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Chronological evaluation of functional changes in neonatal skin: A temporal evaluation of skin maturation

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Abstract

Preterm skin is immature, lacking many functional and structural qualities. Hospital acquired skin breakdown in preterm neonates poses challenges in intensive care and can be a source of pain and infection. Much of the evidence to inform promotion of skin health is adapted from research in adults or older children, leaving a gap in knowledge, which limits care strategies for these vulnerable babies. This research study aimed to identify structural and physiological characteristics of skin in extreme preterm babies and to evaluate these changes with time. This was a longitudinal cohort study of preterm and term infants admitted to NICU in a single large University Hospital. Transepidermal water loss (TEWL), pH. dermal characteristics using high frequency ultrasound imaging (HFUSI) were measured serially and evaluated with gestational age, birth weight, ambient conditions, nutrition, and medications.

Preterm (n=39, median GA 27+4) and Term infants (n=20) were recruited over 18 months. The birth weight of the recruited babies ranged from 500-4230g. TEWL at <48h of age was inversely related to GA (r_s = -0.69, p < 0.01) and birth weight (r_s = -0.53, p = 0.001). Time to reach functional stratum corneum maturity was influenced by GA at birth and incubator humidity. The correlation of skin pH with gestational age was not significant (r_s = -0.30 p = 0.073). Qualitative analysis of ultrasound pictures revealed clear delineation of skin layers in term and late preterm babies (>27+6 weeks GA) soon after birth, but not in extreme preterm babies (GA <28 weeks) till at least 3 weeks of age. Dermal thickness in <28week infants continued to be significantly less than term infants after 8 weeks postnatal age while total skin thickness and dermal echogenicity were matched with those in term infants. This is the first study to show functional maturation in the dermal-epidermal layers in extreme premature infants, by revealing the structural and functional changes in skin in the early weeks of life.

Research and clinical implications

Future research could include use of histological comparison of skin structure with physiological maturity and structural dermal characteristics of skin as noted on ultrasound analysis. This will provide guidance for individualised intensive care strategies for extreme preterm babies for example ambient incubator humidity, ability to endure device related stress produced by the non-invasive device interfaces and other intensive care practices involving nutrition and skin-to-skin care.

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Research Thesis: Declaration of Authorship

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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.

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Abbreviations and definitions

BMI	Body Mass index
CE	Cornified Envelope
ECM	Extracellular Matrix
GA	Gestational Age (calculated from last menstrual period rather than from date of fertilisation which takes place 2 weeks after last menstrual period)
HF	High Flow
HFUS	High frequency ultrasound scan
HFUSI	High frequency ultrasound scan image
IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
LF	Low Flow
LSCS	Lower segment caesarean section
nCPAP	Nasal Continuous positive airway pressure
NG	Nasogastric tube
NICU	Neonatal intensive care unit
MCP 1	Monocyte chemoattractant protein 1
PAH	Princess Anne Hospital
pH	Measure of hydrogen ion concentration
PIs	Pressure injuries

PNW	Postnatal ward
QC	Quality Control
RCT	Randomised controlled trial
RH	Relative Humidity
ROI	Region of interest
SC	Stratum Corneum
SI	Sustained inflammation
TEWL	Trans epidermal water loss
TGF-β	Tumour growth factor beta
TNF-α	Tumour Necrosis Factor alpha
TP	Time point
TSI	Total Skin Thickness
Gestation	Post menstrual gestational age of baby at birth
Term	Baby born at gestational age ≥37 weeks
Preterm	Baby born at gestational age <37 weeks
Late Preterm	Baby born at 34-36 weeks of gestational age
Moderate preterm	Baby born at 32-34 weeks of gestational age
Very preterm	Baby born at <32 weeks of gestational age
Extreme preterm	Baby born at <28 weeks of gestational age
NMF	Natural moisturising factor

Chapter 1 Preterm neonates and skin injury

1.1 Need for evaluation of neonatal skin

Traditionally, the neonatal period has been defined as the first month of life (Edmond et al., 2019). Although neonates seem quite passive and tranquil, they are challenged physiologically as each organ system transitions from the in-utero symbiosis of the materno-foetal interface to a state of functional independence. This 'challenge' is also seen in the skin, the largest organ in the body. In addition, the clinical and biological translation of our knowledge of certain aspects of skin physiology gained from adults and older children may not be appropriate for neonates because of substantial structural and physiological differences from that of older individuals. While some of the problems that these babies encounter is essentially the same as for older patients, for example pressure ulcers, albeit on a smaller scale, their solutions require some understanding of the processes of adaptation that are taking place. Indeed, skin is vital for babies in maintaining homeostasis, and a recognition of the aspects of neonatal physiology which have no correlation in adult medicine seems essential (Ward Platt and Hey, 1992).

1.2 Challenges faced by premature newborn babies

The World Health Organization (WHO) defines preterm birth as the delivery of an infant before 37 weeks of gestation. Mean preterm birth rates have been reported as 9.6 % in the United States, 5–7% in Europe, and 7% in the United Kingdom (Chawanpaiboon et al., 2019). The Office of National Statistics has published preterm birth rate in England and Wales to be 7.6% (Ghosh K, 2023). Recent systematic review has also shown that there is no measurable change in preterm birth rate at global level and approximately 15% of all preterm births occur at less than 32 weeks (Amegah, 2023, Ohuma et al., 2023). Technological advances over the years e.g., non-invasive ventilation and pharmacological advances e.g., use of exogenous surfactant (Schwartz et al., 1994) and use of antenatal steroids (Wapner et al., 2016, Liggins and Howie, 1972) have resulted in increased survival rates at earlier gestations (Costeloe et al., 2012). As a result, survival at 28 weeks gestation is now expected for almost every child. However, while, children born prematurely have improved chances of survival, a significant proportion of them are still at significant risk of developing long-term problems, as a direct result of being born prematurely (Costeloe et al., 2012). Increasing survival of these

vulnerable babies underscores the need to assess and implement care that prevents adverse short-term complications and optimizes long-term outcomes (Humberg et al., 2020).

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1.3 Challenges to neonatal skin

There has been much interest in skin injury, chronic wounds and healing in adults with recent evidence highlighting a cost of £8 billion annually for treating all chronic wounds (Guest et al., 2015) across the NHS. Unfortunately, the economic cost of neonatal wounds and skin injury has not been accounted for in literature. From working in a neonatal intensive care unit as a clinician, there are multiple anecdotal accounts of skin damage and pressure ulcers in neonates. In support of this view, an epidemiologic study of neonates in Southern France by Ligi et al reported that a third of all iatrogenic events (compromising patient safety) were pertaining to skin (Ligi et al., 2008). Skin injuries continue to occur in neonatal age group across all healthcare settings, the majority being preventable (Sardesai et al., 2011). Prevalence rates as high as 23% (Baharestani and Ratliff, 2007) and incidence rates of 16% (Fujii et al., 2010) have been reported in NICUs. A recent review of pressure injuries by Delmore et al also noted that medical devices are the leading cause of pressure injuries in young children and neonates, with devices accounting for 38.5% to 90% of pressure injuries (PIs) in this population (Delmore et al., 2019). A neonatal skin injury is devastating for parents and often results in permanent scarring especially in 22-25 week babies (Lund, 2014), a longer hospital stay and increased hospital costs (Straatman et al., 2015). Moreover, they contribute to an increased risk of infection which is the major cause of infant morbidity and mortality in the NICU (Visscher and Narendran, 2014a) as well as challenges in management of skin injuries (Scheans, 2015).

