL BIOPHYSICAL SKIN PARAMETERS OF HOSPITALISED PATIENTS PRESENTING WITH STAGE I PRESSURE ULCERS

The results of this study have been submitted for publication in International Wound Journal:

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doi:10.1111/iwj.14194

8.1 INTRODUCTION

To reduce the burden of pressure ulcers (PUs) on the healthcare systems and ensure a better quality of life for affected patients, it is of critical importance to detect PUs at an early stage prior to a loss of skin integrity. Indeed, when patients are admitted to care settings and considered at high risk of developing PUs, skin assessments are performed to identify early signs of damage (NCGC 2014). As indicated in section 1.6, this includes a thorough visual examination of at-risk body sites to identify the presence of erythema at the skin surface, followed by a manual test for non-blanching erythema, termed a skin tolerance test (Whitlock 2013). In addition to skin assessments, a PU risk assessment is also performed, using established tools, such as Braden, Waterlow, PurposeT and Norton scales (chapter 1, section 1.5) (Moore et al. 2019). However, these tools have been shown to have limited prognostic value above that of the experience and judgement of the nurse and/or clinician (Chou et al. 2013).

Stage I PU, characterised by non-blanchable erythema over intact skin, corresponds to the most common PU stage and is the first indication that skin health has been compromised (Shi et al. 2020). Detection of PUs at this stage is of critical importance for the implementation of optimal preventive strategies (section 1.4), which could facilitate recovery. However, even experienced clinicians face a significant challenge as a result of inconsistent and subjective diagnoses of skin damage using visual observations, medical history and/or basic physical examination (Payne 2016; Kottner et al. 2010). One such limitation is the reliance on skin redness, which can be misdiagnosed as incontinence-associated dermatitis or moisture lesions (Ayello et al. 2009). In addition, this local redness is impossible to identify in dark skin, resulting in a higher incidence in some care settings (Haavisto et al. 2022).

As illustrated in Chapter 2, Section 2.1, various non-invasive *in vivo* measurement techniques have been introduced within research settings, to monitor biophysical skin parameters associated with the structure and function of the skin (Bader et al. 2018). Of these, the measurement of both Transepidermal Water Loss (TEWL) and Stratum Corneum (SC) hydration has been regularly adopted in studies assessing individuals in long-term care facilities (Kottner et al. 2015) and specific comorbidities e.g. chronic venous insufficiency (CVI) (Angelova-Fischer et al. 2010). Although TEWL values are strongly influenced by different factors, including gender, age, and anatomical sites (Table 2.2), higher values are generally associated with loss of skin integrity (Jansen van Rensburg et al. 2019; Akdeniz et al. 2018). Similarly, a high SC hydration value is reflective of overhydrated skin, which increases the coefficient of friction and weakens the epidermis (Wilson et al. 2022). Conversely, dry skin may be considered a risk factor for heel pressure ulcer development (Lechner et al. 2017). Changes in TEWL and SC hydration were also evident in the skin surrounding venous leg ulcers (Dini et al. 2014), and have been examined in a range of different dermatological studies (Zainal et al. 2020; Montero-Vilchez et al. 2021).

Lab-based studies with prescribed insults to the skin have demonstrated that different biophysical skin parameters could accurately monitor temporal and spatial changes in skin health (chapters 4 and 5). In addition, the combination of these parameters may serve as a more powerful tool to differentiate between healthy and compromised skin sites (Figure 4.4) (Jayabal et al. 2021). There have, however, been a limited number of studies assessing changes in skin parameters over the site of early stage pressure ulcers. One such study involved spinal cord-injured (SCI) patients and compared their baseline biophysical skin values to other controls on both SCI patients and able-bodied cohorts (Scheel-Sailer et al. 2017). Although the findings of the study revealed a significant increase in skin redness and perfusion in SCI patients compared to the controls, however, the study was conducted on a small sample size of patients with stage I PU (n=6). In addition, the 30 years age gap between the intervention and control group might have influenced the results. Furthermore, the authors did not investigate changes in TEWL, which has been well-established as an important parameter reflective of skin barrier impairment (Akdeniz et al. 2018).

Nonetheless, stage I PU remains a challenge to detect and classify (Kottner et al. 2020), despite the growing awareness of the pathogenesis of skin damage. The majority of pressure ulcers are initiated in the superficial skin layers, thus presenting an opportunity to monitor the changes in biophysical skin parameters to reflect the development of the damage.

8.2 AIM OF THE STUDY

The present study aimed to assess skin health using an array of non-invasive measurement techniques, taken over a series of time points, to highlight the spatial and temporal changes in the biophysical skin parameters of hospitalised patients presenting with stage I PU.

8.2.1 STUDY OBJECTIVES

The objectives of the study include the followings: -

1. Recruit hospital inpatients presenting with stage 1 pressure ulcers, defined as non-blanching erythema.
2. Perform data collection in the form of a time series of non-invasive measurements involving biophysical tools and the collection of biofluids and surface skin corneocytes from the site of damage and adjacent healthy skin areas.
3. Investigate the influence of intrinsic factors (age, gender, BMI, etc) on skin health parameters in a clinical setting.
4. Analyse the parameters for their ability to differentiate between health and PU sites and identify time-dependent trends.
5. Assess the sensitivity and specificity of biophysical parameters to diagnose skin damage.

8.3 MATERIALS AND METHODS

The study received ethical approval from the UK Research Ethics Committee (REC) and the Health Research Authority (HRA) (IRAS 301685) (Appendix D). Signed and dated informed consent was received from each participant on the day of screening.

8.3.1 STUDY PROTOCOL

An observational longitudinal cohort study was designed to assess the spatial and temporal differences between a stage I pressure ulcer site, defined by non-blanching erythema to a healthy control skin site in hospitalised patients. The anatomical locations of the investigation included the area of skin compromised by skin damage (PU site) and an adjacent healthy site 10 cm lateral to it (control site) (Figure 8.1A). During this evaluation of spatial differences in skin sites, each enrolled patient was assessed on two separate occasions, namely session 1 (following screening for inclusion/exclusion criteria) and session 2 (24 hours after session 1) (Figure 8.1B). Furthermore, investigations were also conducted on a sub-cohort of patients at an intermediary site 5 cm between the control site and the PU site (Figure 8.1A). To examine the temporal response, a

convenience sample of patients had a third assessment on a selected day, termed session 3, which took place at least 6 days after the first assessment (Figure 8.1B), prior to hospital discharge.

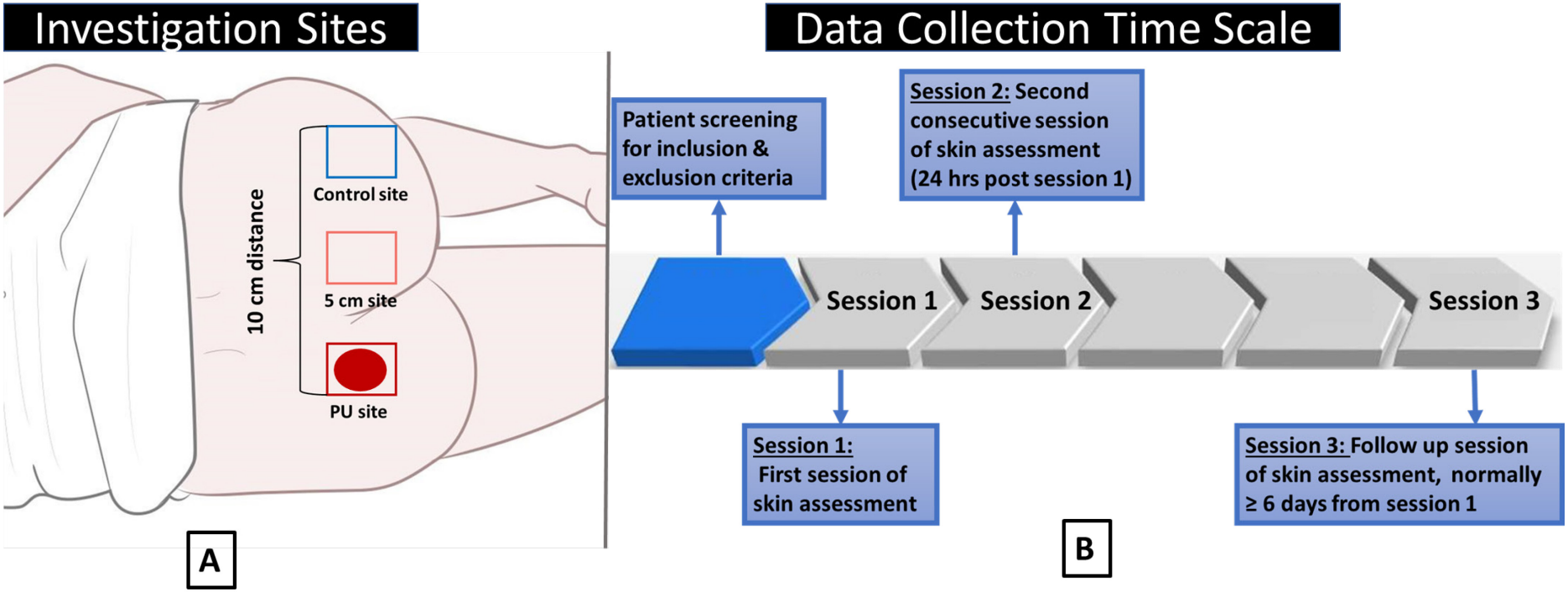


Figure 8.1. (A) Investigation sites associated with the location of the stage I PU and at distances of 5 cm and 10 cm from this location, the latter representing a control site. (B) Study protocol involving three test sessions.

8.3.2 SCREENING AND DATA COLLECTION SETTING

Prior to being enrolled in the study, each patient, identified by a nurse as presenting with redness and erythema on the skin surface, was subjected to a further assessment to ensure that the erythema could be classified as a stage I PU. The PU was verified by performing a test for non-blanching erythema, termed skin tolerance test, as previously reported (Figure 1.14) (Whitlock 2013). To review briefly, a finger was pressed over the area compromised by erythema for 15 seconds. If the skin remained red following the lifting of the finger, the patient was considered to present with a stage I PU. This procedure was carried out during the initial assessment for each patient, but not repeated during subsequent test sessions. Patient assessments were performed in the hospital bay in which patients were admitted, which was maintained at a temperature between 22^{o}C to 25^{o}C. Privacy and dignity of participants were maintained via hospital curtains and patients could request a chaperone (member of clinical staff or relative) where needed.

8.3.3 STUDY COHORT

Participants were purposefully recruited from four geriatric departments at one large University Hospital in the UK. The recruitment process, which lasted 4 months (March – July 2022), was conducted in close collaboration with the relevant clinicians, in particular ward nurses, who approached potential participants with no undue coercion. The study inclusion criteria consisted of; a) patients above 18 years of age, b) patients of all genders and ethnicity and c) patients presenting with a stage 1 PU. The exclusion criteria included a) patients with broken skin and/or presenting with active skin conditions at the sites of interest, b) patients approaching the end of life, c) patients who cannot be repositioned due to medical reasons and/or situated in COVID-19 departments, d) patients unable to provide informed consent and/or unable to understand the study protocol.

8.3.4 SKIN MEASUREMENTS

Spatial and temporal responses were evaluated by employing two skin parameters, namely Transepidermal Water Loss (TEWL) and Stratum Corneum (SC) hydration. These were selected as the most sensitive parameters following the findings of chapters 5 and 7. In addition, the investigation of a limited number of skin parameters was required in order to ensure data collection could be completed in an efficient manner minimising the burden for patients. TEWL was measured using the open chamber Tewameter® TM 300 (section 2.1.1.1), which was gently placed on the skin sites and collected values at 1Hz for one minute with an output in g/h/m² estimated from the mean of the last 10 readings when equilibrium was achieved. SC hydration was assessed using the

Corneometer® CM 825 (section 2.1.2.2), which was gently placed on the skin site and its response was expressed in arbitrary units (A.U.) as the mean of 5 repetitive measurements. Prior to the assessment, the sites of interest were blotted using a paper towel (Tork®, UK) to ensure the removal of body fluids and contaminants. All measurements were performed in accordance with international guidelines (du Plessis et al. 2013) and following described order to minimise influences (Kottner et al. 2014).

In addition, demographic data, medical history and relevant patient notes pertaining to nursing descriptions of the skin damage and relevant information from the hospital PU risk assessments scale were recorded. These included gender, age, ethnicity, height, weight, body mass index (BMI), current medications, routine skincare regimen and any prophylactic measures adopted to minimise the progression of skin damage. Risk factors for the development of PUs were identified based on an adapted risk assessment scale, which classified the patients into three categories namely, green, amber and red, with the latter grouping individuals presenting or at high risk of acquiring skin damage. All patients recruited were deemed to be at high risk of developing pressure ulcers.

8.3.5 SAMPLE SIZE CALCULATION

Due to the observational nature of the study, the sample size was determined to enable the power of statistical tests to be sufficiently high for the primary outcome measures (TEWL) under realistic assumptions. A power sample size calculation was performed (Verma et al. 2020), with an effect size f=1.18, α=0.05, and power=0.95, calculated from a 50% change in TEWL between PU and healthy skin sites as a marker of clinically meaningful effect size (Peer et al. 2022). Thus, an estimated sample size of 50 was calculated. In the likely scenario of loss to follow-up between sessions, an additional 10 patients were included in this estimation. According to information from the Tissue Viability Department at University Hospital Southampton (UHS) Foundation Trust, an average number of 50 patients are referred monthly with presentations of early signs of PUs. As such, 60 patients could be identified with a 10% recruitment rate, sampled over a 12-month period.

8.3.6 DATA ANALYSIS

Data from the biophysical parameters were imported into Microsoft Excel (Microsoft Office 365, USA) and IBM SPSS statistics V28 (IBM, Armonk, New York). Shapiro-Wilk and D'Agostino-Pearson's analyses revealed that the parameters were non-normally distributed and hence non-parametric statistics were employed. Wilcoxon signed-rank test was used to compare differences between the PU and healthy control sites within patients. Mann–Whitney U test analysis was performed to establish the independent effect of variables associated with demographics (i.e., gender), as well

as intrinsic factors (i.e., mobility status, incontinence and comorbidities). A group-level analysis was carried out using a Friedman test to assess the independent effect of time on skin parameters across the three sessions. Furthermore, to establish individual time-dependent profiles, data were normalised to session 1, and the ratio change over time for each patient was estimated, as previously described by the authors (Bostan et al. 2019). Spearman correlation was used to evaluate associations between TEWL and SC hydration parameters. An ordinal logistic regression analysis was performed, with "progression" and "non-progression" into PU set as the binary outcome. Receiver Operating Characteristic (ROC) analysis was used to investigate the sensitivity and specificity of the parameters and the area under the ROC curve was performed to measure the diagnostic ability of each parameter. Statistical significance was defined as $p < 0.05$.

8.4 RESULTS

A cohort of 30 inpatients (15 male and 15 female) presenting with stage 1 PUs were recruited into the study as detailed in Table 8.1. Participants were from a White ethnic background, with ages ranging from 71 to 95 years old (mean ± SD = 85.9 ± 6.6 years). The mean height and weight were 1.66 ± 0.09 m and 65.7 ± 21.3 kg, respectively, with a corresponding mean body mass index (BMI) of 24.3 ± 7.6 kg/m^2. Of the cohort, 67% (n= 20) presented with PU located at the sacrum, 63% (n= 19) were incontinent, 53% (n= 16) were bedridden, while only 17% (n= 5) had a previous history of PUs. Despite interventions including repositioning and support surfaces, 10 individuals developed a stage II PU or greater during their hospital stay. Three patients opted out of the study before session 2 and, as such, their data were used for session 1 analysis only.