Research in this area has been limited to observational studies revealing that risk factors for neonatal skin injury are decreased gestational age and medical device use (Nie et al., 2022, Gray, 2004, Schlüer, 2017). A recent systematic review concluded that the frequency of neonatal skin injury is higher than in the adult population, and all neonatal injuries that are acquired during hospital care, are related to diagnostic or interventional medical devices which are critical to care of the patient (August et al., 2018). This contrasts with injuries seen in the adult population where only around a third have been attributed to devices in the acute care setting (Black, 1969). A recent survey of neonatal nurses at a tertiary neonatal unit PAH (Princess Anne hospital), highlighted that at least a quarter of the nurses perceived that their patients endured skin damage daily (Liversedge et al., 2018). The prevalence of neonatal skin lesions has been reported to range from 9.3% to 43.1% (August et al., 2018) while the incidence of pressure injuries in neonates admitted

to intensive care unit ranges from 3.7% to 21.6% (García-Molina et al., 2018). The prevalence of diaper dermatitis amongst hospitalised infants and children has been reported to be 17 to 43% (Edsberg et al., 2016). The proportion of device related pressure injuries out of all pressure injuries in the neonatal intensive care units have been reported to be 73.5-80% (Fassino et al., 2023, García-Molina et al., 2018, Visscher and Taylor, 2014). Implementation of quality improvement and education programs actually led to apparent increase in incidence of all skin lesions and was thought to be biased by incomplete initial reporting (Fassino et al., 2023). While a number of systematic reviews have investigated pressure injury prevention strategies for adult patients (Lin et al., 2020, Alshahrani et al., 2021), Setchell et al have conducted a paediatric review for the first time and identified that preventive strategies are effective in reducing the number and severity of pressure injuries among critically ill children admitted to intensive care (Setchell et al., 2023).

A prospective multicentre trial showed that most common location of pressure ulcer in neonatal intensive care is the nose (Fujii et al., 2010), which incidentally is the part of skin in contact with medical devices for the longest time in preterm babies (e.g., Continuous positive airway pressure (CPAP) masks, nasal gastric tubes). The survey of neonatal nurses mentioned above noted that CPAP, adhesive tape, and intravenous cannula were the most common causes of skin damage in these babies (Liversedge et al., 2018). There are other examples of skin injury sustained due to medical devices in continuous, close contact under pressure with skin, especially nCPAP applied on extreme preterm babies, with skin breakdown at the site of nasal septum and nasal bridge (Imbulana et al., 2018) (Singh J and D, 2017). Studies are underway to influence the interface pressure between the skin and devices as well as mattresses in order to prevent pressure ulcers (Courtwright et al., 2017, Mallick et al., 2023).

To understand why medical devices, cause injuries to these vulnerable neonates it is critical to characterize the unique features of neonatal skin. This is because the tolerance of skin to device loads is likely to be dependent on its structure and function from a physical and biochemical perspective. Thus, there is great need for knowledge of parameters such as skin barrier function, dermal thickness etc. in neonates, including preterm infants.

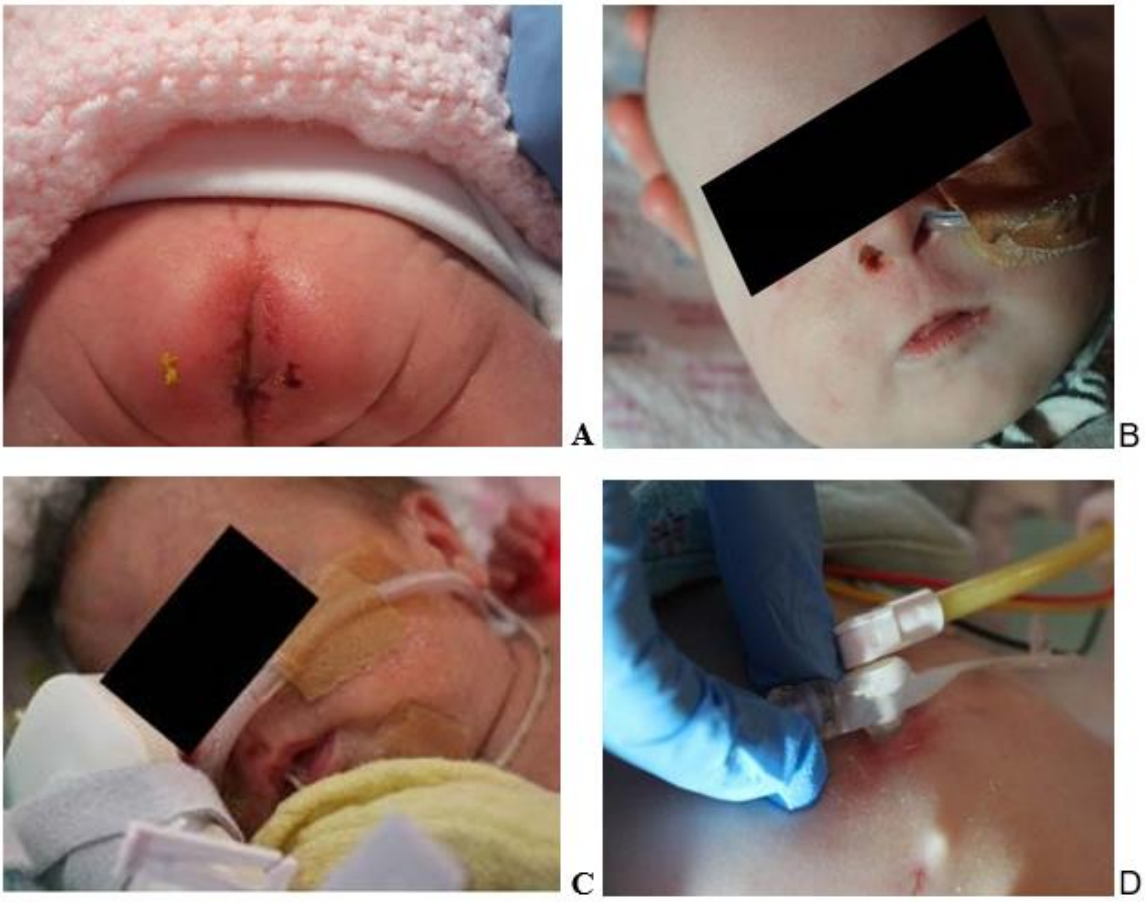


Figure 1-1 Challenges to neonatal skin

(A) Broken skin from diaper dermatitis. (B) Nasal pressure ulcer associated with previous nasal endotracheal tube. (C) Upper lip pressure ulcer from previous oral/endotracheal tube. (D) Skin damage from gastrostomy

Chapter 2 Review of Literature

2.1 Search strategy

A narrative review method was adopted to provide comprehensive background for the purpose of understanding current knowledge and highlighting the significance of new research. Latest update was performed in January 2024.

This review explored for published literature with the aim to review the current understanding of the differences in structure and function of preterm skin compared to term infants. The objectives of this literature search were to establish:

1. Current knowledge about barrier properties of skin in extreme preterm babies and best methods to measure it.
2. Current knowledge of skin structure in preterm babies using invasive and non-invasive techniques and best methods to measure it.
3. Awareness of neonatal skin injury, especially device related skin injury in extreme preterm babies.
4. Identify gaps in knowledge about barrier properties of skin and skin structure in preterm babies which could provide more information to guide neonatal intensive care practices to help avoid skin injuries in these vulnerable babies.

2.2 Search method

Literature search was conducted on electronic data bases and Google Scholar. The period over 1946-2024 was explored on OVID. PubMed, PubMed Central, CINHAL, Ovid MEDLINE, EmBase and PSYCINFO databases were also used for this search. The search terms used in various combinations were as in Table 2-1. In addition to evaluating the current literature surrounding the characteristics of the skin, a focused review of the equipment used to assess the skin was conducted. Data regarding skin measurement device performance e.g., accuracy and reliability, benefits, and limitations of use in clinical scenarios was also identified from the published literature.