Table 8.1. Anthropometric and demographic data of study participants with details of the locations compromised by PU and associated clinical conditions. n/a = data not available; * patients who developed stage II PU or greater

Participant ID	Gender	Age (years)	Body Mass Index (kg/m²)	Body Surface Area (m²)	Location of Pressure Ulcer	History of Pressure Ulcer	Mobility Status	Incontinent	Diabetic	Number of Medications
#1	Female	79	34.6	1.77	Buttock	No	Reliant mobility	No	Yes	12
#2	Male	78	19.1	1.65	Sacrum	No	Reliant mobility	yes	No	5
#3	Male	88	24.0	1.74	sacrum	No	Reliant mobility	yes	No	8
#4	Male	84	23.2	1.71	Sacrum	yes	Reliant mobility	yes	Yes	9
#5	***Male****	***94***	***23.1***	***1.79***	***Sacrum***	***No***	***Bedridden***	***Yes***	***No***	***11***
#6	Male	80	16.3	1.60	Sacrum	No	Reliant mobility	No	No	9
#7	Male	93	20.6	1.69	Sacrum	No	Bedridden	No	No	6
#8	***Male****	***88***	***32.4***	***2.29***	***buttock***	***No***	***Reliant mobility***	***No***	***No***	***n/a***
#9	Female	83	14.8	1.31	Sacrum	No	Bedridden	yes	Yes	5
#10	***Male****	***75***	***27.7***	***1.92***	***Sacrum***	***yes***	***Bedridden***	***yes***	***Yes***	***11***
#11	Male	77	22.1	1.79	Sacrum	No	Reliant mobility	No	yes	12
#12	***Male****	***93***	***17.4***	***1.62***	***Sacrum***	***yes***	***Bedridden***	***yes***	***No***	***9***
#13	Female	95	30.0	n/a	buttock	No	Bedridden	yes	No	8
#14	Male	94	18.3	1.52	Sacrum	No	Bedridden	yes	No	12
#15	Male	84	27.8	2.01	Sacrum	No	Reliant mobility	No	No	12
#16	Male	95	21.3	1.74	buttock	yes	Reliant mobility	No	No	9
#17	Male	89	21.4	1.95	Sacrum	No	Reliant mobility	yes	No	7
#18	Female	71	26.8	1.50	Sacrum	No	Bedridden	No	Yes	14
#19	***Female****	***93***	***19.5***	***1.46***	***Sacrum***	***No***	***Bedridden***	***No***	***No***	***16***
#20	Female	82	n/a	n/a	buttock	No	Bedridden	yes	yes	18
#21	***Female****	***83***	***26.7***	***1.72***	***buttock***	***No***	***Reliant mobility***	***No***	***Yes***	***14***
#22	***Female****	***92***	***45.9***	***2.33***	***Sacrum***	***No***	***Bedridden***	***yes***	***No***	***15***
#23	***Female****	***91***	***30.3***	***1.67***	***Sacrum***	***No***	***Reliant mobility***	***yes***	***No***	***6***
#24	***Female****	***85***	***35.4***	***1.84***	***buttock***	***No***	***Reliant mobility***	***yes***	***No***	***11***

Participant ID	Gender	Age (years)	Body Mass Index (kg/m²)	Body Surface Area (m²)	Location of Pressure Ulcer	History of Pressure Ulcer	Mobility Status	Incontinent	Diabetic	Number of Medications
#25	***Female****	***82***	***16.6***	***1.54***	***buttock***	***No***	***Bedridden***	***yes***	***Yes***	***15***
#26	Female	86	17.0	1.37	buttock	No	Reliant mobility	yes	Yes	7
#27	Female	89	16.4	1.45	Sacrum	No	Bedridden	No	No	4
#28	Female	90	19.4	1.46	buttock	yes	Bedridden	yes	No	12
#29	Female	88	39.4	2.02	Sacrum	No	Bedridden	yes	No	12
#30	Male	75	17.6	1.61	Sacrum	No	Bedridden	yes	No	n/a

8.4.1 SPATIAL DIFFERENCES IN SKIN PARAMETERS

8.4.1.1 TEWL DIFFERENCES BETWEEN PRESSURE ULCER SITE AND CONTROL SITE

Data revealed that the TEWL values at the control site of all patients were at normative levels (Akdeniz et al. 2018), ranging between 3.2 to 16.8 g/h/m² and 3.1 and 19.0 g/h/m² on sessions 1 and 2, respectively (Figures 8.2A and 8.2B). By contrast, the corresponding TEWL values at the PU sites ranged from 21.4 to 118 g/h/m² and 18.4 to 157.5 g/h/m². The differences in values between the two sites were statistically significant ($p<0.001$), with a median difference between sites of 39.9 and 62.3 g/h/m^2 on sessions 1 and 2, respectively. There was considerable variation in TEWL values at the PU sites between sessions 1 and 2, although these were not statistically significant ($p = 0.07$). Indeed, while some patients exhibited a progressive increase in TEWL values in session 2, with values exceeding twice that of session 1 (#6, #7, #8, #11 and #28), this trend was not evident with other patients who showed similar TEWL values between sessions (#1, 2#, #5 and #14).

8.4.1.2 SC HYDRATION DIFFERENCES BETWEEN PRESSURE ULCER SITE AND CONTROL SITE

Spatial changes in skin status as measured by SC hydration revealed a high degree of inter-patient variation at both the control and PU site (Figures 8.2C and 8.2D). Indeed, there were no clear differences between control and PU sites ($p>0.05$). The values at the control site were generally consistent between sessions with SC hydration varying between 13.0 – 64.5 and 22.0 – 68.5 A.U. at sessions 1 and 2, respectively. The corresponding ranges at the PU sites were 4.3 – 86.1 and 5.7 – 83.4 A.U. On closer examination of the data, a sub-group of patients (#8, #13, #18, #22 and #28) presented with elevated skin hydration values at the PU site at both test sessions. By contrast, several patients presented with very dry skin at the PU site (#6, #16 and #29). It was also noted that at the PU site, a number of patients (#1, #5, #6, #7, #9, #10, #14, #16, #21, and #26) revealed SC hydration values greater than a 1.5 fold change from session 1 to 2, with a maximum fold change of 3.4 (#10).

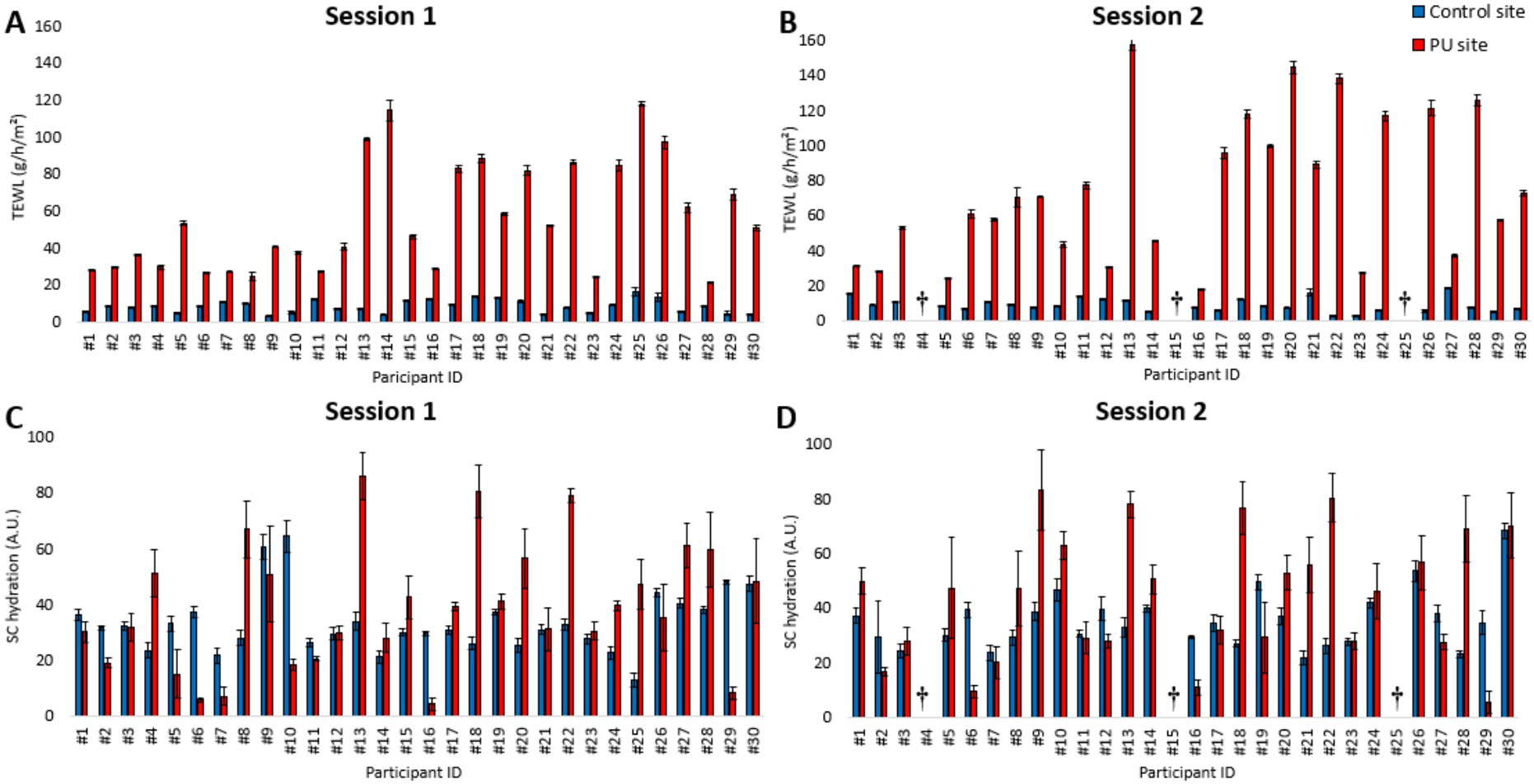


Figure 8.2. Absolute changes in TEWL (A and B) and SC hydration (C and D) values at the PU compromised and adjacent 10 cm healthy control sites for each participant. Significantly increased TEWL values are evident at the PU-compromised sites and a considerable inter-subject variation is observed with SC hydration in both sessions. † indicates missing data

8.4.2 VARIATIONS IN TEWL AT VARYING DISTANCE FROM THE PU SITE

Further analyses were performed on a sub-cohort of 19 patients to only assess changes in skin TEWL response at a distance of 5 cm from the PU site. The data for both sessions are detailed in Table 8.2. Similar to the values at the 10 cm control site, TEWL responses at the 5 cm site were generally at the normative level, with values ranging from 2.7 to 18.7 g/h/m² and 1.7 to 20.0 g/h/m² on sessions 1 and 2, respectively. It was noted, however, that 6/19 patients (#13, #14, #20, #21, #25 and #27) exhibited TEWL values > 20.0 g/h/m² at the 5 cm site in one or both of the test sessions. Although there were significant differences ($p<0.001$) between PU and 5 cm sites, this was not evident either when the 5 cm sites were compared to the 10 cm controls or when the values at the 5 cm sites of the two sessions were compared ($p>0.05$).

Table 8.2. TEWL absolute value at the three investigation sites for the two consecutive test sessions

Participant ID	Session 1			Session 2		
	Control site (10cm)	5 cm site	PU site	Control site (10cm)	5 cm site	PU site
#11	12.5	18.7	27.7	14.1	20.0	77.5
#12	7.2	9.4	40.6	12.3	14.0	30.6
#13	7.6	10.1	99.0	11.6	41.1	157.5
#14	4.1	62.6	114.3	5.8	23.3	45.9
#15	12.1	15.2	46.2	n/a	n/a	n/a
#16	12.9	11.3	28.9	7.8	11.6	18.4
#17	10.0	10.4	82.9	6.3	10.4	95.8
#18	14.0	13.8	88.4	12.0	13.2	118.1
#19	13.4	16.2	58.4	8.5	9.3	99.8
#20	11.0	10.7	81.9	7.3	29.8	144.7
#21	4.3	7.2	52.5	16.1	35.6	89.3
#22	7.6	8.0	86.5	3.2	7.2	138.3
#23	5.2	7.1	24.5	3.1	1.7	27.2
#24	9.3	9.5	84.8	6.4	8.0	116.9
#25	16.8	53.1	118.0	n/a	n/a	n/a
#26	13.5	12.2	97.1	5.6	14.4	121.4
#27	6.1	2.7	61.9	19.0	24.0	37.2
#28	8.7	7.4	21.4	8.1	8.1	126.1
#30	4.7	5.4	50.6	6.9	6.3	72.8
Median	9.3	10.4	61.9	7.8	13.2	95.8
Range	12.7	59.9	96.6	15.9	39.4	139.1

n/a = data not available

8.4.3 TEMPORAL DIFFERENCES IN SKIN PARAMETERS

Ten patients who had an extended hospital stay were included in a follow-up assessment (session 3) to evaluate temporal changes in the two skin parameters. For practical reasons, the day of data collection for session 3 varied between patients, ranging from 6 to 18 days (7.9 ± 3 days) after session 1. To assess time-dependent changes in skin response, the values of the two parameters at both control and PU sites from sessions 2 and 3, were normalised to the corresponding TEWL and SC hydration values on session 1. The absolute values for each session in conjunction with the fold changes are detailed in Table 8.3.

With reference to TEWL at the control site, although there were no significant time-dependent changes ($p=0.7$), however, there was generally a small increase, which was ≤ 1.7 fold in both sessions. However, three patients i.e. #21, #27 and #30, exceeded this threshold in at least one session. Participant #21 was a particular outlier at the control site, with over a three-fold increase in sessions 2 and 3. Four of the ten patients showed a decrease in control site TEWL, denoted by a ratio of <1 from baseline. It is of note that the absolute TEWL values across all participants and sessions remained within the normative range i.e. < 20.0 g/h/m².

No statistical differences ($p>0.05$) were detected at the PU site, with fold changes generally ≤ 1.8. However, three patients (#11, #21, and #26) exhibited increases in TEWL which were $>$ 2 fold on one or both sessions (Table 8.3). By contrast to the control site, the absolute values revealed that the PU-compromised site was >20.0 g/h/m² in all patients for each test session. Indeed, at least 70% (7/10) of the cohort had PU TEWL values >50.0 g/h/m².

With reference to skin hydration, no significant ($p= 0.2$) time-dependent changes were detected both at the control and the PU site. However, 6/10 and 7/10 of the patients exhibited a decrease (< 0.8) or no change at the control site during sessions 2 and 3. By contrast, a small increase in fold change for SC hydration was evident at the PU site, although these values were generally less than 2 fold. The one exception to this trend was patient #10, who presented with a 3.4 and 3.5 fold increase on sessions 2 and 3, respectively.

Table 8.3. Fold changes in TEWL and SC hydration values at the 10 cm healthy control and PU compromised site for test sessions 2 and 3, with associated days of session 3 assessment.

TRANSEPIDERMAL WATER LOSS TEMPORAL PROFILE															
	Session 1		Session 2		Session 3 (Day of assessment)			Fold changes from Session 1							
								Session 2				Session 3			
Participant ID	Control site	PU site	Control site	PU site	Control site	PU site		Control site		PU site		Control site		PU site	
#10	5.3	37.5	8.6	43.9	8.7	52.5	(18)	1.6	↑	1.2	**nc**	1.6	↑	1.4	↑
#11	12.5	27.7	14.1	77.5	14.5	163.8	(6)	1.1	**nc**	2.8	↑↑	1.2	**nc**	5.9	↑↑↑
#12	7.2	40.6	12.3	30.6	7.0	40.9	(7)	1.7	↑	0.8	**nc**	1.0	**nc**	1.0	**nc**
#20	11.0	81.9	7.3	144.7	14.0	74.1	(7)	0.7	↓	1.8	↑	1.3	↑	0.9	**nc**
#21	4.3	52.5	16.1	89.3	18.1	108.4	(6)	3.7	↑↑	1.7	↑	4.2	↑↑↑	2.1	↑
#22	7.8	86.5	3.2	138.3	3.1	71.6	(6)	0.4	↓	1.6	↑	0.4	↓	0.8	**nc**
#24	9.3	84.8	6.4	116.9	4.0	109.9	(8)	0.7	↓	1.4	↑	0.4	↓	1.3	↑
#26	13.5	97.1	5.6	121.4	4.7	78.2	(8)	0.4	↓	1.3	↑	0.3	↓	0.8	**nc**
#27	6.1	61.9	19.0	37.2	8.7	48.0	(7)	3.1	↑↑	0.6	↓	1.4	↑	0.8	**nc**
#30	4.7	50.6	6.9	72.8	9.5	134.2	(6)	1.5	↑	1.4	↑	2.0	↑	2.6	↑↑
STRATUM CORNEUM HYDRATION TEMPORAL PROFILE															
#10	64.5	18.5	46.8	63.2	46.4	64.5	(18)	0.7	↓	3.4	↑↑	0.7	↓	3.5	↑↑
#11	26.4	20.4	30.7	29.2	25.9	32.6	(6)	1.2	**nc**	1.4	↑	1.0	**nc**	1.6	↑
#12	29.6	29.8	39.8	27.9	40.2	43.5	(7)	1.3	↑	0.9	**nc**	1.4	↑	1.5	↑
#20	25.4	56.5	37.2	53.1	26.5	57.4	(7)	1.5	↑	0.9	**nc**	1.0	**nc**	1.0	**nc**
#21	30.9	31.1	22.0	55.8	40.4	22.6	(6)	0.7	↓	1.8	↑	1.3	↑	0.7	↓
#22	32.9	79.3	26.3	80.5	29.6	78.3	(6)	0.8	**nc**	1.0	**nc**	0.9	**nc**	1.0	**nc**
#24	22.8	39.6	42.0	46.2	42.0	68.8	(8)	1.8	↑	1.2	**nc**	1.8	↑	1.7	↑
#26	44.4	35.2	53.7	56.7	45.4	68.5	(8)	1.2	**nc**	1.6	↑	1.0	**nc**	1.9	↑
#27	40.5	61.1	38.2	27.6	35.6	27.0	(7)	0.9	**nc**	0.5	↓	0.9	**nc**	0.4	↓
#30	47.4	48.4	68.5	70.4	54.3	84.1	(6)	1.4	↑	1.5	↑	1.1	**nc**	1.7	↑

↓= < 0.8-fold change; n.c. (no change) = 0.8 – 1.2; ↑ = > 1.2-fold change; ↑↑ = ≥ 2.5-fold change; ↑↑↑ = ≥ 3.5-fold change

8.4.4 CLUSTER ANALYSIS OF SKIN PARAMETERS WITH AGE AND BMI

In order to assess the implication of patients' age and BMI on TEWL and SC hydration values spearman correlation analyses were carried out. Both at the control sites as well as at the PU sites, data revealed no trends with either TEWL or hydration (Appendix E). Cluster analyses between the sessions revealed consistently high TEWL values for some patients (#13, #18, #22 and #24) in the overweight (BMI ≥ 25) and obese (BMI ≥ 30), with values above 80 and 115 g/h/m^2 on sessions 1 and 2, respectively (Appendix F). No interesting trends were detected at the control sites for both sessions. Similar analyses were performed with regard to the age of the patients with no remarkable findings (data not shown).

8.4.5 INFLUENCE OF INTRINSIC FACTORS ON TEWL AND SC HYDRATION

There was no significant influence on any of the seven intrinsic factors at the control site either on session 1 or 2 (Figures 8.3A and B). By contrast, some of these factors influenced the TEWL values at the PU site (Figures 8.4A and B). There were significant differences in TEWL values between genders, with female patients expressing significantly higher TEWL values compared to males in session 1 ($p < 0.05$) and in session 2 ($p < 0.01$). Similar significant differences were evident in session 1 regarding mobility status and incontinence, with bedridden and incontinent patients presenting elevated TEWL values ($p < 0.05$), compared to those with reliant mobility (able to mobilise with assistance) and those who were independent with bladder and bowel function (Figure 8.4A). However, mobility and incontinence did not influence session 2 values i.e. $p > 0.05$. The impact of the other intrinsic factors revealed no statistically significant trends although the anatomical locations presenting with PU, namely sacrum and buttocks, differed with TEWL values being higher at the buttocks on both sessions (Figures 8.4A and B).

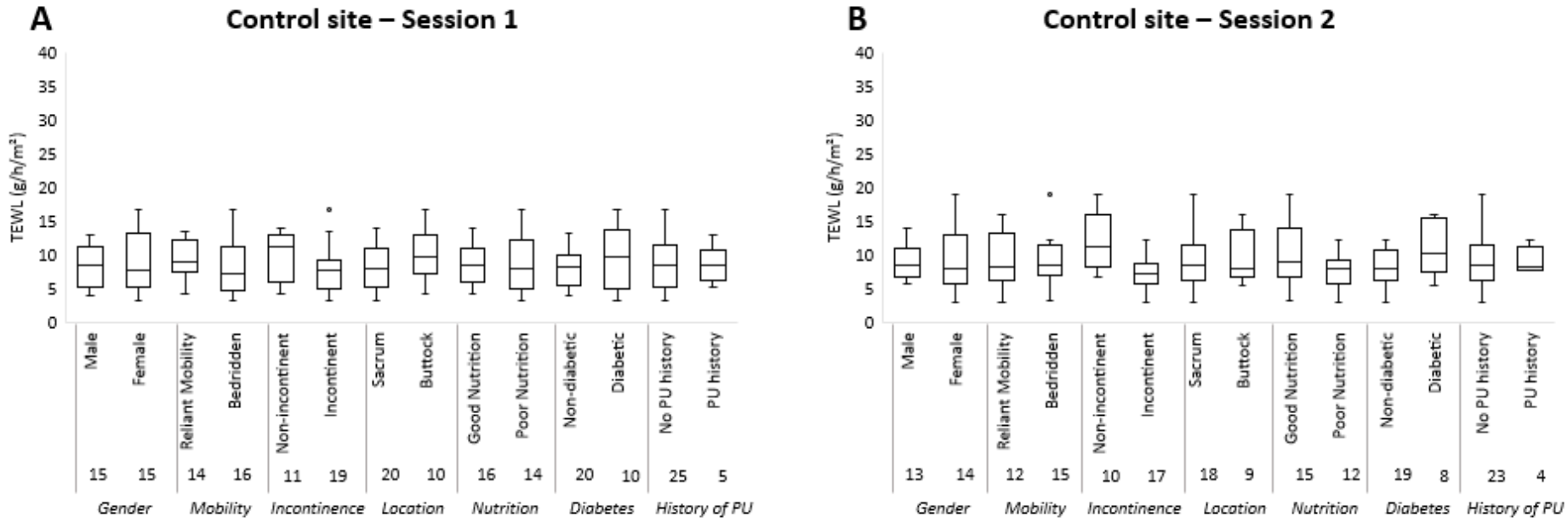


Figure 8.3. Impact of intrinsic factors on TEWL output values at the control site on session 1 (A) and session 2 (B). No interest trends detected with any of the intrinsic factors. The data labels on the categories indicate the number of participants per group. For the purpose of data clarity, the Y-axis upper limit in this figure has been put to 40 g/h/m².

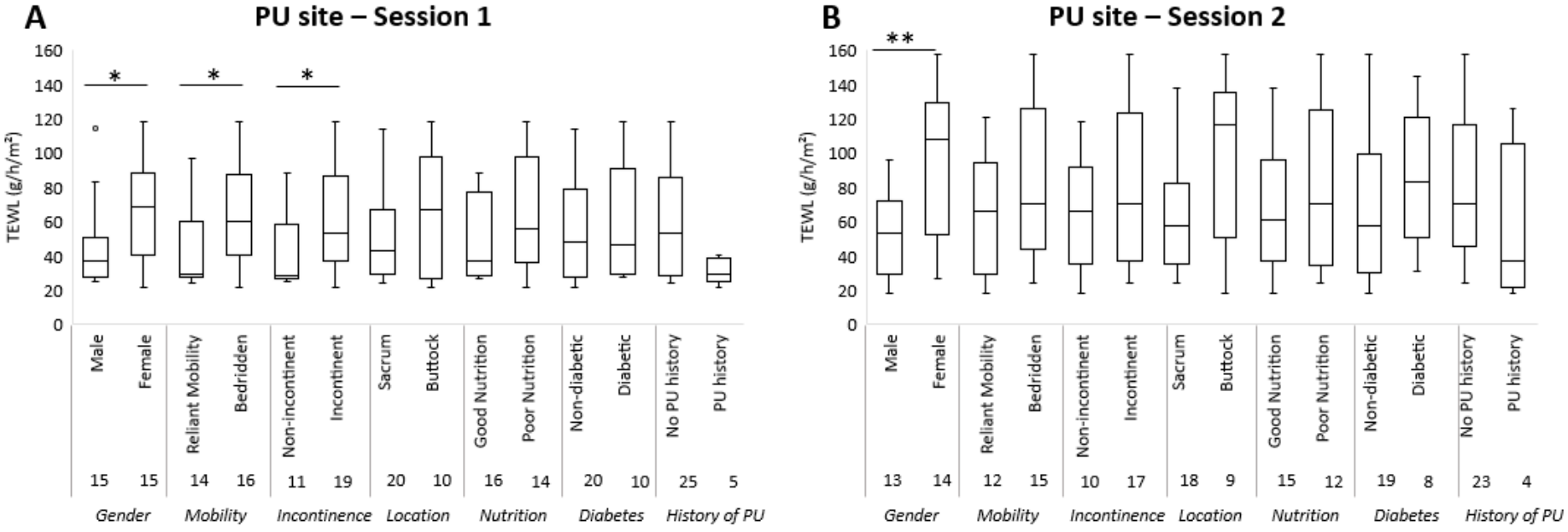


Figure 8.4. Impact of intrinsic factors on TEWL output values at the PU site on session 1 (A) and session 2 (B). Significant impact associated with gender, mobility and incontinence. The data labels on the categories indicate the number of participants per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

There was no significant influence on SC hydration for any of the seven intrinsic factors at the control site (Figures 8.5A and B). In a similar manner to TEWL, gender was the main factor influencing skin hydration values at the PU site during sessions 1 ($p < 0.01$) and session 2 ($p < 0.05$) (Figures 8.6A and B). No other significant trends were evident in session 1 in relation to the other factors. In session 2, there were significant differences ($p<0.05$) observed in mobility status and diabetes on SC hydration values. Although there were no significant SC hydration changes with incontinence ($p = 0.07$), it was noted that patients presenting with incontinent episodes tended to express higher values compared to those who had control of bladder and bowels. The PU sites associated with the buttocks presented higher skin hydration values compared to the sacrum on both sessions (Figures 8.6A and B).

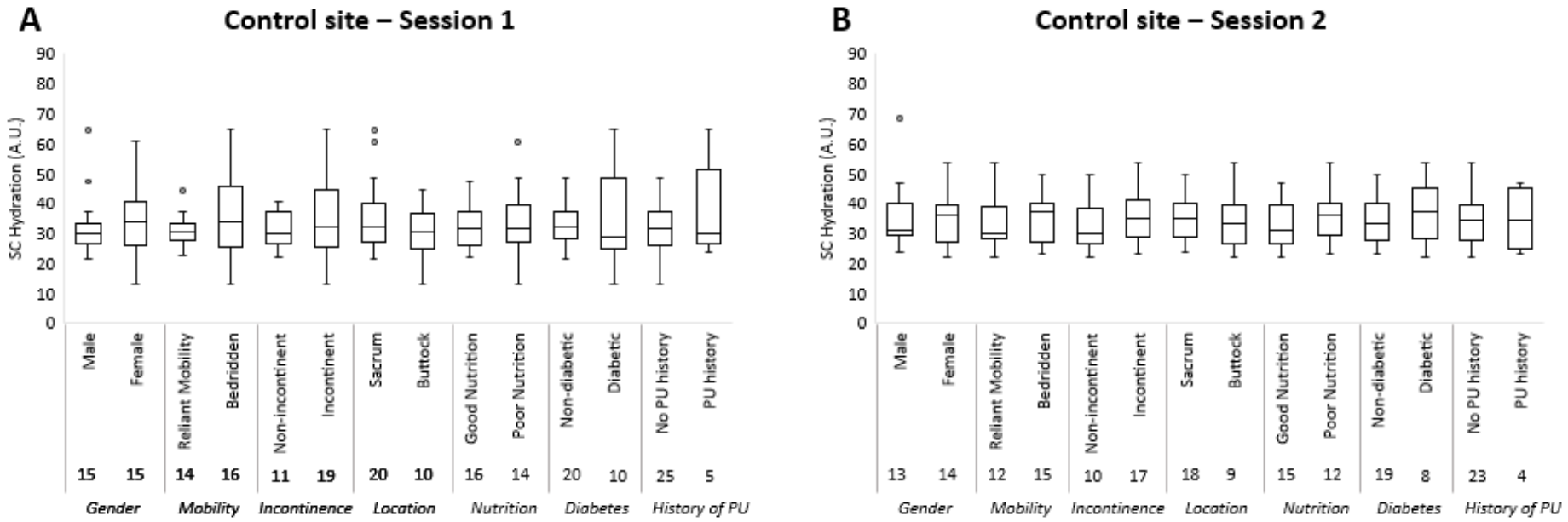


Figure 8.5. Impact of intrinsic factors on SC hydration output values at the control site on session 1 (A) and session 2 (B). The data labels on the categories indicate the number of participants per group.

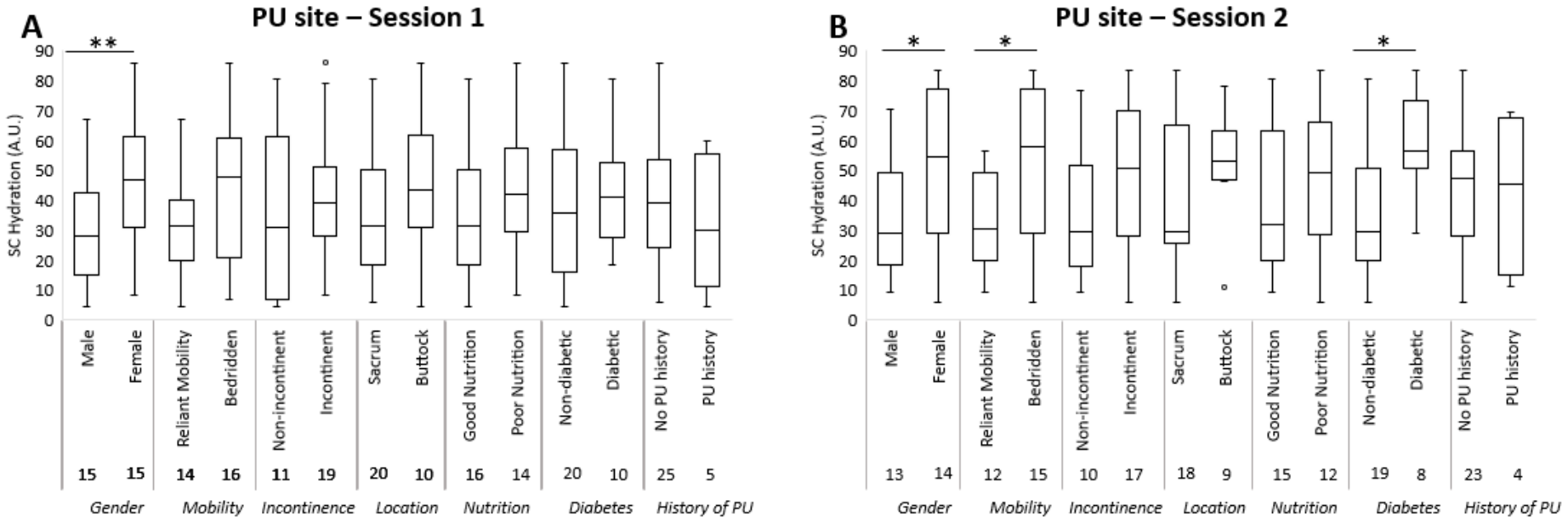


Figure 8.6. Impact of intrinsic factors on SC hydration output values at the PU site on session 1 (A) and session 2 (B). The data labels on the categories indicate the number of participants per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

8.4.6 CORRELATION BETWEEN SKIN PARAMETERS

Association between the TEWL and SC hydration parameters at the PU site revealed interesting trends, which are presented in Figure 8.7. Close examination of the data suggested that the individual TEWL values for session 2 were often higher than the corresponding values for session 1 for each category of SC hydration. In addition, it was observed that increasing skin hydration values corresponded to higher TEWL values. Although the parameters were not significantly correlated on session 1 ($r = 0.3$, $p = 0.07$), a statistically significant positive association was evident for session 2 ($r = 0.6$, $p < 0.001$).

Further analysis involved introducing SC hydration thresholds (Figure 8.7) to conveniently divide the skin into categories namely, dehydrated (< 35 A.U.), hydrated (36-69 A.U.) and overhydrated (> 70 A.U.) (Constantin et al. 2014). A number of participants who demonstrated dehydrated skin values presented TEWL values for both sessions which were < 30 g/h/m². By contrast, the few participants i.e. #13, #18 and #22 who demonstrated overhydrated skin values presented TEWL values for both sessions which were > 80 g/h/m². The patients in the hydrated category (35 ≤ SC hydration ≤ 70 A.U.) revealed considerable variability in TEWL values with a range of 21 – 145 g/h/m² for both sessions.