Table 2-1 Search terms and Boolean operators for the narrative review

infan* or newborn* or neonat* or full term* or preterm* or premature”
Skin* or Epiderm* or Derm* or Stratum Corneum or SC or Cutan* or cutis or cuticle* skin or dermis or epidermis
pH or hydrogen ion* or insensible water loss or TEWL
Hydrogen-ion Concentration
Transepidermal water loss*
Thickness
skin ulcer or pressure ulcer damage* or sore* or ulcer* or injur* or integrity or wound* or matur* or Barrier* or immature* “wounds and injuries”
Ultrasound
Imaging
Humans

2.3 Characteristics of the skin

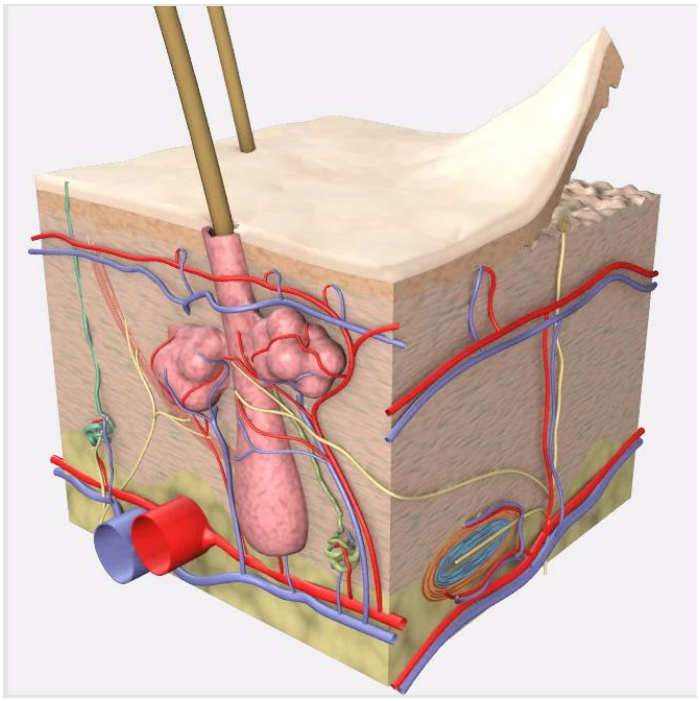
Literature review on embryology, structure and function of skin is very important to recognise the gaps in knowledge. Vernix caseosa is a unique feature in human life and has been studied in detail.

2.3.1 Structure of skin

The skin is the largest organ of the human body (Leider, 1947). It is comprised of the epidermis, dermis, and subcutaneous tissue or hypodermis(Gao et al., 2013) as in Figure 2-1. The outermost layer (epidermis) is approximately 75–150 μm thick in adults, although it is considerably thicker in the palms of the hands and plantar aspects of the feet, and it is 5-35 μm in term newborn period (Blume-Peytavi et al., 2016),. The epidermis is divided into five strata, however only the deepest layer contains the metabolically active cells. The dermis is the deeper layer of skin containing dense connective tissue and provides strength and elasticity to the skin. The layers of skin are labelled in the diagram below and each layer is discussed in further detail in this section.

2.3.1.1 Epidermis

The epidermis is traditionally subdivided into stratum corneum (SC) and the viable epidermis (layers composed of living cells) as in Figure 2-1. The basal layer is composed of a single layer of keratinocytes and is the site where keratinocyte cell division occurs. Cells from this layer undergo differentiation as they progress upwards through the strata of the epidermis, with the terminal stage of keratinocyte differentiation being represented as flattened corneocytes without nuclei in the SC (Lai-Cheong and McGrath, 2017). In this way, the epidermal tissue is continuously renewed (Baroni et al., 2012). During the corneocyte maturation process, the profilaggrin protein is dephosphorylated to filaggrin, which is proteolyzed to amino acids, which furthe combine with ions, organic acids, and sugar to make the Natural Moisturising Factor (NMF) (Rawlings and Matts, 2005). In addition to keratinocytes and corneocytes, there are other specialised cell types in the epidermis, see Table 2-2.



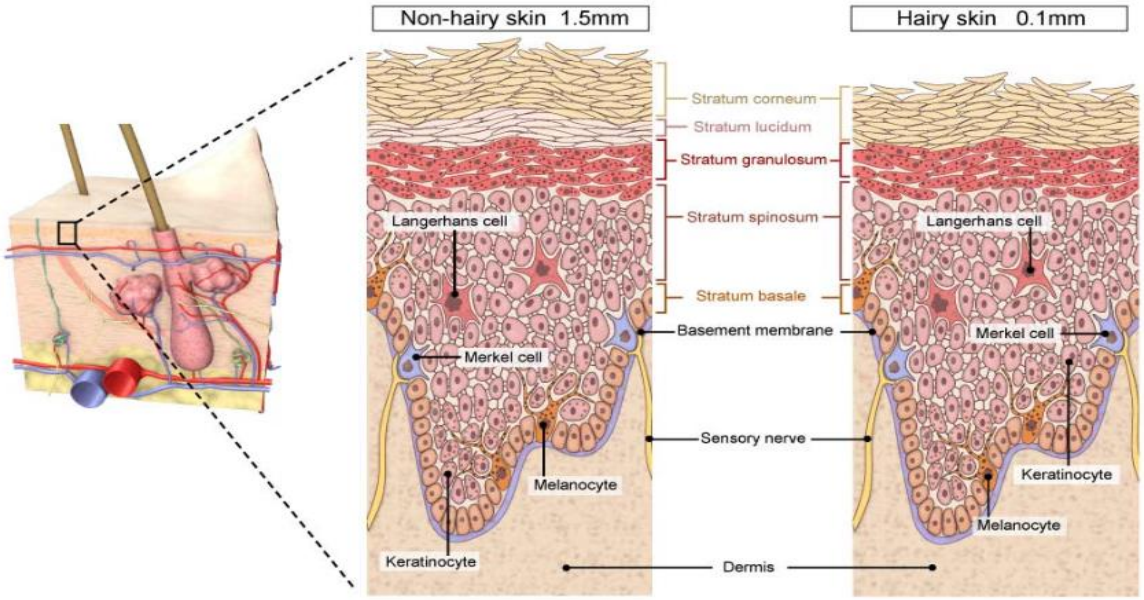


Figure 2-1(A) Skin morphology (B) Epidermal differentiation

2.3.1.2 Dermo-epidermal junction

The integrity of the epidermal–dermal junction, an undulating structure, is critical for the normal transport and communication of biomolecules between the epidermis and the underlying dermis. The dermo-epidermal junction zone is comprised of the basal layer of epidermal keratinocytes, the dermo-epidermal basement membrane, and the upper (papillary) layer of the dermis (Villone et al., 2008). The basement membrane is made up of the lamina lucida and the lamina densa and allows molecules to diffuse between the epidermis and dermis (Rocken, 2012). The epidermis and dermis are both anchored to the basement membrane. Anchoring filaments connect the hemidesmosomes of the basal keratinocytes to the lamina densa, and anchoring collagen XVII fibrils connect the basement membrane to the papillary dermis (Leyva-Mendivil et al., 2015).

Skin homeostasis depends on the stable cohesion between the epidermis and the dermis, which are tightly interconnected through the dermo epidermal junction, as described earlier. The anchoring complex within the dermo epidermal junction is responsible for the stability of the dermo-epidermal cohesion and consists of the hemidesmosomes of basal keratinocytes, the anchoring filaments linking the hemidesmosomes to the basement membrane, and the anchoring fibrils connecting the basement membrane with the underlying dermis (Villone et al., 2008, Ellison and Garrod, 1984).