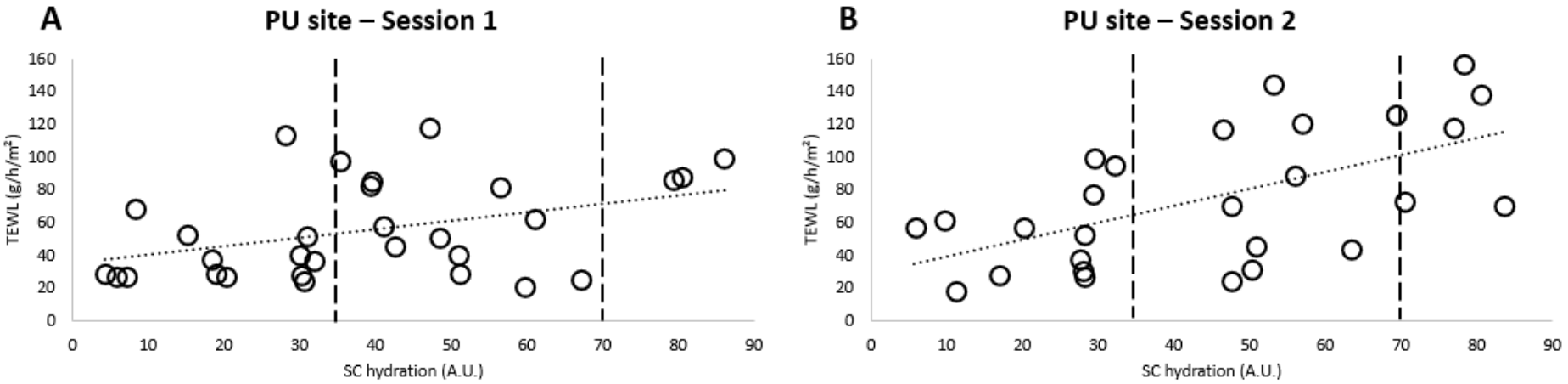
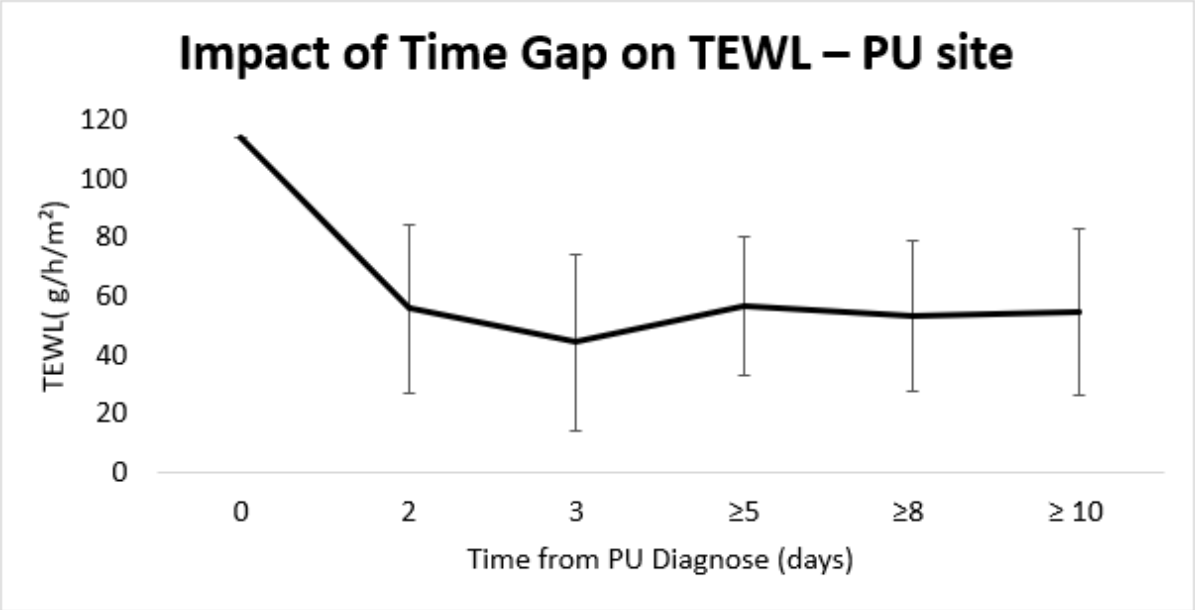


Figure 8.7. Correlations between SC hydration and TEWL values at the PU site on A) session 1 ($r = 0.3$, $p = 0.07$) and B) session 2 ($r = 0.6$, $p < 0.001$). Figure shows an increase in TEWL with increasing skin hydration levels. This is particularly evident in session 2.

8.4.7 RELATIONSHIP BETWEEN THE TIME OF PRESSURE ULCER DETECTION AND SKIN ASSESSMENT ON TEWL

In order to evaluate the possible implications of the time gap between PU detection and the TEWL parameter at the PU site, patients were conveniently divided into 6 groups namely 0, 2, 3, ≥ 5, ≥ 8, and ≥ 10 days post-PU diagnoses from session 1 skin assessment. Data showed no clear implications of the time gap on TEWL parameter output (Figure 8.8), with minimal variations in TEWL values, irrespective of the day of skin assessment from the day of PU diagnosis.



Time	*0 Day*	*2 Days*	*3 Days*	*≥ 5 Days*	*≥ 8 Days*	*≥ 10 Days*
N° of patient	1	2	3	5	8	11

Figure 8.8. Fluctuation of TEWL values at the different time points of skin assessment from the date of PU diagnose. Figure highlights no clear implication of time on TEWL expression.

8.4.8 RECEIVER OPERATING CHARACTERISTIC CURVE AND ODDS RATIO ANALYSIS

The sensitivity and specificity of TEWL and SC hydration parameters, to distinguish between healthy and PU-compromised locations are detailed in the receiver operating characteristic (ROC) curve analysis (Figure 8.9). In relation to TEWL, closer examination showed that the area under the curve (AUC) was 0.99 (95% confidence interval [CI], 0.999 – 1.000, *p<0.001*). Indeed, the TEWL parameter exhibited a sensitivity of 100% and a specificity of 98.5% at a cutoff value of 18.22 g/h/m². By contrast, SC hydration displayed a smaller AUC (0.604; 95% CI, 0.503 – 0.705, *p= 0.04*) and

presented with a sensitivity and specificity of 62.7% and 44.8%, respectively, at a cutoff value of 31.03 A.U.

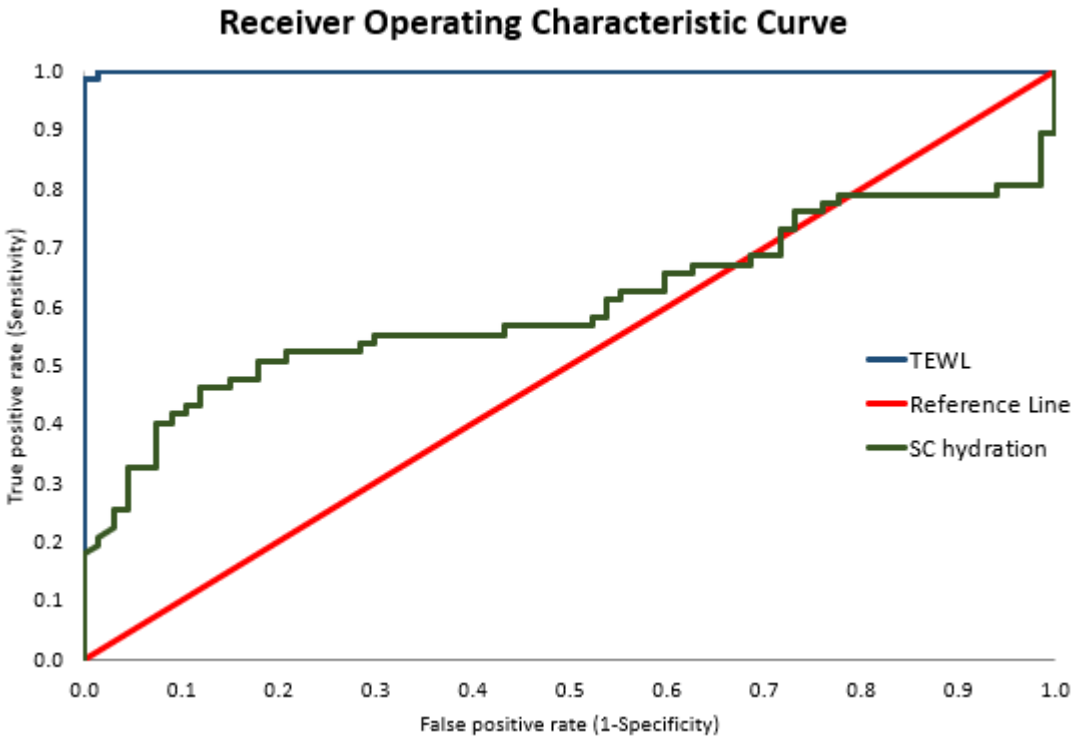


Figure 8.9. Receiver Operating Characteristic curve for TEWL and SC hydration for all the sessions of data collection.TEWL presented a better sensitivity compared to SC hydration, with an AUC of 0.99

Given the strong diagnostic value of the TEWL parameter, further analysis was conducted to assess its prognostic value in relation to the progression of the PU i.e. to stage II as observed in n=10 patients (Table 8.1). Patients who developed greater stages of PU were categorised as “progressed” into PU, while the remanent of the participants was allocated to the “non-progressed” group. Close examination of the data revealed that the odds ratio (OR) for patients to develop (progress into) stage II PU was 0.997 (95% CI; 0.977 – 1.017, $p = 0.75$), with a probability of 50%. Thus, the likelihood of these patients developing or not further stages of PU was similar.

8.5 DISCUSSION

The present study was designed to assess both spatial and temporal changes in skin parameters over the site of a stage I PU. Following their good performance in previous studies (chapters 5 and 7), two biophysical parameters reflecting skin barrier properties (TEWL) and SC hydration were monitored in the present study. The results revealed that the control site TEWL values remained in a normative range throughout. By contrast, distinct local increases in TEWL values over the site of the PU, which for some patients varied over time. A high degree of variability was observed in SC hydration values, with the PU site demonstrating both over-hydrated and dry skin properties. Intrinsic factors of gender, mobility and incontinence affected some of the biophysical values at the

PU site during distinct sessions. The majority of the elderly cohort had mobility restrictions and required multiple pharmacological drugs (Table 8.1), indicative of multiple comorbidities and pathologies associated with aging. Further analysis revealed a strong diagnostic value of TEWL (ROC AUC =0.99), but limited prognostic value in determining further damage (stage II PU) to the local skin sites.

The site-specific differences in TEWL values were evaluated by comparing the responses of the PU-compromised anatomical location with that of control sites 5 cm and 10 cm away. In particular, significant increases in TEWL responses were detected at the PU site on both test sessions (Figures 8.2A and 8.2B). By contrast, the 5 cm and 10 cm values generally conformed to normative values (Akdeniz et al. 2018) suggesting that, for the majority of patients, the upregulation was highly localised (Table 8.2). Similar TEWL upregulation has been reported for patients presenting with chronic venous leg ulcers (Dini et al. 2014), with the site of investigation presenting a significant ($p<0.001$) increase in mean TEWL value (167 ± 6 g/h/m²) compared to the control site (38 ± 4 g/h/m²). The values reported at both the control and investigation sites are higher compared to the findings of this research and of the previous studies in the current thesis (chapters 5 and 7), nevertheless, these authors investigated a site in close proximity to an open wound and used the forearm as the control site, which was at a significant distance from the site of investigation. Indeed, it is well established that TEWL outputs vary considerably depending on the anatomical sites of investigation within the individual (Kottner et al. 2017). To the best of our knowledge, no other study has investigated variations in the TEWL parameter fairly adjacent to a pressure-damaged skin location.

In contrast to TEWL, SC hydration did not yield clear differences, with highly variable values detected at the PU and control sites on both sessions (Figure 8.2C and 8.2D). These findings were consistent with previous studies where median skin hydration values were similar at a stage I PU compared to control sites in a small cohort of spinal cord injured patients (Scheel-Sailer et al. 2017) and following the application of sustained mechanical loading on the heel and sacral skin of healthy participants (Kottner et al. 2015). Nonetheless, a recent systematic review reported an association between skin hydration and the development of PU (Wilson et al. 2022), although the authors highlighted both a high degree of variation in hydration values and a focus on its predictive capability. For example, a study in Indonesia reported inconsistent values attributed to ambient conditions which often reached 30° C (Yusuf et al. 2015), whereas the present study was conducted in more moderate temperatures (22°C to 25°C). However, in another study, a significant correlation between increased skin hydration and PU development was established, although the authors attributed these findings to the prolonged exposure of skin to moisture derived from fecal incontinence and sweating (Sanada et al. 2007). In addition, a significant decrease in skin hydration

was reported in patients who healed from recurrent PUs (Shibata et al. 2020). The unremarkable findings of the present study could also result from the presence of potentially confounding variables, such as incontinence and impaired mobility (discussed below), as presented by many of the patients (Table 8.1). It is worthy of note that the practice in each of the geriatric departments was to employ absorbent pads for each patient during their inpatient stay, regardless of incontinence state. This will have affected skin hydration values, as the use of incontinent pads can induce changes in the microclimate of an occluded area (Falloon et al. 2018).

The study also evaluated temporal changes in TEWL and SC hydration values after, at least, 6 days from the initial skin assessment in a sub-set of the cohort. Although no significant findings were detected across the sites of investigation for both parameters, however, at the control sites, analyses revealed that the fold changes in both parameters did not exceed 1.7 for the majority of patients during the three sessions (Table 8.3). The corresponding fold changes at the PU sites only exceeded a 2.0 fold change in 3/10 of patients. Similar fold changes were also evident for SC hydration when values for sessions 2 and 3 were compared to session 1 (Table 8.3). These small temporal changes in skin barrier function over time could be attributed to reduced integrity in stratum corneum, although this suggestion could only be confirmed in an extended longitudinal analysis, which could assess the prognostic value of biophysical parameters to determine further skin damage or remodelling behaviour. It is important to further understand the relationship between TEWL and the structure and function of the stratum corneum. Indeed, cell-based studies have implicated the important role of stratum corneum corneocytes which continually turnover within the stratum corneum following mechanical stimulation which may explain the increased TEWL values (Évora et al. 2021; Tokumura et al. 2005). However, further research is needed to elucidate this concept.

The current study also examined the implications of intrinsic factors on biophysical outputs. Impaired mobility, poor nutrition and constant skin exposure to moisture have all been implicated as causal factors for PU development (Coleman et al. 2014). Results suggest that female patients and those who were either bedridden or incontinent generally expressed higher TEWL and SC hydration values at the PU sites during one or both sessions (Figures 8.4 and 8.6). The gender differences can be compared to a previous study at skin sites of healthy volunteers aged > 50 years, which reported higher female SC hydration values but similar TEWL values (Luebberding et al. 2013). In addition, significantly higher TEWL and SC hydration values have been reported in bedridden and incontinent patients (Fujimura et al. 2016), as well as for healthy individuals subjected to moisture in combination with mechanical loading (Bostan et al. 2019). As such, patient intrinsic factors represent important confounding factors that need to be addressed prior to translating these parameters into clinical practice.

PU skin sites demonstrated a significant correlation between the two biophysical parameters, which have not been identified previously (Figure 8.7). A similar correlation has been described previously in individuals affected by atopic dermatitis (Hon et al. 2008). Nonetheless, the present trend was not apparent for all participants in the cohort, with some demonstrating an impaired barrier function (TEWL >50 g/h/m^2) in the presence of dry skin (SC hydration <35 A.U.). The categorisation in terms of skin hydration levels represented an extension of that previously reported (Constantin et al. 2014). The added category incorporating overhydration values of >70 A.U. correspond to those patients regularly exposed to moist interfaces, for example, an incontinence pad or excess sweating. Impaired skin barrier function, namely an increase in TEWL, could be indicative of SC vulnerability and therefore risk of further damage i.e. stage II-IV PU in which skin integrity has been lost, resulting in a wound.

The robustness of the candidate parameters assessed using ROC curve analysis, revealed that TEWL was more sensitive and specific in determining spatial changes in skin health (Figure 8.9). It is of note that 10 of the 30 patients developed progressive skin damage regardless of the preventative measures which had been prescribed (Table 8.1). Due to the limited number of participants, any associations with respect to either TEWL or SC hydration values could not be fully evaluated, although preliminary analysis revealed an odds ratio = 1, which demonstrated limited prognostic value. It is of note that regardless of the number of days between the first assessment and the day skin redness was reported by the clinicians, there were no influences on the TEWL parameter profiles. Thus, its use for diagnostic purposes appears most relevant.

The study is limited by the relatively small sample size and a homogenous cohort of elderly Caucasian individuals, which limits the generalisability of the results to younger individuals and those with other ethnic backgrounds. Indeed, non-blanchable erythema has been reported to be difficult to detect in patients with dark skin, with a corresponding increase in the rate of pressure ulcers incurred in this sub-population (Bauer et al. 2016). Although skin measurements were standardised following internationally published guidelines (du Plessis et al. 2013), the researcher adopted a pragmatic approach where the circumstances of hospital departments (i.e. room temperature, humidity, etc) might have influenced the absolute value of the parameters. Indeed, the study tried to minimise external influences by, for example, performing the assessments at the same hours each day and the recruitment process was completed during warm periods of the year in order to avoid circadian rhythm and seasonal influences. Furthermore, skin parameters were assessed only at the sacrum and buttocks of the patients and not at other skin areas e.g. heels often vulnerable to PU damage. In addition, the individual diagnoses of the patients were not considered as it would have been difficult to interpret the impact of specific pathologies and comorbidities on skin parameters.