Table 2-2 Epidermal cell types and functions

Cell type	Function	Source
Keratinocytes	95% of epidermal cells are keratinocytes in different stages of differentiation	(Lai-Cheong and McGrath, 2017)
Corneocytes	Corneocytes are terminally differentiated enucleated cells that make up the SC and contribute to the barrier integrity	(Lai-Cheong and McGrath, 2017)
Melanocytes	Melanocytes synthesise melanin-containing organelles (melanosomes), which are then transferred to basal keratinocytes	(Lai-Cheong and McGrath, 2017)
Lymphocytes	Involved in specific immune responses.	(Baroni et al., 2012)
Langerhans cells	Immune function	(Baroni et al., 2012)
Merkel cells	Provide sensory information to the central nervous system.	(Hao et al., 2015)

2.3.1.3 Dermis

The dermis is dense connective tissue composed of cellular and extracellular components. The cell types and functions are outlined in Table 2-3. The extracellular matrix (ECM) is composed of collagen, elastin, and proteoglycans. Collagen accounts for 70% of the dry weight of the dermis, with the primary collagen types present in the dermis being types I and III, though at least 16 types are present (Rocken, 2012). Collagen molecules cross-link with one another, forming a strong and stable network that provides the skin with increased tensile strength (Agache and Humbert, 2004) and stiffness (Hussain et al., 2013). Elastic fibres make up 2-3% of the dry weight of the dermis and are primarily composed of elastin, imparting elasticity, and resilience to the skin (Rocken, 2012). As with collagen molecules, elastin molecules crosslink with similar molecules to form a stable network. Proteoglycans and glycosaminoglycans (GAGs) form the amorphous gel which surrounds the collagen and elastin fibres (Tracy et al., 2016).

The dermis can be divided into papillary and reticular strata. The upper papillary layer comprises approximately 20% of the dermis. The dermal papillae contain capillary loops, with some also containing nerve endings sensitive to heat or touch (Tortora and Derrickson, 2014). The reticular dermis is composed of thicker, denser collagen fibres than the papillary dermis, and contains complete elastic fibres (Haydont et al., 2019, Rocken, 2012, Tracy et al., 2016). In preterm neonates, the papillary dermis underlying the dermo epidermal junction is oedematous, collagen fibrils are smaller than those of the term newborn or adult, and the anchoring structures are decreased, with wide spaces between connecting points (Holbrook, 1982). During skin maturation, cell attachments and epidermal cellularity increase, and the dermo epidermal junction becomes undulated. These undulations increase the surface contact between the two layers, facilitating the exchange of oxygen, nutrition, and waste products (Evans and Rutter, 1986).

The variations in the neonatal and adult skin fibroblasts, collagen and TGF-β explains the drastic difference in scar tissue in the two age groups. There is scarless healing in wounds of foetal and newborn skin (Živicová et al., 2017, Haydont et al., 2019) and fibrous scars in adults. It is interesting to also note that the pioneering antenatal neurosurgical management of meningomyelocele has been greatly successful (Sacco et al., 2019, Leung et al., 2012, Kabagambe et al., 2018).

The dermis is highly vascularised, with cutaneous blood flow contributing significantly to thermoregulation. For example, when the body is subjected to thermal stress, the resulting vasodilation increases the loss of body heat via convection, preventing the internal organs from overheating. Sweat glands are also involved in thermoregulation, and these are hosted in the dermis, as are sebaceous glands and hair follicles. The cell types of the dermis and their functions are described in Table 2.3.

Table 2-3 Dermal cell types and functions

Cell type	Function	Source
Fibroblasts	Synthesise and deposit collagen, elastin, and proteoglycans	(Wong et al., 2007)
	Promote keratinocyte proliferation and release cytokines and growth factor	(el-Ghalbzouri et al., 2002)
	Facilitate re-epithelialisation as part of wound healing	(el-Ghalbzouri et al., 2002)
Macrophages	Phagocytic immune cells that scavenge foreign substances, including cell debris and damaged tissue	(Baroni et al., 2012)
Mast cells	Specialised secretory cells containing granules with contents such as histamine and prostaglandins	(Burns and Graham-Brown, 2011)

2.3.1.4 Subcutaneous tissue/Hypodermis

The subcutaneous fat tissue, below the dermis, is present as white adipocytes separated by fibrous septae, loosely attached to the dermis. The hypodermis varies with anatomical site, age, gender, race, endocrine and nutritional status of the individual. Subjacent to this layer can be a muscle layer, which overlies either bony prominences or internal tissues and organs.

White adipocytes in subcutaneous tissue develop from embryonic stage (Poissonnet et al., 1988), but majority of the differentiation occurs after birth (Burdi et al., 1985). From 20 weeks of pregnancy brown adipocytes start developing in various parts of subcutaneous tissue and comprises 1% of body fat by term (Cannon and Nedergaard, 2004). Preterm babies lack mitochondria rich brown adipose tissue which protects the internal organs against mechanical trauma and provides insulation against external extremes of hot and cold (Baroni et al., 2012).

2.3.2 Embryology

It is important to understand the development of skin from the embryonic period to understand the clinical challenges faced by these cells and tissues daily.

2.3.2.1 Embryology of Epidermis

A single epithelial layer initially forms from the ectoderm during embryogenesis under the influence of fibroblast growth factors, bone morphogenic proteins, and Notch signalling (Fuchs, 2007). A basal epidermal layer and one periderm layer are created by gestational week 4 (Lane et al., 1985). The periderm covers the basal layer and forms tight junctions during foetal development. Melanocytes appear in the basal layer during weeks 5–8. Early foetal development (up to 10 weeks) corresponds to initial epidermal stratification and formation of third 'intermediate' layer between the basal cell layer and the periderm. The cells in this layer remain highly proliferative, such that by week 22-24 epidermis contains 4-5 layers in addition to periderm which is terminally differentiated with formation of cross-linked cornified envelope. This envelope is formed by foetal suprabasal cells adhering to other cells to create a barrier structure (Sumigray and Lechler, 2015). By week 26, the epidermis is fully keratinised and consists of one basal layer, 2-3 spinous layers, one granular layer, and 5-6 stratum corneum layers (Holbrook et al., 1989). Eight distinct phases of differentiation occur over gestational weeks 5 and 26 (Holbrook and Odland, 1980). The periderm protects the developing epidermis from amniotic fluid till 23 weeks when it disappears. Keratinisation (terminal differentiation in stratum granulosum and SC) initiates in the skin appendages at 11-15 weeks and extends to interfollicular epidermis by 22-24 weeks. The cornification of interfollicular epidermis occurs in a programmatic fashion from head (week 23) to toe and dorsal ventral abdomen (week 25) across the foetus The basal cuboidal cells in early fetal epidermis express keratin markers (K1/K10) and desmosomal protein desmoglein 3 also known as pemphigus vulgaris antigen (Lane et al., 1985, Woodcock-Mitchell et al., 1982) indicating potential for keratinisation. The cornified cell layers increase in number aiding the formation of water-impermeable barrier (Moll et al., 1982, Dale et al., 1985). Both these studies were conducted in human foetuses. In the second of the eight phases described by Holbrook (Holbrook and Odland, 1980) the epidermis thickens in certain regions giving rise to hair pegs (Duverger and Morasso, 2009) and the hair extends out through the skin in stage 6. The sebaceous glands develop near the upper hair follicle at gestational weeks 13-14. Eccrine glands appear at about the same time and continue to develop through gestational week 24 (Cui and Schlessinger, 2015). The basal cell layer also begins to secrete collagen V and VII, the latter being the major component

of anchoring fibrils of the dermis. Foetal rats have been used widely for studies of mammalian ontogenesis. Both the structural organisation and amount of the lamellar lipids in the epidermis aide in formation of a competent barrier just before birth in rat pups (Aszterbaum et al., 1992). Whereas no measurable barrier was demonstrated in the pups at 19 days of gestation, by 21 days a competent barrier was noted to be uniformly present (normal gestation in rat pups is 22 days). This study also demonstrated that with the development of skin barrier the total lipid content of stratum corneum had doubled while cholesterol and non-polar ceramides had increased by 5-fold. Also, while secreted lamellar body sheets were present in extracellular spaces of SC on day 19, a well organised lamellar bilayer structure was only noted by 21 days. A similar study has not been replicated for human skin.

Hanley et al in the University of California developed an in-vitro model using rat pups to identify the factors critical for foetal epidermal barrier formation. It was clear that the foetal epidermal development is accelerated in presence of dexamethasone and triiodothyronine (Hanley et al., 1996). Steroids are routinely used in threatened preterm labour to increase the chance of survival of the baby and reduce the risk of health problems (NICE, 2010). However the mechanism of epidermal maturation in the preterm infant is likely to be different from the rat, as it is not influenced by antenatal steroids in human beings (Jain et al., 2000). Similar findings have been reported in a systematic review more recently (August and Kandasamy, 2017). As the use of antenatal glucocorticoids in preterm pregnancies has become part of standard clinical practice, it would not be possible to carry out an ethical large, randomised trial, so an indirect assessment of skin barrier function is needed.

Filaggrin is a large, histidine-rich protein localized in the newly formed corneocyte layer above the granular layer in the epidermis (Harding and Scott, 1983). Its function is to aggregate epidermal and inner root sheath keratin filaments into highly ordered linear arrays. These aggregated fibres are part of the envelope that surrounds cells entering the stratum corneum. Filaggrin starts out as a high-molecular-weight precursor called profilaggrin, located in the keratohyalin granules of the granular layer (Harding and Scott, 1983). As the granular cells differentiate into cornified cells, the profilaggrin is dephosphorylated and degraded into the highly basic, lower molecular weight filaggrin.

Filaggrin continues to be degraded almost immediately after the keratin fibres are formed. One of the first steps in this degradation process is the conversion of arginine residues in the filaggrin molecule to citrulline. This process increases the acidity of the filaggrin molecule, resulting in the loosening of the filaggrin/keratin complex and increasing the access of proteolytic enzymes. At this point, the filaggrin molecules are completely degraded into their respective amino acids and derivatives, making up 70-100 percent of the free amino acids and their derivatives present in the stratum corneum (Scott et al., 1982)

Natural moisturising factor (NMF) is composed of by-products of filaggrin and includes amino acids, urea, lactate and pyrrolidone carboxylic acid. NMF exists in normal skin, packaged in the corneocytes. These components are highly efficient humectants (bind and hold water). The hydrated NMF forms ionic interactions with keratin fibres, reducing the intermolecular forces between them thus increasing the elasticity of the stratum corneum, making it more resilient to mechanical stress (Jokura et al., 1995, Rawlings and Matts, 2005). Though non-viable the stratum corneum is a dynamic structure with enzyme activity. These enzymes need water to function. NMF provides this necessary water (Harding et al., 2000). NMF is reduced in xerosis, atopic dermatoses, psoriasis, ichthyosis vulgaris. In fact, reduced level of NMF is seen after bathing and exposure to UV light (Rawlings and Harding, 2004).

A recent study has shown that NMF levels when measured within the first week after birth are significantly less in all preterm babies born before 31 weeks in comparison with the late preterm infants, term infants and adults. However, the levels are significantly higher than adults in all three groups of infants when measured at about 100 days after birth. Also, the early preterm babies show much less expression of NMF in the first week as compared to both late preterm and term infants and start to upregulate the processes that drive production of NMF by 2-3 months of age as a response to transition after birth (Visscher et al., 2021). Thakoersing et al generated human skin equivalents under submerged conditions mimicking the aqueous in-utero environment and investigated the morphology and differentiation process of the formed epidermis. The submerged human skin equivalents showed comparable tissue morphology, several differentiation markers and SC lipid composition compared with human skin equivalents grown at the air-liquid interface and native human skin. The SC of the submerged human skin equivalents, however, contained less natural moisturizing factors (NMF) compared with the air-exposed counterparts (Thakoersing et al., 2010), indicating that NMF is less in-utero.

Tight junctions are present in cell-cell borders in periderm and epidermis. Historically, it was thought that the tight junctions play only a minor role in the epidermal water permeability barrier (Elias et al., 1978). However more recently common skin diseases like atopic dermatitis have been linked with acquired tight junction dysfunction and involucrin-cldn6 transgenic mice have been shown to display skin barrier defects (Enikanolaiye et al., 2010, Furuse et al., 2002, Morita et al., 2011). These results suggest that tight junctions also play an important part in forming epidermal permeability barrier.

Congenital diseases of Epidermis

Gross defects in early epidermal organogenesis are rarely observed in neonates probably because they are incompatible with survival. In contrast, the congenital defects in epidermal

maturation are not uncommon like lamellar ichthyosis which is inherited in autosomal recessive manner. In 30% of patients this is caused by mutations in the gene encoding epidermal transglutaminase, an enzyme that cross-links the sub-membranous proteins that form the insoluble cornified envelope of the SC. Similar presentation is seen in patients with homozygous mutations in the ABCA12 gene which encodes for ATP-binding cassette transporters which are important for lipid trafficking across keratinocyte membranes. Harlequin ichthyosis is an extreme variant with mutation in the ABCA12 gene.

In contrast to these permanent genetic defects, the inadequate epidermal keratinisation and maturation of preterm epidermis are transient. Immaturity of SC places infants born before 28 weeks GA at risk of dehydration, excessive penetration of drugs/chemicals and infection (Evans and Rutter, 1986, Harpin and Rutter, 1983). The SC in full term newborn is structurally and functionally equivalent to an adult over first few weeks whereas maturation of SC in premature infant is accelerated after birth, though the duration to being equivalent to adult skin is longer in extremely premature infants (Kalia et al., 1998).

Specialized cells in the epidermis

Melanocytes and Langerhans cells are two major specialised cells that populate the epidermis in early embryonic development. The melanocytes are derived from neuroectodermal cells which also give rise to many tissues of the face and peripheral autonomic neurons (Anderson, 1994). The neural crest cells destined to become melanocytes migrate away from the neural tube within the mesenchyme, adjacent to presumptive epidermis. They migrate as semi coherent clones laterally and then ventrally around the trunk to the thoracoabdominal midline, scalp, and face and distally along the extremities. The embryonic paths taken by these cells follow the Blaschko's lines that can be readily visualised in patients with pigmentary dyscrasias, such as hypo melanosis of Ito and linear/whorled hyper melanosis. The failure of adequate number of melanocytes to completely supply distal points on their embryonic migration path occurs in different types of Waardenburg syndrome and piebaldism resulting in depigmented patches in central forehead, central abdomen, and extremities. Genetic basis of both these conditions have been established. (Boissy and Nordlund, 1997). In albinism however, the development of melanocytes is normal, but production of melanin is inadequate. The varying severity of this condition are due to mutations in the gene encoding for tyrosinase, the rate limiting step in production of melanin (Rees, 2011).

Melanocytes are first detected in epidermis by day 64 estimated GA (Holbrook et al., 1989). The density of melanocytes is noted to be quite high, approximately 1000cells/mm2 (Holbrook et al., 1989). The melanocytes are noted to transfer melanosomes to keratinocytes from 5 months of age (Breathnach and Wyllie, 1965, Zimmermann and Cornbleet, 1948). All melanocytes are in place

and melanogenesis is well underway, but the skin of newborn infant is not fully pigmented and will continue to darken over the first several months, especially apparent in individuals with darker skin.

Langerhans's cells are detectable in the epidermis by 6-7 weeks GA but do not yet possess specialised organelles characteristic of mature cells (Foster et al., 1986). Once transitioned to fetus they produce characteristic granules of mature Langerhans cells. Also, while they resemble the adult phenotype by the second trimester, the total number of cells only increases to adult numbers in the third trimester (Foster and Holbrook, 1989) when the immune system is fully activated. Merkel cells, which are associated with the epidermal appendageal structures and involved in mechanoreception can be morphologically detected in the palmoplantar epidermis at 10-14 weeks GA. Studies suggest that the Merkel cells are derived from pluripotent keratinocytes rather than neural crest, but results and not conclusive (Saxod, 1996).