TEWL and SC hydration parameters have been widely used in dermatological skin research as markers of skin health. Nonetheless, their implementation in acute and/or long-term care settings, as objective means of predicting changes in skin status, has been limited. Of the two parameters utilised in the study, TEWL was highly sensitive to differentiate between healthy and damaged skin. Indeed, several studies have reported changes in TEWL values as clinical early markers of skin barrier disturbances prior to the presence of visible alterations (Jayabal et al. 2021; Schario et al. 2017). Nevertheless, due to the complexity of the skin architecture, a single biophysical parameter may be insufficient to detail changes in skin health, particularly given the diverse nature of the pathoaetiological factors implicated in PU development (section 1.3.1). This has motivated recent research focusing on biochemical strategies to monitor changes in skin status. Among these, biomarkers have recently gained attention as an innovative approach to identifying early signs of skin compromise (Bader et al. 2018). Indeed, there is growing evidence that cytokines such as IL-1alpha and IL-1RA play an important role in the early stages of skin damage (Worsley et al. 2018; Soetens et al. 2019). Nonetheless, more studies are required to establish the clinical utility of these biomarkers and establish how complimentary skin health parameters can be used to provide predictive or prognostic data. For example, the sub-epidermal moisture scanner (SEM, Bruin Biometrics, USA) has been reported to be sensitive to detect early signs of skin damage prior to clinical observation (Moore et al. 2017) but has been shown to have a limited positive predictive value (14%) (Okonkwo et al. 2020). It is of note that in the present study, biomarkers were collected by sampling the skin sebum of the patients. An overview of the extraction and analysis of the concentrations of inflammatory markers is offered in the general discussion section of this thesis (chapter 9, section 9.3). It is of strong believe that the combination of biophysical and biomarker parameters may provide an optimal solution to establish an objective means of predicting and monitoring pressure ulcers, supporting clinical practice and differentiate diagnosis.

8.6 CONCLUSIONS

Two biophysical parameters, reflecting skin barrier function and hydration, were evaluated to identify differences in responses on a stage I PU when compared to healthy adjacent sites. The results based on a cohort of 30 patients showed spatial and temporal changes in TEWL, with gender, mobility and incontinence representing factors which can influence the outputs of the parameters. The biophysical parameters revealed that stage I PUs represent localised damage with healthy skin situated as close as 5 cm away from the site of injury. The findings of this study demonstrate that increases in TEWL can be used as an objective parameter associated with early development of pressure ulcers and could support clinicians in providing an improved diagnostic assessment of skin compromise thereby identifying patients who require effective preventive measures. Further

research is required to determine if this approach would prove applicable to other patient groups at risk of developing pressure ulcers.

Chapter 9 GENERAL DISCUSSION

9.1 ADDRESSING THE RESEARCH QUESTIONS

Human skin is known to fulfill a variety of important functions, ranging from protection against pathogens to environmental insults, such as physical or chemical irritants. When the skin is subject to prolonged exposure to these challenges, it might result in the development of various types of damage and a loss of skin integrity (wounds). Among these, those that are chronic in nature, such as PUs and IAD, have been associated both with a considerable economic burden on healthcare systems and a detrimental impact on the individual quality of life (sections 1.2.1 and 1.2.2). To identify the individual risk of these chronic conditions, various types of assessment strategies (e.g., risk assessment scales and skin observations) have been introduced both in acute and long-term care facilities. However, such strategies lack objectivity and present poor predictive value (sections 1.5 and 1.6). It is thus imperative to establish objective measures which detect skin damage at an early stage, in order to allow the implementation of personalised preventive strategies, which will benefit both patients and healthcare services.

These concepts have driven previous research designed to detect early signs of skin barrier impairment using an array of non-invasive objective tools (chapter 2). Indeed TEWL, pH, sub-epidermal moisture, skin hydration and erythema measures have all been employed to examine the response of skin to clinical challenges which precede the formation of PUs and IAD, typically following exposure to various degrees of pressure, shear, friction and moisture. However, to date, none of these techniques, alone or in combination, have proven to either detect skin damage at early stages or differentiate between the different conditions. These limitations have restricted their translation into the clinical setting.

This has motivated the current doctoral fellowship, which is designed to acquire an improved understanding of the biophysical pathways underpinning skin damage using different strategies. It encompassed a range of approaches from survey questionnaires of healthcare workers managing patients with COVID-19, to the monitoring of skin parameters on both healthy cohorts and vulnerable patients. The PhD project had to adapt to the circumstances in which it was undertaken, with the worldwide pandemic restricting access to patients presenting with skin damage. Thus, the focus was translated to others at risk of skin damage, namely healthcare professionals wearing PPE and methodologies which could be applied during restrictions (retrospective analysis and survey methodologies). This approach has led to the generation of a considerable volume of analysed research data, from a portfolio of lab and clinical-based studies which aim to facilitate the translation into clinical settings.

In particular, the thesis was able to provide, to a certain degree, answers to the research questions detailed in chapter 2, section 2.2.

1. ***Can parameters from biophysical measurements differentiate between different skin damage mechanisms?***

Retrospective and prospective data from the lab-based studies were employed to investigate the possible identification of skin parameters which could be sensitive and specific to a range of insults. To this end, established insult models, able to mimic clinical scenarios, were employed to challenge skin health and consequently evaluate skin parameters' behaviour. In particular, analyses of the data from the retrospective studies revealed sub-groups of individuals within the cohort who responded distinctly to specific insults. Accordingly, the overall sensitivity of the parameters derived from the biophysical techniques was limited in detecting temporal changes evoked by the skin insults. Nevertheless, in some cases, increased TEWL and SC hydration values were detected when the skin of abled-bodied volunteers was subjected to mechanical and chemical irritation (chapter 4), as well as to moisture exposure and pressure in the form of high sitting (chapter 5). Although changes in skin parameters were detected, the parameters investigated were not able to distinguish between the skin insult models. Thus, specificity to differentiate between skin damage types was not achieved. Indeed, future research should focus on finding strategies able to identify the main driving force leading to skin impairment when the latter is subjected to multiple insulting agents.

2. ***Do extrinsic and intrinsic factors affect the output of biophysical skin parameters?***

The influence of intrinsic factors on biophysical parameter outputs was clearly evident in acute care facility patients (chapter 8). Indeed, cluster analysis revealed that gender, mobility, comorbidities (e.g., incontinence, diabetes) and anatomical location might have significant implications on the outputs of skin parameters, with female, bedridden, incontinent and diabetic patients exhibiting upregulation in biophysical parameters, namely TEWL and SC hydration. In addition, a degree of differences was detected in relation to the anatomical location of PU appearance, with the buttocks presenting higher parameter values compared to the sacrum.

With regards to the lab-based research, both retrospective and prospective studies, as well as the preclinical studies, highlighted distinct differences in responses following chemical and mechanical insults even within a cohort of healthy volunteers and at-risk individuals. Indeed, some degree of association was detected with participants' BMI, age and working patterns. This reinforces the idea of cluster analysis, which has been employed in the host lab when examining the response of able-bodied cohorts to skin challenges (Soetens et al. 2019; Bostan et al. 2019). Indeed, the implications of both intrinsic (BMI, age and gender) and extrinsic factors (type, magnitude and duration of insult) have been associated with the risk of acquiring skin damage (Coleman et al. 2013).

Interestingly, in these lab-based studies, except for TEWL and SC hydration, no remarked correlations in most of the biophysical parameters were observed with intrinsic and extrinsic factors. The unremarkable findings might be due to the use of a homogenous (e.g. similar age group, same gender, etc..) participant sample which limited the ability to assess differences. Nonetheless, there appear to be some associations between participants' age and BMI with the SEM parameter (Figures 4.6 and 4.7), as well as a considerable implication of anatomical sites on SEM basal outputs (chapter 4, section 4.2.4, Table 4.3).

In addition, some degree of influence of participants' intrinsic and extrinsic factors was detected in the preclinical study of HCWs using RPE during the outbreak of the coronavirus pandemic. Indeed, the bridge of the nose was the anatomical location most compromised by respirator wearing (Figure 7.4), with individuals working longer hours expressing higher folds changes in skin parameter values (Figure 7.11, Table 7.4). Interestingly, the outputs of the parameters following PPE usage were influenced by participant BMI, with individuals in the obese range of weight being more prone to skin changes compared to healthy-weighted participants. A complementary meta-analysis from the literature review revealed that females and HCWs from ethnic minority backgrounds were the most affected by PPE usage. Although this might be associated more with fitting process issues than individual intrinsic factors, however, HCWs from this sub-group reported more skin damage (Yu et al. 2021). This was not surprising as the standards by which FFP3 masks are manufactured (EN-149) are based on anthropometric panels taken from white males in the 1970s. As a result, the current limited designs of FFP3 using the "one-size-fits-all" principle favour features of male Caucasians to the detriment of females and those of non-Caucasian ethnic backgrounds. It was disappointing to note that although the majority of the UK healthcare force consists of female professionals (Tables 6.1 and 6.3), no action was taken to develop suitable PPE and protect the skin health of these individuals (NHS England 2020). Given the potentially damaging effects of these face protectors and the lack of consolidated guidelines for their usage during the first peak of the pandemic, the multicentre survey study (chapter 6) represented a major study to delineate information pertaining to the time frame of mask application after which adverse skin reactions and/or damage could occur (Figure 6.4, Figure 6.5).

3. Are biophysical parameters sensitive in differentiating spatial changes in skin health?

Among the parameters examined, TEWL was the only one particularly able to distinguish between compromised and healthy skin, as evidenced by increased parameter values at the site of a stage I PU in hospitalised patients (Figure 8.2). The robustness of this parameter was further emphasised with ROC curve analysis, which highlighted a 100% sensitivity in differentiating between healthy and damaged skin (Figure 8.9). Differently from TEWL and the previous studies in this thesis, where a degree of variation was detected, SC hydration was unable to clearly differentiate between PU-

compromised and healthy skin locations, with a high degree of intersubject variation observed and a low sensitivity detected. This highlights the need for more evidence-based research pertaining to understanding the potential of this parameter and how it could be employed in the detection of early signs of skin damage. Indeed, different from the findings of the study in chapter 8, a recent review claimed the potential of SC hydration as a marker for PU development (Wilson et al. 2022). Nevertheless, the results of this review highlighted a high degree of skin hydration value.

In addition, TEWL was able to highlight spatial differences following the challenges of the skin with mechanical loads in the form of face protectors (chapter 7). Indeed, increased TEWL values were detected at the sites under the perimeter of mask application, whereas, the unchallenged control site showed minimal parameter variations. Furthermore, in the lab-based studies, differences in most of the biophysical parameters were detected when the insulted anatomical locations were compared to the control sites. Indeed, increased values of TEWL and SC hydration and decreased erythema values were detected at the sites challenged with moisture, in the form of synthetic urine, and pressure, in the form of high sitting (chapter 5). No meaningful changes were evident at the adjacent unchallenged control sites during the test sessions.

4. Can biophysical parameters detect temporal changes in skin health?

Temporal changes in biophysical parameters were detected in the lab-based studies, with most parameters (except for erythema) showing consistently increased values at the skin sites subjected to challenges over the period of experiment. However, the skin response to these challenges was transient in nature, as evidenced by a return of parameter values towards basal levels, reflecting recovery. This is congruent with the findings of other studies, which explored a range of mechanical and chemical insults including moisture and irritants (Angelova-Fischer et al. 2012; Soltanipoor et al. 2018). Similarly, insults involving chemical irritants have been observed to evoke damage to the skin barrier, although the effects have been reported to be reversible with time (Han et al. 2017).
Time-dependent changes were also observed in both preclinical (chapter 7) and clinical (chapter 8) studies. However, these changes were only detected by the TEWL parameter, which highlighted a significant increase following two consecutive days of RPE usage. Furthermore, although not significant, temporal trends in TEWL were observed at the sites compromised by stage I PU when assessments were performed 24 hours apart. In addition, longitudinal observation of the parameter highlighted upregulation, downregulation and no changes in its values following ~ 1 week of patients' skin assessment (Table 8.3). However, as these findings were observed in a limited patient cohort (n=10), more research involving a greater sample size is needed to fully elucidate the prognostic value of these changes in relation to skin health.

9.2 GENERAL LIMITATIONS

There are different factors which contributed to some of the unremarkable findings presented in the thesis, as well as limiting the ability of this doctoral fellowship to fully offer a comprehensive answer to specific research questions. In particular, the small sample size used in the various studies limited the potential to generalise the findings. However, such a limited number of participants per study was deemed necessary given the nature of the research protocols, where each individual acted as its own control, as such eliminating the need for a separate control group. Another considerable limitation was the lack of participant diversity. Indeed, except for the clinical patient study, there was generally a limited number of male participants. In addition, most of the participants involved in each research study were of similar age ranges, limiting the possibility to acquire meaningful data pertaining to the implication of this intrinsic factor on biophysical parameters. Furthermore, the lack of ethnic background diversity, with most participants from the White Caucasian ethnicity, played a considerable role in impeding the acquisition of further knowledge relating to the relationship between this factor and skin biophysical parameters. Indeed, a motivating factor for this research was to identify tools which could assess changes in skin health irrespective of colour, a research question which remains to be answered. One major limitation was also the selection of the parameters to investigate, which was not consistent across the studies of the thesis. For example, following the lab-based studies, skin surface pH was not among the parameters assessed within the other studies. In addition, given the unremarkable results both in the lab-based (chapter 5) and preclinical (chapter 7) studies, the assessment of erythema using Mexameter, was deemed unnecessary for subsequent studies. In addition, both skin pH and erythema were not adopted in the clinical study (chapter 8) due to the need to reduce the amount of time spent at the patient's bedside during data collection. The SEM scanner was also not implemented beyond the retrospective analysis due to commercial restrictions, it would have been particularly interesting to have assessed this device given its prominence in the field in recent years. In addition, the effect of the environmental condition was not considered fully considered in the data analysis. Indeed, although the Tewameter TM300 used for the assessment of changes in skin parameters presents with a temperature and humidity sensor, which assesses the ambient conditions during measurements, the implication of the output values of these sensors on the results obtained was not investigated. However, a case-controlled design approach was employed in the thesis, where measurements were made at the same time in two or more skin sites, thus, minimising the effects of ambient conditions on the measurement.

It is also important to highlight that in the studies of the current thesis, the use of biophysical tools to assess the skin health of the participants was performed by the doctoral research fellow, who has been extensively trained by experienced researchers. It would be interesting to acquire the

perspective of clinical staff (e.g., nurses, healthcare assistants, etc…) whilst using these tools to assess the feasibility of their use in practice. Furthermore, these biophysical tools were used on participants either in a lab-controlled environment or in acute care facilities, where the heat index was monitored at all times. Further studies are needed to examine the implication of using the tools in participants housed in other settings, such as long-term care facilities.

9.3 COMPLIMENTARY ANALYSES

In collaboration with two Marie Curie Fellow, supplementary data analysis was conducted on both the lab-based and clinical cohort data sets. Here I led the acquisition of biofluids and cells and collaborated with data analysis, interpretation and publication.

9.3.1 SKIN BIOMARKERS FROM SEBUM

Alongside biophysical parameters, in order to interrogate the wider physiological implications of external loading, moisture exposure, shear and frictional forces on skin health, we sampled biomarkers from sebum on the skin surface and quantified inflammatory cytokines. This approach has been pioneered by the research group to reflect the state of the epidermis and underlying tissues following external insults (Bader et al. 2018). A non-invasive method for the collection of biomarkers consists of using a lipophilic commercial tape, Sebutape patches (CuDerm, Dallas, TX, USA), held on the sites of interest for 2 minutes using gloved hands and tweezers. The tapes were subsequently removed, placed in appropriately labelled containers, and stored at -80^{o}C until biochemical analysis. The subsequent extraction of inflammatory cytokines was performed following a modified protocol developed within the host lab (Jayabal et al. 2022). Both the extraction process, as well as the analysis of the biomarkers, were performed by another doctoral fellow, part of the STINTS consortium (Hemalatha Jayabal). In relation to the current thesis, the quantification of inflammatory markers was performed for the studies described in chapters 5, 7 and 8 (Table 9.1).

Table 9.1. Summary of the biomarkers investigated in the thesis with associated relevant findings.