2.3.2.2 Embryology of Dermis

Development in the Embryonic period

The cell of origin for the presumptive dermis depends on its anatomic location. The dermis of the face is derived from neural crest cells; that of the dorsal trunk is derived from the dermatomyotome portion of the differentiated somite; and the dermis of the limbs is derived from the lateral plate (somatic) mesoderm (Noden, 1992). Regional patterning of the skin and differences in the type and quality of the epidermal appendages produced in the older fetus is thought to reflect these early differences in dermal cell precursors. By 6–8 weeks GA, the presumptive dermal cells already underlie the epidermis. Most protein components of collagen fibres and some microfibrillar components of elastin fibres (fibrillin) are synthesized by the embryonic dermal cells. Lane and Holbrook showed that the patterns of distribution of types I, III, and V collagen in skin are established in embryonic stages of development (Lane et al., 1985, Holbrook, 1998). With increasing gestational age, the fibrils enlarge and organise themselves into larger fibres. Also, the density of fibrous collagen in the extracellular matrix increases leading to expansion of dermis (Smith et al., 1986). Moreover, the ratio of collagen III to collagen I is 3:1, the reverse of that in the adult (Smith et al., 1982, Smith et al., 1986, Epstein, 1974, Sykes et al., 1976). The collagen type I builds a scaffold with thick fibres that have low turnover (Smith et al., 1982, Smith et al., 1986, Epstein, 1974, Sykes et al., 1976, Singh et al., 2023). The maturation of collagen type I however depends on collagen type III, which produces thin, less durable fibres with high turnover. Mature collagen type I is primarily responsible for mechanical stability while type III collagen that produces

thin strands is mostly considered juvenile collagen of the early wound healing phase (Revell et al., 2021).

Development in the foetal period

After embryonic–foetal transition, the presumptive dermis is distinguishable from the underlying skeletal condensations. Within the dermis, electron microscopy shows that there is a progressive change in matrix organization and cell morphology, such that by 12–15 weeks, the fine interwoven mesh of the papillary dermis adjacent to the epidermis can be distinguished from the deeper, more fibrillar reticular dermis (Smith et al., 1986). Studies on human foetuses have shown that total number of cells per mm^2 dermis and Ki-67 (nuclear protein associated with cell proliferation) are significantly higher in foetal dermis at 16-22 weeks GA than adult dermis. These reports are from the studies conducted using the light and electron microscopes. Large collagen fibres accumulate in the reticular dermis during the second and third trimesters. Collagen type I is the principal component of the extracellular matrix in both foetal and adult skin, but foetal skin contains a higher ratio of collagen type III to collagen type I than adult skin albeit in the sheep and murine studies (Knight et al., 1993, Merkel et al., 1988). From 13 weeks of gestational age, the structural protein fibronectin is present in all the layers of the dermis in the foetal skin but only in the dermo epidermal junction and around blood vessels in the adult skin (Coolen et al., 2010). Definitive elastin fibres first become detectable around 22–24-week GA (Deutsch and Esterly, 1975, Coolen et al., 2010).

By the end of gestation, the dermis is thick and well organized but is still much thinner than in the adult and has a higher water content, reminiscent of the foetal dermis. Studies have shown higher level of glycosaminoglycans (GAGs) in the foetal as compared to adult dermis. The amount of both hyaluronic acid (rabbit foetal skin) (Mast et al., 1991) and chondroitin sulphate (foetal mice) (Whitby and Ferguson, 1991) are higher in the foetal skin than in the adult skin. Coolen et al have used immunohistochemistry and immunofluorescence on human foetal skin samples obtained from the limbs and shown that glycosaminoglycan chondroitin sulphate is detected in the papillary dermis and in the upper part of the reticular dermis from 16 weeks GA (Coolen et al., 2010). It has been suggested that glycosaminoglycans play a role in foetal scarless wound healing (Mast et al., 1993).

Dermal maturation is marked by increasing tensile strength and the transition from a nonscarring to a scarring response after wounding (Coolen et al., 2010, Leung et al., 2012). Studies have shown that permissive scarless wound healing in foetal skin is due to intrinsic properties of the

skin and independent of the in-utero environment. (Longaker et al., 1994, Larson et al., 2011). The studies describing the composition of skin (Coolen et al., 2010) have proved helpful in understanding the processes of wound healing in mid-gestation. Translation of this knowledge is evolving the novel clinical therapeutic modalities to improve scarring in adults (Leavitt et al., 2016).

Congenital defects in dermis

Most congenital defects in development of dermis are incompatible with life. Infants born with restrictive dermopathy have thin, flat dermis with lack of elastic tissue fibres. They survive to birth but die in the neonatal period because of respiratory insufficiency. It is an autosomal recessive disorder (Nijsten et al., 2002). Focal dermal hypoplasia is an X-linked dominant disorder. The females who are functional mosaics develop bands of dermal hypoplasia that follow Blaschko's lines (Wang et al., 2007).

Nerves and vasculature in the skin

The development of cutaneous innervation closely parallels that of vascular system in terms of its pattern, rate of maturation and organisation. There are two types of nerves in skin, somatic sensory and sympathetic autonomic. They are small and unmyelinated. A 17.5-week foetus can demonstrate localised somatosensory reflex in the perioral region using von Frey hairs (Bradley and Mistretta, 1975, Hogg, 2004).(Bradley and Mistretta, 1975, Hogg, 2004). The developing nerves as a whole function as receptor in early development and are superseded in later development by more definitive end organs (Cauna and Mannan, 1961). Among the neuroendocrine functions of postnatal skin, cortisol has been shown to be manufactured de novo by human hair follicle and is present in preterm stratum corneum (Narendran et al., 2010, Ito et al., 2005). These findings highlight the skin-brain connection during normal development.

Blood vessels have been identified in foetal skin at 11 weeks GA. They have been reconstructed at this age by computer graphics that illustrate the complexity of vascular plexus. The blood vessels help delineate dermal-hypodermal junction (Johnson and Holbrook, 1989). By 3 months the distinct horizontal and vertical networks are formed. By 5 months vasculogenesis has largely ceased. Formation of complex vascular plexuses after this time are initiated by angiogenesis, budding and migration of endothelium from pre-existing vessels. The superficial architecture continues to be further organised and is responsible for the skin redness often observed in the newborn. The complexity of the vasculature decreases over the first few months as skin surface area increases, lanugo hairs are lost and sebaceous gland activity decreases. It is at this time that the rate of skin growth is the greatest. By 3 months the vascular pattern resembles those of the

adult (Lawrence Eichenfield, 2014). Errors of neurovascular morphogenesis can lead to common syndromes such as Klippel-Trenaunay, Sturge Weber and PHACE syndromes.

2.3.2.3 Embryology of Hypodermis

Hypodermis can be delineated by 8-10 weeks GA (Polin RA, 1998). It is a distinct region separated from overlying cellular dermis by a plane of thin-walled vessels. Towards the end of first trimester, the sparse hypodermis can be distinguished from slightly denser, more fibrous matrix of dermis. In the second trimester mesenchymal derived preadipocytes begin to differentiate and accumulate lipids. By third trimester the more mature adipocytes are aggregated into large lobules of fat divided by fibrous septa. In addition to being the passive fuel reserves for the body, adipose tissue has been shown to have an active endocrine role (Poulos et al., 2010). An example is the gene that encodes leptin, whose abnormal regulation has been implicated in the pathogenesis of obesity.

2.3.3 Vernix caseosa

Vernix is a viscous, waxy, whitish material that coats the foetal skin surface during the last trimester. It is a mixture of water containing cells covered by a mixture of lipids (Pickens et al., 2000a, Rissmann et al., 2006). It first appears at 16-18 weeks postconceptional age over the eyebrows and over time, it covers the foetal skin surface, advancing from head to toe and back to front (Hoath et al., 2006, Marissen et al., 2023). Its role begins in the delivery room and forms the foundation for the ‘‘golden hour’’ concept of newborn resuscitation (Lamary et al., 2023).

2.3.3.1 Process of vernix formation

By week 11 the periderm starts to provide a temporary barrier suitable for aqueous in-utero environment with active transport mechanism between the amniotic fluid and the embryo. Periderm cells are replaced continuously and are mixed with sebum secretions from the sebaceous glands within the epithelial walls. It is within this combination that vernix caseosa formation occurs (Khonsary, 2023, Agorastos et al., 1988).

An endocrine-based mechanism for vernix production has been proposed (Narendran, 2002) based on the earlier work by Holbrook (Holbrook and Odland, 1980) who had shown that the epidermal barrier occurs first in the anatomical vicinity of the hair follicles (Hardman et al., 1999). Placental or hypothalamic corticotropic-releasing factors (CRF) may signal the pituitary gland to release adrenocorticotropic hormone (ACTH), causing the adrenal gland to release androgenic steroids (Figure 2-2). These androgens facilitate the release of lipid rich sebum from the sebaceous glands. Foetal cells from the hair follicles and infundibular part of sebaceous glands, mix with sebaceous lipids, extrude through the hair shaft, and continue to form and spread over the interfollicular epidermis during latter gestation (Hardman et al., 1998, Rissmann et al., 2006). Vernix lipids cover the hydrated vernix cells to create a hydrophobic coating during latter gestation, thereby protecting the underlying foetal epidermis from exposure to amniotic fluid (Youssef et al., 2001). Production of superficial lipid film (sebum) in the immediate vicinity of the hair follicle changes the trans epidermal water gradient, which facilitates cornification of the underlying epidermis. In both animal models and cultured human skin, control of the trans epidermal water gradient has been demonstrated to be pivotal in regulating stratum corneum formation (Akiba, 1955, Narendran et al., 2000, Visscher et al., 2005, Visscher and Narendran, 2014b).

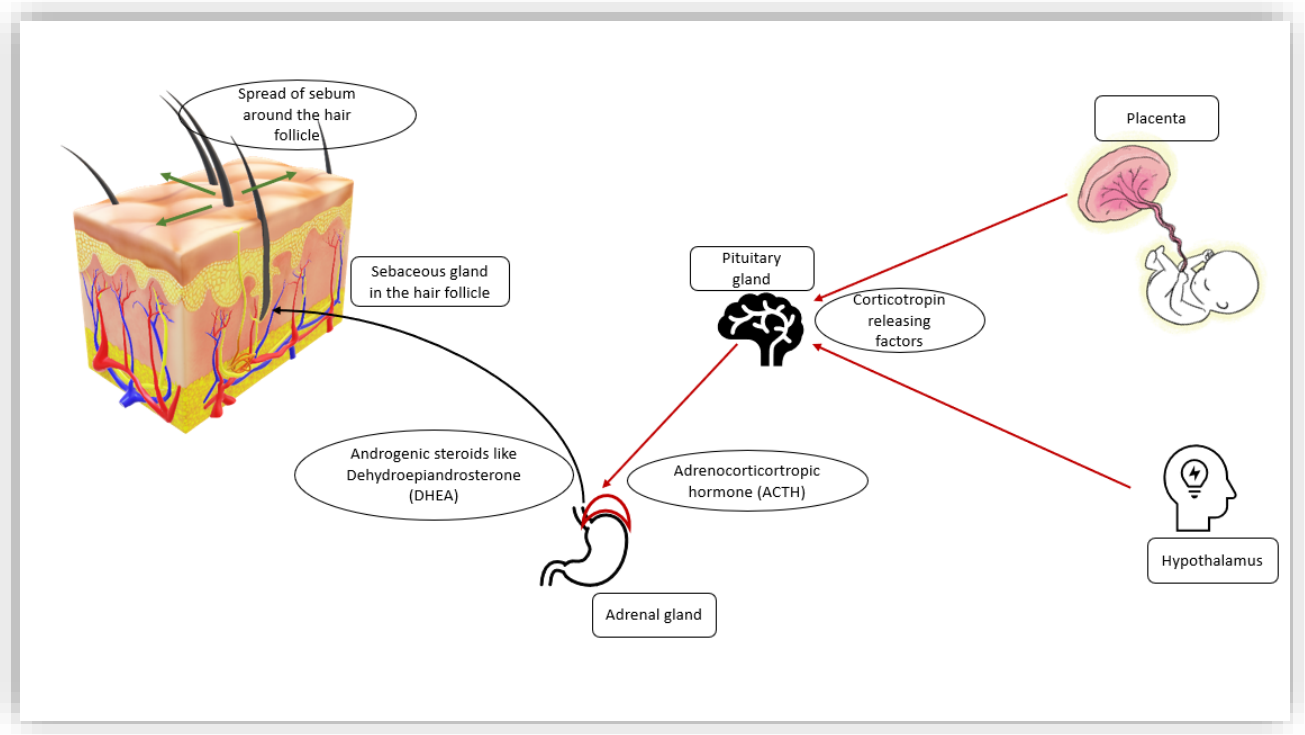


Figure 2-2 Hypothetical endocrine based theory for vernix production and epidermal barrier maturation (Narendran, 2002).

Enzymes required in this process, hydroxyl steroid dehydrogenases and 5 alpha reductase are present from 16 weeks (Zouboulis and Degitz, 2004). The glands reach a peak of activity in the third trimester and their secretion together with desquamated corneocytes into the overlying lipid matrix results in the formation of vernix.

2.3.3.2 Distribution of vernix

Observational studies in the past suggested no gender effect on vernix coverage and the coverage to be inversely related to birth weight, with maximum for infants less than 2000g as birth weight (Akiba, 1955). More recently amongst 430 babies (GA 33-42 weeks) the vernix coverage was noted to be higher for lower GA, those born via C-section, females and Caucasian infants and lower for those born following meconium exposure (Visscher et al., 2005). Coverage was significantly higher on the back than chest, indicating regional differences. The percent area covered was 72% for 24 infants 33-37 weeks, 38% for 372 babies 37-41 weeks and 12% for 34 infants 41-42 weeks. There was variable thickness of coverage in the whole group(Visscher et al., 2005). Vernix coats the foetus until birth (Elias, 1996).

Late in the second trimester and particularly in the third trimester, the increased physiological concentration of pulmonary surfactant emulsifies surface vernix (Holbrook, 1998). There is 'roll up' and detachment of vernix and consequent increase in amniotic fluid turbidity. The effect of pulmonary surfactant to emulsify and detach vernix from an inert plastic support under in vitro conditions has been demonstrated (Narendran et al., 2000) supporting the mechanism of increasing amniotic fluid turbidity secondary to surfactant-mediated detachment of skin surface vernix.

2.3.3.3 Constitution of vernix

Early work on vernix characterization focused largely on its lipid component (Nicolaides, 1971). More recently it has been established that vernix consists of water (81%), lipid (9%), and proteins (10%) (Hoeger, 2011). Vernix is highly viscous, as the water resides within a highly structured state as water-filled foetal corneocytes. These foetal corneocytes in utero act as "cellular sponges" to facilitate and maintain cornification by preventing water moving across the foetal skin, while the sebaceous lipids in vernix provide a hydrophobic barrier (Pickens et al., 2000a). The ultra-structural studies of vernix show hydrated corneocytes devoid of nuclei and other organelles with sparse network of keratin filaments, about 1-2 micrometres in thickness, lacking desmosomal connections, and surrounded by a thick layer of amorphous lipid without lamellae. The intercellular lipids contain unidentified inclusion bodies, presumably proteinaceous material of keratinocyte origin (Agorastos et al., 1988). This structure of vernix is described as "pasta and cheese" morphology with a "mobile" architecture.

Specific lipid composition of vernix is free lipids, cholesterol esters, wax esters, ceramides derived from stratum corneum, and sebaceous origin squalene, cholesterol, triglycerides, free fatty acids and phospholipids, besides others bound to cell envelope consisting of fatty acids ω-hydroxy acids and ω-hydroxy ceramides. The lipids in vernix are predominantly nonpolar lipids with cholesterol, ceramides and free fatty acids making 10% of lipids. In contrast cholesterol, ceramides and free fatty acids constitute 80% of lipids in stratum corneum (Rissmann et al., 2006).

Over two hundred proteins have been identified in vernix (Holm et al., 2014). The most abundant protein types were hydrolases (14%), proteases (11%), enzyme modulators (11%), cytoskeleton proteins (10%). A proteome analysis of vernix caseosa has shown the presence of potent antimicrobial polypeptides. 39% of identified vernix proteins are components of innate immunity and 29% have direct antimicrobial properties (Tollin et al., 2006). Origin of vernix proteins seems to be amniotic fluid, foetal lungs, blood contamination, dermal origins and activated keratinocytes.