CHAPTER n°	THEME OF THE CHAPTER	BIOMARKERS INVESTIGATED	RELEVANT FINDINGS
5	Evaluate temporal changes in skin cytokines following moisture exposure and pressure application	IL-1α, IL-1RA, IL-6, IL-8, INF-γ and TNF-α	Considerable variability within and between the inflammatory biomarkers. Variable temporal changes in cytokines relative to basal values, with INF-γ, IL-6 and TNF-α ratios increasing during moisture and pressure exposure.
7	Explore changes in biomarker expression at the sites under the perimeter of RPE application in HCWs	IL-1α, IL-1RA, IL-8 and TNF-α	Considerable intra- and inter-subject variability. Some individuals expressed consistently higher responses in inflammatory biomarkers throughout the test sessions, following RPE usage.
8	Examine spatial and temporal differences of biomarkers at sites compromised by stage I PU and 10 cm healthy adjacent control site	IL-1α and IL-1RA, IL-6, IL-8, TNF-α, INF-γ, IL-33, IL-1β and G-CSF	Spatial changes with upregulation of IL-1α, IL-8 and G-CSF as well as downregulation of IL-1RA recorded at the site of stage I PU compared to the adjacent control sites.

During the lab-based and preclinical studies, there were considerable variations in the inflammatory biomarkers, with no consistent trends observed when the skin was challenged with external loading or with moisture exposure. Nevertheless, an upregulation in high abundance proteins, namely IL-1α and IL-1RA, was observed in distinct individuals following skin exposure to both dry and synthetic urine-saturated pads and after consecutive days of respirator application. In addition, as a result of the use of an improved extraction protocol, it was possible to quantify low-abundance proteins namely TNF-α, IL-6 and IL-8 (Abiakam et al. 2023).

More interesting was the quantification of biomarkers sampled from the anatomic locations of interest of individuals presenting with stage I PU. Indeed, nine different cytokines including high (IL-1α and IL-1RA) and low (IL-1beta, TNF-α, IL-8, INF-γ, IL-33, IL-6 and G-CSF) abundance proteins were extracted and quantified. Despite considerable inter-subject variability, distinct significant spatial differences were observed, with the PU-compromised locations expressing higher and lower concentrations of pro- and anti-inflammatory cytokines, respectively, compared to the control sites. The follow-up session showed varying biomarker responses, with the majority of patients exhibiting continued upregulation of the pro-inflammatory levels and a remarked downregulation of the anti-inflammatory cytokine (IL-1RA) at the PU site. Similarly, data showed also upregulations of some of the low abundance proteins, namely IL-8, G-CSF and IL-1beta, at the PU sites, while the other cytokines, such as INF-γ, TNF-α and IL-33, showed considerable variability and no evident differences between the sites of investigation. Furthermore, the ratio between the two IL-1

proteins (IL-1α/IL-1RA), which have been reported to counterbalance each other during the initial phases of an inflammatory process, showed a resulting ratio below 5 at the control sites and between 1 – 98 at the PU sites. This result gives confidence that the large variation at the PU site is indicative of the inflammatory process occurring at the epidermal-dermal layers of the skin. The robustness of the biomarkers was examined using the ROC curve analysis (Figure 9.1). The data showed that IL-1α, IL-1RA and their ratio IL-1α/IL-1RA presented with excellent performance compared to the other cytokines, such as TNF-α, IL-6 and IL-33 (Table 9.2). The implications of patient intrinsic factors on biomarker responses were also examined. While no significant associations were found at the control sites, interestingly, at the PU sites, gender, PU location and nutritional intake significantly affected IL-1α expression.

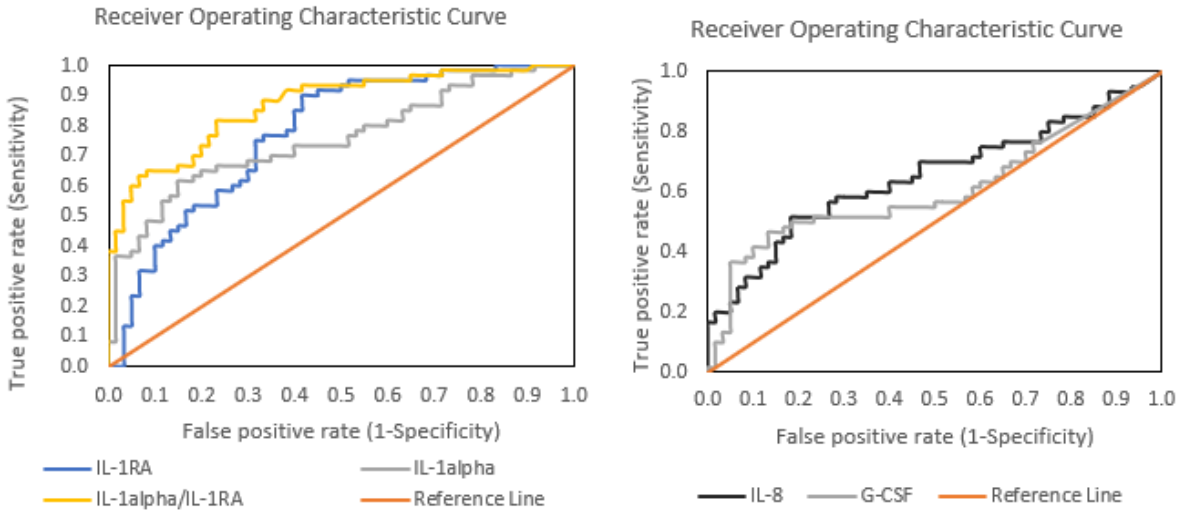


Figure 9.1. Representative Receiver Operating Characteristics (ROC) Curves for (a) high abundant and (b) low abundant proteins with AUC greater than 0.6. The ratio IL-1alpha/IL-1RA is the most sensitive among the examined biomarkers. Image courtesy of Jayabal H. early stage research part of the STINTS project (Jayabal et al. 2023)

Table 9.2. Biomarkers and the corresponding area under the curve of the ROC curve. (*** -p<0.005, ** - p<0.01, *-p<0.05)

Biomarker	AUC
IL1α/IL1RA	0.87***
IL-1RA	0.77***
IL-1α	0.75***
IL-8	0.65**
G-CSF	0.61*
IL-1beta	0.58
INF-γ	0.57
IL-33	0.54
IL-6	0.52
TNF-α	0.51

More detailed findings of inflammatory cytokines with regards to the studies in chapters 5 and 7 can be found in published manuscripts namely:-

- *Abiakam, N., Jayabal, H., Filingeri, D., Bader, D.L., Worsley, P. R. The effects of moistened incontinence pads on loaded skin with reference to biophysical and biochemical parameters. Journal of Wound, Ostomy and Continence Nursing 2023.*
- *Abiakam, N., Jayabal, H., Mitchell, K., Bader, D.L., Worsley, P. R et al. Biophysical and biochemical changes in skin health of healthcare professionals using respirators during COVID-19 pandemic. Skin Research Technology. 2022.*

In addition, the biomarkers findings from the hospitalised patients' study have been edited into a journal manuscript, which is currently under review for publication in the International Wound Journal.

- *Jayabal, H., Abiakam, N. S., Filingeri, D., Bader, D. L., & Worsley, P. R. (2023). Inflammatory biomarkers in sebum for identifying skin damage in patients with a Stage I pressure ulcer in the pelvic region: A single centre observational, longitudinal cohort study with elderly patients. International Wound Journal.*

9.3.2 ANALYSIS OF SUPERFICIAL CORNEOCYTES

Although biomarkers and biophysical parameters are the most investigated in studies examining changes in skin integrity, it is also critical to evaluate the implications of external insults on the properties of the stratum corneum (SC) and its main cellular component, corneocytes. The latter is known to undergo a complex process of maturation, namely the cornification process (Évora et al. 2021), which includes the cross-linking of certain precursor proteins (e.g. involucrin and loricrin), and the covalent attachment of lipids to produce a rigid and hydrophobic cornified envelope (CE) structure (Candi et al. 2005). In addition, a gradual degradation of corneodesmosomes (CDs) occurs in the central region of the cell, originating a honeycomb formation of cell junctions. This honeycomb pattern is believed to underpin the barrier function resulting in lower values of TEWL (Goto et al. 2020), as well as in improved flexibility of the whole SC layer by attenuating mechanical insults and allowing minimal relative sliding of the outer SC over the inner SC (Kitajima 2015). Corneodesmosomes are observed indirectly by immunostaining of desmoglein 1 (Dsg1) (Goto et al. 2020), which is a cadherin-type cell-cell adhesion molecule found in stratified epithelial desmosomes, expressed in the suprabasal layer of the epidermis.

The analysis of the properties of the SC corneocytes was conducted following skin challenges as described in chapters 5, 7 and 8. This was performed using a minimally invasive tape strip

technique, which consisted of applying and gently holding commercial cellotape tape on the investigation sites for 5 seconds prior to removal. The principal investigator (NA) was responsible for corneocytes harvesting and storage. Following removal, the tapes were placed in a closed Eppendorf tube sealed with Parafilm and stored at -20 °C until further analysis. This was performed by collaborating STINTS researcher (Ana Evora), based at the school of chemical engineering at the University of Birmingham. To review briefly, before further manipulations, tape strips were thawed and allowed to equilibrate for 30 minutes in a facility housing the Atomic Force Microscope (AFM), where the temperature is controlled at 19 °C. Assessment of CE maturation and indirect visualization of CDs by the immunostaining of desmoglein-1 (Dsg1) was then conducted. The findings highlighted a considerable inter-subject variation for both immature CEs and Dsg1. Nonetheless, a high level of CDs and immature CEs were detected at the insulted location compared to the control sites and low levels of immature CEs were associated with higher TEWL values after prolonged pressure application. In addition, a greater amount of Dsg1 was detected at the compromised skin sites. Atomic Force Microscopy (AFM) analysis revealed changes in the surface characteristics of the corneocytes over grade I PUs. Indeed, while cells at the healthy control sites were mostly smooth, characterised by cross-over ridges, peaks, and valleys, which are typical of normal cells, by contrast, corneocytes harvested from the compromised locations were rough and characterised by the presence of circular nano-objects (CNOs) (Figure 9.2). These findings suggest the need for more evidence-based research pertaining to the possibility of using surface corneocytes as biomarkers for the early detection of skin compromise.

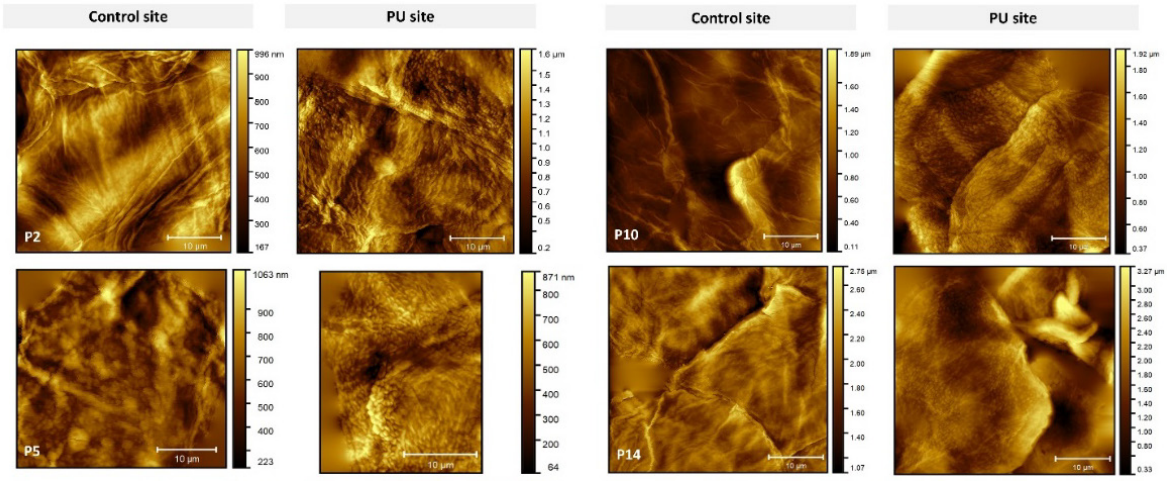


Figure 9.2. AFM analysis of superficial corneocytes revealed differences in the topography of superficial corneocytes between control and stage I PU-compromised sites. While at the control site, cells presented typical features of ridges, valleys, and peaks, by contrast. cells at the PU site presented an abundance of CNOs. (Image is courtesy of Ana Evora, Early Stage Researcher from the University of Birmingham)

The corneocyte results from the study in chapter 7 have been edited into a journal manuscript published in the Journal of Tissue Viability (*Évora, A. S., Abiakam, N., Jayabal, H., Worsley, P. R., Zhang, Z., Johnson, S. A., ... & Bader, D. L. (2023). Characterisation of superficial corneocytes in skin areas of the face exposed to prolonged usage of respirators by healthcare professionals during COVID-19 pandemic. Journal of Tissue Viability*), detailed findings of the corneocytes in stage I PU (chapter 8) are under preparation for journal submission.

9.4 CONTRIBUTION OF THE THESIS TO THE CURRENT STATE OF KNOWLEDGE

Various invisible complex changes occur at the epidermal-dermal layers, which if interrogated, could provide an indication of the skin status, prior to breakdown. Thus, the potential to investigate the biophysical properties of the skin could aid in avoiding further damage from occurring. Although physical sensors have proved useful in assessing tissue status, the findings of the current thesis suggest certain technical limitations, detailed in Table 9.3. These need to be addressed before they could be incorporated into routine screening protocols to be used in conjunction with traditional skin and risk assessment scales in clinical settings.

The findings of the current thesis not only contribute to extending the current knowledge of early detection of change in skin healthy parameters in relation to PU and IAD but also provides empirical evidence pertaining to the possible adoption of some of these parameters in acute care settings. In particular, this thesis highlighted the potential of TEWL parameter to support routine clinical skin assessment due to its ability to detect early signs of skin barrier impairment, as well as the capacity to distinguish between healthy and damaged anatomical locations. In addition, its ability to rapidly acquire real-time data highlighting changes in the skin barrier, at the bedside, makes TEWL parameter a good candidate for clinical environment. Another parameter which is worth further investigation is SC hydration. Although in the studies of the thesis, skin hydration was not able to differentiate between healthy and damaged skin, it did highlight clear variations from baseline when the skin was exposed to moisture for prolonged periods. In addition, the studies presented in this thesis powered the creation of new knowledge namely:-

- A methodology was developed to assess spatial and temporal changes in skin health using biophysical, biomarker and cell analysis across a range of study designs.
- Skin sites exposed to pressure and saturated incontinence pad revealed changes in skin barrier and hydration parameters, with moisture being the primary driving force causing changes in skin health.
- For the first time, significant associations were identified between the duration of personal protective equipment use and frequency of relief with reported skin reactions. In addition, it was remarked on the importance of fit testing, particularly with ethnic minority groups.
- The data derived from the survey provided the basis for evidence-based guidelines to inform the application and design of protective equipment (NHS England 2020).
- A novel methodology was developed to objectively monitor skin health in a real-world scenario of healthcare professionals exposed to prolonged use of PPE.

- Changes in biophysical markers of skin integrity following prolonged and repetitive use of RPE by HCWs, with the largest changes associated with the nasal bridge after two consecutive days of RPE usage.
- The acute care patient study represented the largest cohort evaluation of stage I PU utilising biophysical measurement parameters
- There were significant spatial and temporal changes in skin biophysical parameters, shown to be localised to within 5cm of the damaged site.
- The outputs of skin parameters were influenced by intrinsic factors associated with pressure ulcer risk.
- The diagnostic potential of TEWL was demonstrated with ROC value (>0.9).

Table 9.3. Strengths and limitations of skin biophysical parameters investigated in the thesis

PARAMETER	STRENGTH	LIMITATION	RECOMMENDATIONS
TEWL	Able to identify spatial changes, capable of detecting early signs of compromise following insults, potential to detect time-dependent changes, able to distinguish between stage I PU and adjacent healthy sites	Inability to differentiate between the causatives of skin damage, influenced by the patient underlying conditions (e.g. presence of incontinence), lack of threshold defining the state of skin, considerable inter-subject variation, influenced by intrinsic factors	Could be used to detect changes in the barrier function across the different anatomic locations. Possess the potential to differentiate between pre-clinical (chapter 5 and 7) and clinical skin damage (chapter 8). Requires improvements in relation to data acquisition and interpretation to inform clinical decision-making
Stratum Corneum Hydration	Capable of detecting changes following prolonged skin exposure to moisture, ability to detect temporal changes following skin challenges	Inability to detect spatial differences, unable to differentiate between damaging factors, unable to distinguish between healthy and early signs of PU-compromise sites, lack of threshold defining skin hydration status (e.g. dehydrate, overhydrate, etc), large inter-subject variability, influenced by intrinsic factors	Sensitive to moisture-related skin insults (e.g. incontinence). However, unable to distinguish between PU and healthy adjacent skin sites. Therefore, presents with limited diagnostic capability but could serve as an adjunct to monitoring skin hydration in incontinent patients.
Erythema	Represents one of the primary changes in skin which corresponds to visual inspection	Large inter-subject variation, influenced by anatomical sites (difficulties in assessing curved locations), unable to detect spatial and temporal differences, influenced by subject skin tone and surrounding factors (e.g. presence of moisture)	Provided limited diagnostic and monitoring value
pH	Acid mantle is a critical part of the skin barrier	Unable to detect early signs of damage	Limited ability to detect changes in skin surface status

PARAMETER	STRENGTH	LIMITATION	RECOMMENDATIONS
Subepidermal Moisture	Demonstrated some specificity to mechanical insult in the retrospective data	Influenced by anatomical locations (can be used only on the heels and sacrum), designated threshold non-reflective of skin status, influenced by subject intrinsic factors (e.g. BMI)	Offers user-friendly data acquisition and interpretation. Limited by anatomic locations of assessment. Requires further research to confirm diagnostic accuracy of proposed delta threshold

9.5 CLINICAL IMPLICATIONS

The programme of research has reinforced the notion that non-invasive biophysical and biomarker approaches can be used to monitor local changes in skin health. This objective approach has the potential to overcome the limitation of current subjective skin assessments and could be used as an adjunct in clinical practice. The result revealed that some parameters are more sensitive than others, in particular, TEWL provided the opportunity to detect changes in skin barrier properties both at an early stage, as well as in an established damaged state (stage I PU). A degree of relevant results was also obtained with SC hydration, although the parameter displayed considerable variability across study subjects with no clear evidence of spatial differentiation for PUs. The erythema parameter yields no consistent or considerable findings in the current thesis. This was disappointing given that skin surface colour changes are among the clinical signs observed prior to gross damage occurring. Due to the limited time-dependent assessments performed in the thesis, more empirical data is needed to validate the prognostic values of these parameters. This is of critical importance to enable clinicians to identify the current status of the patients' skin health (e.g whether it is healing or worsening). Furthermore, the tools used for the examination of the skin status need to be adapted to be more user-friendly and enable quick clinical decision-making. In addition, as most of the tools used are able only to interrogate changes occurring at the superficial layers of the skin (e.g. stratum corneum and epidermis), there is a compelling need to modify them to allow the understanding of events occurring at the subepidermal level.

More importantly, for these parameters to be used in clinical practice, it is necessary to outline distinct thresholds which could inform on skin status. An attempt to use a threshold approach has been recently adopted by a novel point-of-care device (section 2.1.2.3), resulting in a lack of sensitivity and specificity, and poor predictive value.

9.6 FUTURE WORK

In December 2020, a longitudinal experimental protocol, designed to assess spatial and temporal changes in the skin status of individuals affected or at high risk of acquiring PUs, diabetic foot ulcers (DFUs) and IAD, was submitted to the Research Ethics Committee (REC) and Health Research Authority (HRA) seeking approval. The study was designed to recruit participants from the University Hospital Southampton Trust (UHST), as well as from community care homes and podiatric clinics. Although the project received the University of Southampton (UoS) ethical clearance, it received a non-favourable opinion from the REC and HRA committee due to uncertainties surrounding COVID-19 restrictions, which did not allow human-based studies especially if the participants were elderly individuals living in care homes or acute care settings. As a result, a major revision of the experimental protocol formed the basis of the study in chapter 8,

which was allowed to be conducted only after the peak of the pandemic. Nevertheless, given the improvement in COVID-19 circumstances and the uplifting of the restrictions, there is greater confidence that the project would receive a favourable ethics opinion if re-submitted. This will allow for an extended longitudinal analysis, which is necessary in order to assess the prognostic value of biophysical parameters in identifying the risk of skin damage and a better understanding of the time-dependent changes indicative of recovery or worsening skin health. In particular, given the promising result from TEWL (see chapter 8), future studies should focus on the design of protocols in which TEWL could be used for the assessment of the skin of patients or individuals at risk of acquiring PU and IAD over several months period. With a particular focus on patients who are at risk of presenting with stage I PU. In addition, interesting findings pertaining to pro- and anti-inflammatory cytokines and corneocytes, call for a better understanding of the role of these biomarkers in the early stages of skin damage. By assessing different types of skin damage (PU, IAD and DFUs), a better understanding of the specificity of specific parameters can also be established.

Although the assessment of some of the parameters (e.g. TEWL) can be easily performed at the patient's bedside in acute care settings by a researcher, it is necessary to perform feasibility studies with clinicians and healthcare workers. This could include comparing traditional skin assessment methodologies against the employment of bioengineering devices, to acquire more understanding of the nurses' perceptions in using these biophysical tools, as well as the implications of introducing novel devices into clinical practice. This could be achieved via the employment of mixed methodology studies consisting of skin assessments performed by HCWs in addition to interviews which will allow clinicians to report any barriers and facilitators to adopting the technologies (Smith et al. 2020). Furthermore, as the skin presents a very complex architecture, there is a compelling need to design more research focused on novel systems able to combine measurements from multiple parameters in order to obtain sensitive, specific and reliable assessments of early signs of loss in skin integrity. This could be achieved by following the methods proposed in chapter 4, where the results of different measurements were combined and weighted depending on their respective value in diagnosing skin damage (Figure 4.4). In addition, the recent exponential advancement in the field of artificial intelligence (AI) warrants more research in which machine learning could be potentially adopted to identify meaningful trends within robust datasets, whereby thresholds can be selected in skin parameters. However, prior to conducting these types of studies, further research is required pertaining to the identification of skin biophysical parameters and large data sets are required to train the models. Indeed, a deeper understanding will enable the distinction between the various states of the skin, i.e, healthy, damaged and/or undergoing a healing process.

A crucial but pivotal economic study pertaining to the cost-effectiveness of the use of these bioengineering devices, with associated parameters, is needed before these parameters could be

added to routine practice in care facilities. Leaving aside the discussion of whether the implementation of preventive measures is more expensive than the management of wounds, if the cost implications associated with the introduction of these parameters exceeded the expenditures currently incurred with standard skin and risk assessment strategies, this might impede possible translation into care settings. Studies have reported that current risk assessments classify up to 41% of the patient population as being at risk of developing skin chronic conditions, such as PU, with an associated cost of nursing time to perform a visual assessment of at-risk skin reported to be $8 per patient (Padula et al. 2019). Yet, only a limited number of designated patients develop these conditions, whereas a remarkable number of those designated as not at risk incur PU and/or IAD. Given the unacceptable escalation of the incidences of both conditions, with the associated elevated cost of treatment and management, there is growing confidence that the use of objective means of skin assessment has the potential to save costs to healthcare systems.

Appendix A MULTICENTRE SURVEY QUESTIONNAIRES

MINIMUM DATA SET FOR PPE ACQUIRED SKIN DAMAGE

PARTICIPANT'S INFORMATION

*Required

1. Date of Survey? *

_____

Example: 7 January 2019

2. What is your age? *

_____

3. What is your gender? * *Mark only one oval.*

- ◯ Female
- ◯ Male
- ◯ Prefer not to say
- ◯ _____

4. I would describe my ethnic origin as follows: * *Mark only one oval.*

- ◯ White
- ◯ Asian/Asian British
- ◯ Black / African / Caribbean / Black British
- ◯ Mixed / Multiple ethnic groups
- ◯ _____

5. What is your current role during COVID-19?

_____

6. On average, how many days do you work in a week?

_____

Skip to question 7

PPE FOR EYES AND FACE PROTECTION

7. What type of eye protection are you using when attending your duties?

Choose all that apply

☐ ☐

☐ ☐

☐ _____

8. What type of face protection are you using when attending your duties?

Choose all that apply

☐ Cardinal mask
☐ Valmy respirator mask
☐ Alpha respirator mask
☐ M respirator mask
☐ Easifit respirator mask

☐ _____

9. Did you attend any fit testing appointment before adopting eye and face protection equipment?

Mark only one oval.

- Yes
- No

10. If yes, what was the date of your last fit testing appointment?

_____

Example: 7 January 2019

11. When did you start to use protective equipment?

_____

Example: 7 January 2019

12. On average, how many hours do you wear this protective equipment in a day?

Mark only one oval.

- Less than 6
- 6-8
- 8-10
- 10-12
- 12-13
- Other: _____

13. Do you wear this protective equipment for consecutive days?

Mark only one oval.

- Yes
- No

14. If yes, what is the maximum number of consecutive days?

_____

SKIN CARE WHEN USING PPE

15. Please select your answer

16. If yes, what type of dressing material are you using?

- [ ] Prophylactic
- [ ] Silicone
- [ ] Foam
- [ ] Hydrocolloid
- [ ] Other: _____

17. Do you remove the PPE equipment regularly to relieve your skin?

Mark only one oval.

- ◯ Yes
- ◯ No

18. If yes, how often do you remove this protective equipment?

Mark only one oval.

- ◯ Every 1 hour
- ◯ Every 2 hours
- ◯ Every 3 hours
- ◯ Every 4 hours
- ◯ Other: _____

HOW COMFORTABLE IS THE PPE

19. Please select your answer

protective equipment?		
Are you able to breathe easily when wearing		
Do you feel safe and in control when wearing		

20. If 0 is no pain and 10 is the worse pain imaginable, can you rate your pain, when wearing PPE, on the scale below:

Mark only one oval.

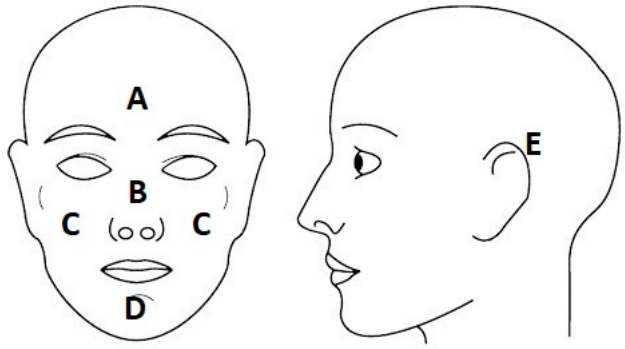
0	1	2	3	4	5	6	7	8	9	10

FACE SKIN HEALTH

21. Please select your answer

protective equipment is placed?		
Do you have any indentation marks where		
protective equipment is placed?		

22. Using the face diagram, please annotate the sites of your skin presenting with redness, itchiness, rash or pressure damage due to the use of PPE:



	Itchiness	Rash	damage	Spots	No skin	
Site A	☐	☐	☐	☐	☐	☐	☐
Site B	☐	☐	☐	☐	☐	☐	☐
Site C	☐	☐	☐	☐	☐	☐	☐
Site D	☐	☐	☐	☐	☐	☐	☐
Site E	☐	☐	☐	☐	☐	☐	☐

23. How good or bad did you perceive the health of your face skin BEFORE STARTING USING PROTECTIVE EQUIPMENT *

Mark only one oval.

0	1	2	3	4	5	6	7	8	9	10
◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

24. We would like to know how good or bad you perceive skin health TODAY *

Mark only one oval.

0	1	2	3	4	5	6	7	8	9	10
◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

25. Would you be happy for us to monitor the health of your skin on a weekly basis using a very brief follow-up questionnaires?

Mark only one oval.

Yes, please

No, thanks

26. If yes, do you mind sharing your contact details with us?

Please write your work email address

MINIMUM DATA SET FOR EYES AND FACE PPE ACQUIRED SKIN DAMAGE FOLLOW-UP QUESTIONNAIRE Date of follow-up survey

1. How many days did you work last week?

2. Are you still using the same protective equipment for the face and skin as of last week?

Yes No

If no, what has changed?

I have added extra protective equipment. Please specify which …… I have removed some protective equipment. Please specify which ……

3. On average, how many hours do you wear this protective equipment in a day?

6-8

8-10 10-12

12-13

Other: please specify ……..

4. Over the past week, did you wear this protective equipment for consecutive days?

Yes No

If yes, what was the maximum number of consecutive days?

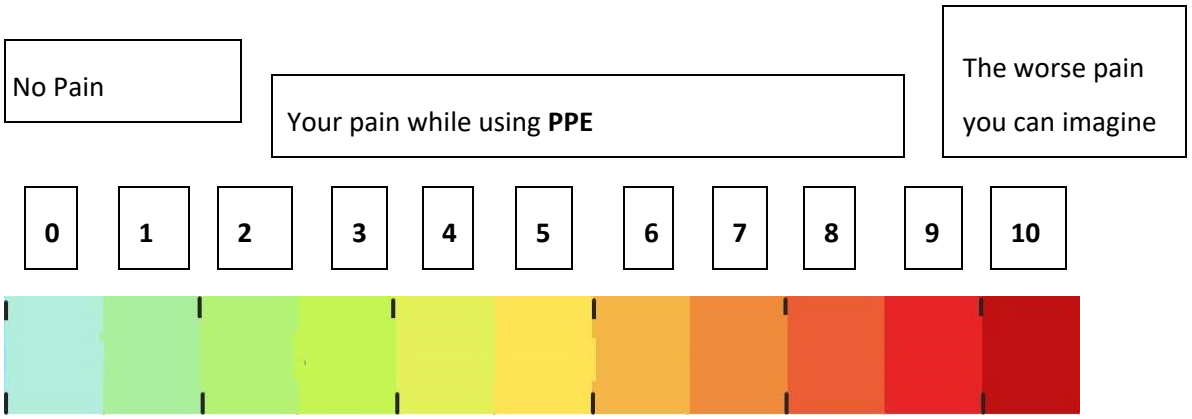
5. Over the past week, did you apply any specific cream or moisturizer before wearing this protective equipment?

Yes No

6. Over the past week, did you use any dressing material between your face and this protective equipment?

Yes No

7. Over the past week can you rate your pain, if 0 is no pain and 10 is the worse pain imaginable, on the scale below:



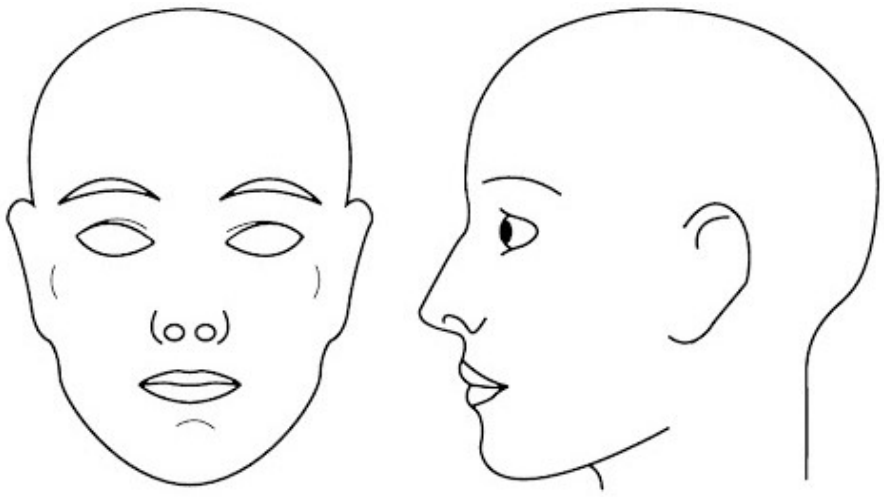
8. Over the past week, has pain been bad enough to interfere with your daily activities?

Yes No

9. Over the past week, have you felt worried or low in mood because of this pain?

Yes No

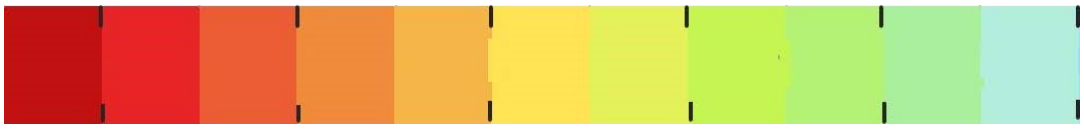
10. Using the face diagram, please annoate the areas of your skin presenting with Redness [RDS], Itchness [ITS], Rash [RSH] or Breakdown [BKN] due to the use of PPE



11. We would like to know how good or bad you perceive your face skin health **TODAY**.

The worst skin health you can imagine	Your face skin health **TODAY**	The best skin health you can imagine

0 **1** **2** **3** **4** **5** **6** **7** **8** **9** **10**



THANK YOU SO MUCH FOR TAKING THE TIME TO COMPLETE THIS SURVEY. WE APPRECIATE YOUR INTEREST AND HELP.

A.1 MULTICENTRE SURVEY ETHICS APPROVAL

From: ERGOII
To: Abiakam N.S.
Subject: Approved by Research Integrity and Governance team - ERGO II 57311
Date: 10 July 2020 08:22:14

Approved by Research Integrity and Governance team - ERGO II 57311

ERGO II – Ethics and Research Governance Online https://www.ergo2.soton.ac.uk

Submission ID: 57311
Submission Title: Monitoring skin health of clinical staff wearing PPE during COVID-19 pandemic
Submitter Name: Nkemji Abiakam

The Research Integrity and Governance team have reviewed and approved your submission.

You may only begin your research once you have received all external approvals (e.g. NRES/HRA/MHRA/HMPPS/MoDREC etc or Health and Safety approval e.g. for a Genetic or Biological Materials Risk Assessment).

The following comments have been made:

-
- Please be advised that the new documents contain typos that may be corrected without resubmitting the documents:

  Consent form item 5: Except *form* my photographic images should read except *from*

  Information sheet track change: Samples will be *disposed immediately* should read *disposed of immediately*.

Appendix B IRAS LETTER OF STUDY APPROVAL

Mr Nkemjika Abiakam
Marie Sklodowska-Curie Research Fellow
University of Southampton
Clinical Academic Facility, Level A, Room AA97,South Academic Block (MP11)
Southampton General Hospital, Tremona Road
Southampton
SO16 6YD

Email: approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

09 September 2020

Dear Mr Abiakam

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Monitoring skin health of clinical staff wearing PPE during COVID-19 pandemic**
IRAS project ID:	**285764**
Protocol number:	**1**
REC reference:	**20/NI/0105**
Sponsor	**University of Southampton**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report

(including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **285764**. Please quote this on all correspondence.

Yours sincerely,
Helen Penistone
Approvals Specialist

Email: approvals@hra.nhs.uk

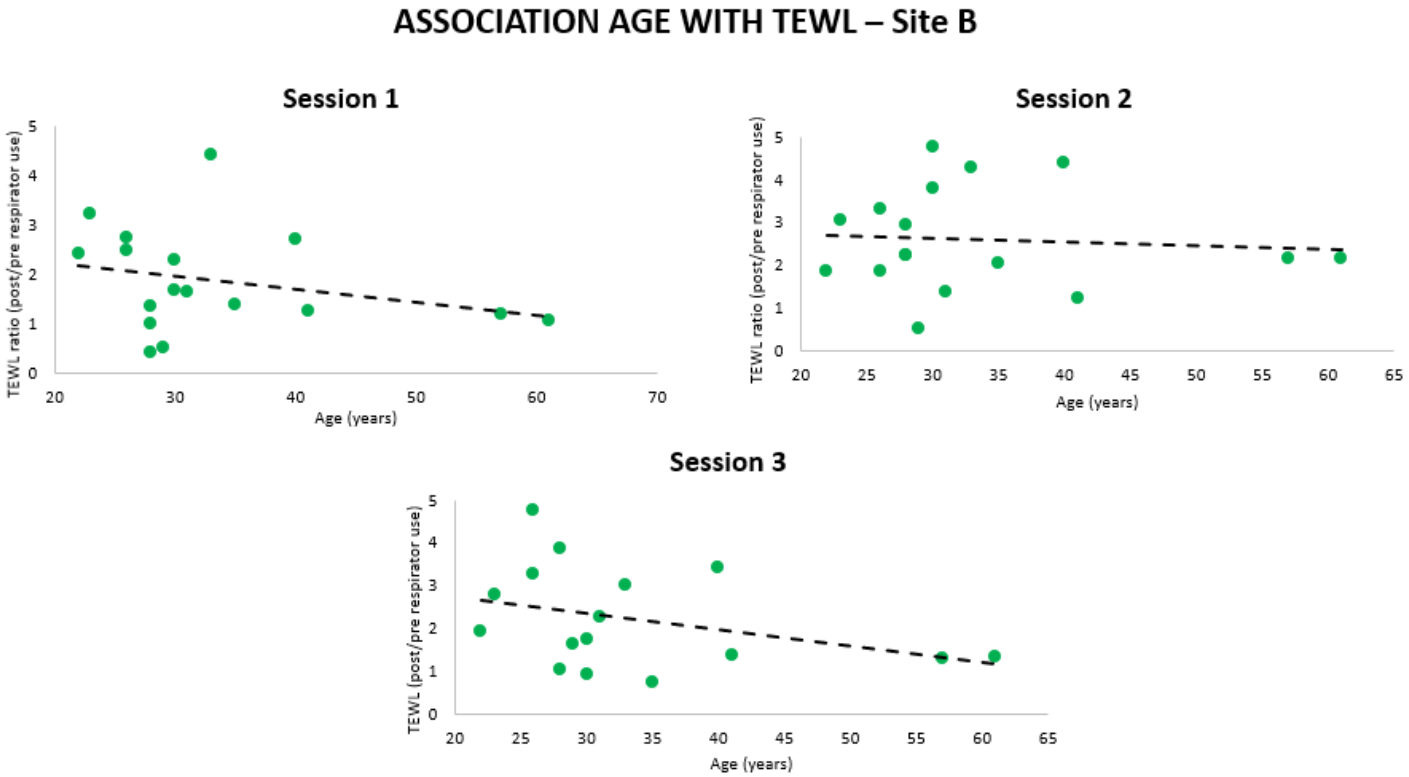
Copy to: *Dr Alison Knight*

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Copies of advertisement materials for research participants [study advertising poster]	1	04 June 2020
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Evidence of Sponsor insurance]	version 1	20 August 2020
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		28 July 2020
IRAS Application Form [IRAS_Form_21082020]		21 August 2020
Letter from sponsor [Letter from Sponsor]	version 1	20 August 2020
Other [Letter of response to REC]	version 1	20 August 2020
Participant consent form [Participant informed consent form]	version 2	20 August 2020
Participant information sheet (PIS)	4	08 September 2020
Research protocol or project proposal [Research proposal protocol]	version 2	20 August 2020
Summary CV for Chief Investigator (CI) [Chief investigator CV]		05 May 2020
Summary CV for student [Student CV]		05 May 2020
Summary CV for supervisor (student research) [Supervisor CV]	1	04 June 2020
Summary CV for supervisor (student research) [Supervisor Curriculum vitae]	version 1	20 August 2020

Appendix C RELATIONSHIP OF TEWL WITH PARTICIPANTS AGE AT THE NASAL BRIDGE ACROSS THE 3 SESSIONS



Appendix D IRAS LETTER OF STUDY APPROVAL

Mr Nkemjika Abiakam

Marie Curie Research Fellow University of Southampton

Email: approvals@hra.nhs.
HCRW.approvals@wales.n

Clinical Academic Facility, Level A, Room AA97, South Academic Block (MP11)

Southampton General Hospital, Tremona Road Southampton

SO16 6YD

16 November 2021 Dear Mr Abiakam

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Detecting changes in Skin status over the site of a stage 1 pressure ul using biophysical sensors and biomarkers**
IRAS project ID:	**301685**
Protocol number:	**1**
REC reference:	**21/SC/0322**
Sponsor	**University of Southampton**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **301685**. Please quote this on all correspondence.

Yours sincerely,
Damilola Odunlami

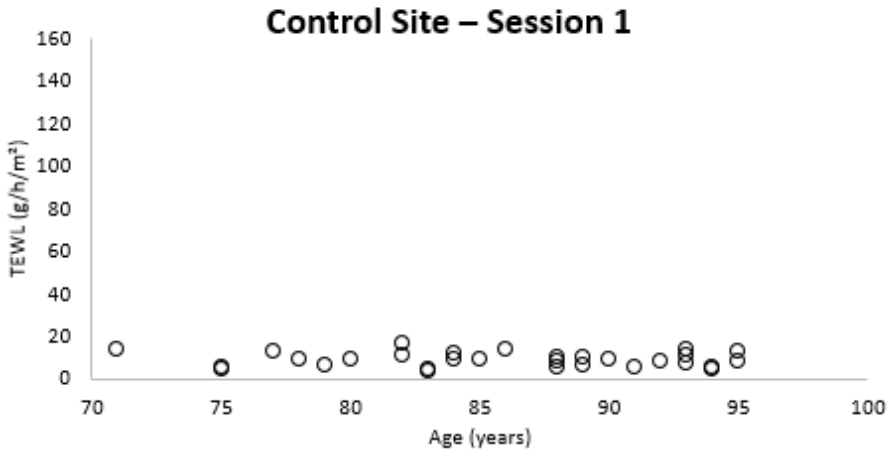
Approvals Manager

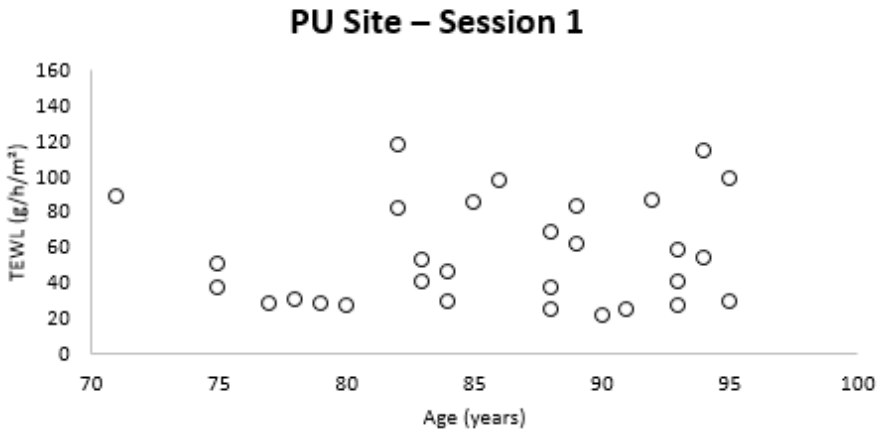
List of Documents

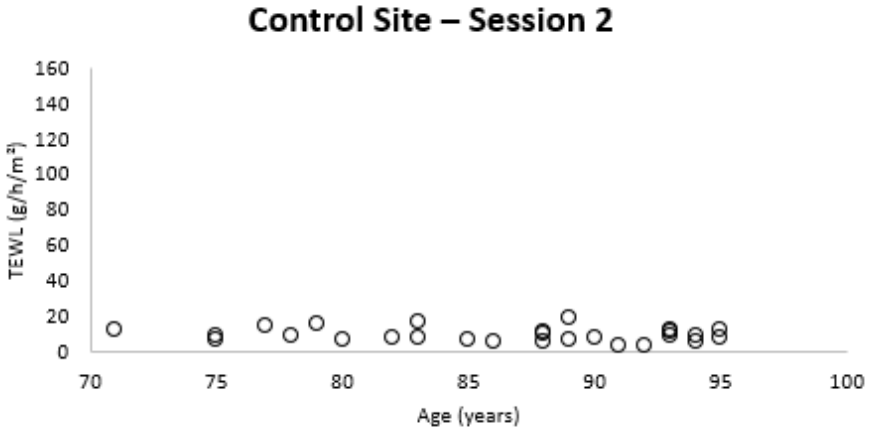
The final document set assessed and approved by HRA and HCRW Approval is listed below.

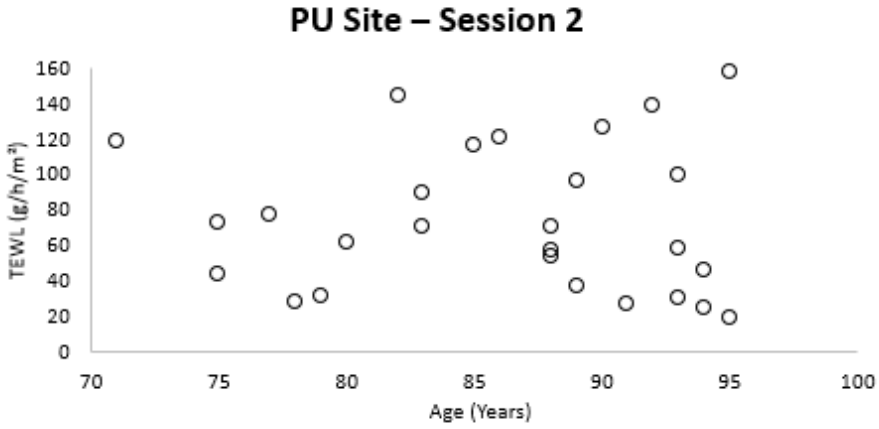
Document	*Version*	*Date*
Copies of materials calling attention of potential participants to the research [Poster advertisement]	1	01 June 2021
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Sponsor insurance letter]	1	08 September 2021
IRAS Application Form [IRAS_Form_09092021]		09 September 2021
Letter from sponsor [Sponsor Letter]	1	08 September 2021
Organisation Information Document [Organisation information document]	1	08 September 2021
Other [Response letter to REC unfavourable opinion]	1	08 September 2021
Other [Funding Budget]	1	08 September 2021
Other [Unfavourable opinion letter]	1	27 September 2021
Other [Sponsor's certificate of insurance]	1	27 September 2021
Other [Lone Working Policy]	1	29 October 2021
Other [Response to the Ethics Committee]	1	29 October 2021
Participant consent form [Participant consent form]	1.1	29 October 2021
Participant information sheet (PIS) [Participant information sheet]	1.1	29 October 2021
Research protocol or project proposal [Research protocol]	1.1	29 October 2021
Schedule of Events or SoECAT [Schedule of events]	1	01 June 2021
Summary CV for Chief Investigator (CI) [Chief investigator CV]	1	08 September 2021
Summary CV for student [Student CV]	1	08 September 2021
Summary CV for supervisor (student research) [Supervisor CV]	1	08 September 2021
Summary CV for supervisor (student research) [Supervisor 2 CV]	1	08 September 2021

Appendix E CLUSTER ANALYSIS OF TEWL WITH PARTICIPANTS' AGE

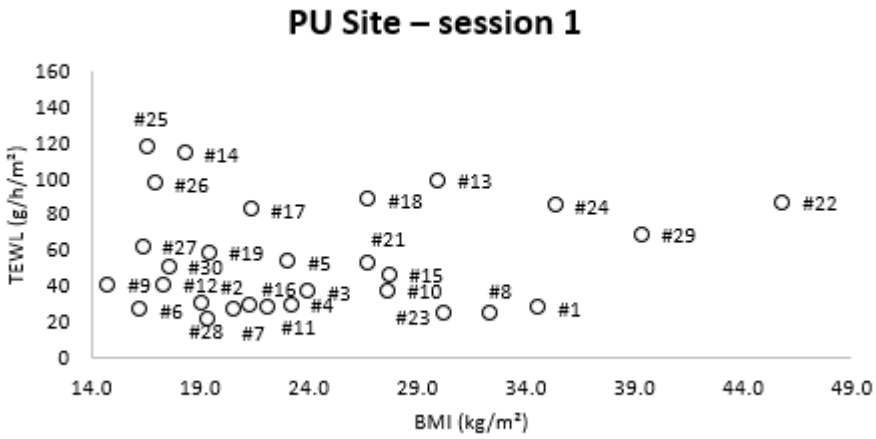


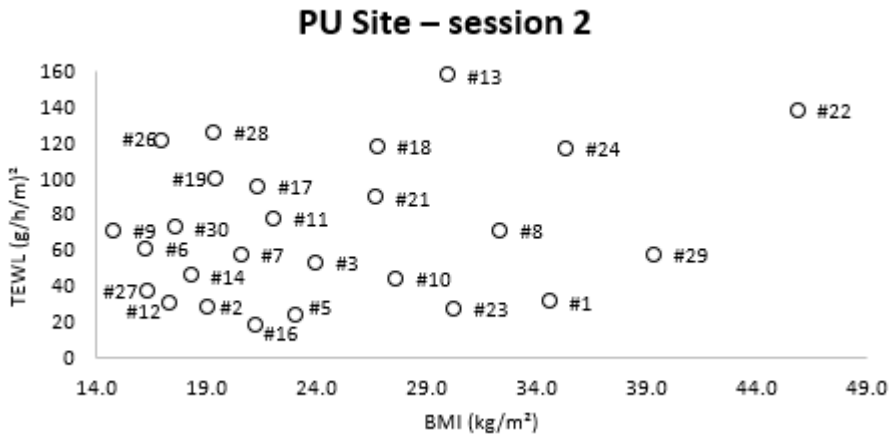






Appendix F CLUSTER ANALYSIS OF TEWL WITH PATIENTS' BMI





GLOSSARY OF TERMS

LIST OF REFERENCES

Abiakam, N., Jayabal, H., Mitchell, K., Bader, D., and Worsley, P. 2023. 'Biophysical and biochemical changes in skin health of healthcare professionals using respirators during COVID-19 pandemic', *Skin Research and Technology*, 29: e13239.

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