Stratum corneum proteins keratin 1, 10, 11, involucrin and filaggrin were also found in vernix (Visscher et al., 2011, Narendran et al., 2010).

2.3.3.4 Functions of Vernix Caseosa

Vernix is a naturally occurring barrier cream with multiple salubrious effects, which support its retention on the skin surface at birth (Visscher et al., 2005). Research on vernix supports the World Health organisation's recommendations that vernix remain on the skin at birth and that bathing be delayed for six hours.

Vernix performs an epidermal barrier function in utero to facilitate epidermal growth underneath it and acts as a hydrophobic barrier against the amniotic fluid maceration and loss of fluids and electrolytes toTEWL (Visscher and Narendran, 2014b).Vernix also acts as a protective biofilm by minimizing friction of foetal parts during delivery and as an antimicrobial cover against the bacteriologically rich environment of the mother's genital tract along with the insulating effect on the foetus (Haubrich, 2003).

Despite modern methods of preterm nursing using incubators, temperature control during first few hours of life in low birth weight very preterm babies remains challenging since these infants have immature stratum corneum and high TEWL. While it has been observed that the hydrophobic layer of vernix is retained after birth till it separates in a natural way by day 5 on most of the skin and day 10 in the folds of skin (Saunders, 1948), the extreme preterm babies do not have the vernix cover unlike full term infants. Also, there is considerable debate on whether vernix has effect on body temperature regulation. Vernix retention had no significant effect on thermal regulation at birth in term infants in a control trial undertaken by Visscher et al, though additional studies are warranted to determine the temperature effects of vernix in younger premature babies (30-32 weeks) (Hoath et al., 2006) (Visscher et al., 2005, Pickens et al., 2000a) .

The vernix within the amniotic fluid when swallowed by the foetus has potential effects on the developing gut and prepares the intestine for ex-utero feeding. Amino acid glutamine and asparagine in the vernix were considered as trophic factors for the developing gut however despite encouraging in vitro and animal model studies, clinical trials do not support their use as intestinal supplements Branched chain amino acids are rare in internal human tissues but are present in high concentrations in skin, vernix caseosa, meconium and in the intestinal tract of full term newborns (Ran-Ressler et al., 2008). They are also major components of bacterial membranes across many genera. Experiments using premature rat pups fed on diets containing branched chain fatty acids showed decreased occurrence of necrotising enterocolitis and microbial flora were altered in this group versus placebo control suggesting protective role of vernix (Ran-Ressler

et al., 2011). Further human trials are needed to explore the role of various amino acids in vernix which affect gut epithelial maturation.

Skin surface adaptation

Vernix films are non-occlusive and permit water vapor transport through them (Tansirikongkol et al., 2007a) In-utero, cornification of the foetal epidermis is incomplete thereby permitting a high water flux potential driven by osmotic gradients. Vernix may serve as a semi-regulated barrier and/or physiological gradient for trans epidermal water and nutrients in utero. This process, in turn, prompts epidermal cornification.

Newborn infants undergo progressive adaptation immediately after birth, including slow reduction in surface hydration, decrease in skin pH and stratum corneum dehydration/desquamation with formation of a dry skin surface. Vernix may have a role in modulating these processes as evidenced by some studies(Visscher et al., 1999) (Hoeger, 2011). Vernix has been noted to lose its exogenous water slowly (Pickens et al., 2000a) and vernix retention after birth has been shown to result in significantly more hydrated skin surface via water binding free amino acids (Visscher et al., 2005) (Visscher et al., 2011). Regional variations have been noted in skin hydration and formation of dry skin surface, like variation in vernix distribution.

The decrease in pH following birth has been attributed to maturation of enzymes responsible for the synthesis of acidic components (Hoeger and Enzmann, 2002) and triglycerides in vernix could be a source of acidic fatty acids. The skin surface acidification appears to occur earlier in the presence of vernix retention (Visscher et al., 2005). An acidic stratum corneum has been shown to inhibit the growth of pathogenic bacteria (Fluhr and Darlenski, 2018) and facilitate colonization with commensal organisms on the skin surface.

Vernix is said to have antioxidant properties by virtue of the presence of antioxidants vitamin E and melanin in it (Pickens et al., 2000b, Youssef et al., 2001). As birth marks a time of high oxidative stress, the antioxidant properties of vernix may help in coping with the pro-oxidant environment as suggested by a decrease in vitamin E levels in vernix on exposure to ultraviolet light (pro-oxidative stressor) (Thiele et al., 1998, Visscher et al., 2005)

In-vitro studies have described mechanical barrier properties of vernix with respect to bacterial invasion (Joglekar, 1980). Vernix has also been shown to effectively block penetration of exogenous chymotrypsin present in the amniotic fluid from meconium contamination, while retaining endogenous(epidermal) chymotrypsin (Tansirikongkol et al., 2007b). Also, it has been shown that vernix, contains antimicrobial peptides and is active against candida albicans and effective against growth of group B streptococcus, listeria monocytogenes and klebsiella pneumoniae(Tollin et al., 2006, Akinbi et al., 2004).

Vernix is also associated with surfactant-associated protein A and surfactant-associated protein D implicated in maintenance of airway bacterial homeostasis and also against intra-uterine infection (Narendran et al., 2000). Lysozyme and lactoferrin are the other innate immune proteins present in vernix (Agorastos et al., 1988) .

Because of its high-water content, vernix acts as an agent to moisturize the stratum corneum. Comparison with various barrier creams like petrolatum, Aquaphor, and Eucerin, has shown vernix to be having higher water content (Narendran, 2002). Application of vernix to adult volar forearm results in an increased capacity to bind exogenous water (Bautista et al., 2000).

Vernix contains filament aggregating protein (filaggrin), which when broken down forms water-binding Natural Moisturizing Factor (NMF), which operates to maintain suppleness and plasticity of stratum corneum. Further research and innovation could enable vernix caseosa to be used effectively as a natural emollient, with all its naturally endowed properties (Singh and Archana, 2008).

The regulation of trans epidermal water gradient is known to be important in the epidermal barrier formation and regeneration following wounding. These factors may account for healing properties of vernix in treating perineal wounds following delivery(Narendran, 2002) It was hoped that it may also be used in atopic dermatitis against bacterial skin infections (Roos et al., 2004). Similarly, a superficial layer of vernix has been investigated for grafting of burn areas over laboratory-cultured skin surfaces (Haubrich, 2003).

Premature infants less than 28 weeks lack the coverage of vernix and have underdeveloped SC. The TEWL in these babies is consistent with wounded skin surface (Hammarlund and Sedin, 1980). Attempts to improve barrier function with petrolatum and oils have resulted in increased hospital acquired infections and delayed barrier repair. Damaged stratum corneum treated with semipermeable films recovers more quickly than under complete occlusion or no occlusion, as reported after tape stripping in premature infants. Vernix films are semipermeable and allow passage of water vapour with transmission rates depending on thickness of the film (Tansirikongkol et al., 2007a). Therefore, vernix films may promote SC development in premature infants and repair damaged skin (Visscher and Narendran, 2014b).

In addition, experiments performed using human skin soiled with carbon particles, vernix had comparable efficacy to standard commercial skin cleansers (Moraille et al., 2005). And unlike commercial soaps, it could provide physiologically relevant lipids to the skin surface which are compatible with cutaneous lipids and follicular pores.

2.3.4 Skin Function

Functionally, the highly organised skin is designed to permit gas and fluid transport across its surface and, critically, maintain the internal body homeostasis, via the sweat glands and blood vessels (Knobel-Dail, 2014, Charkoudian, 2003). Other functions include protection of underlying tissues and organs (Zimmerman et al., 2014), immunity and synthesis of vitamin D.

Many barrier functions attributed to the epidermis are localised in the stratum corneum, especially providing a physical barrier against invasion by foreign bodies and mechanical trauma. Stratum corneum also has an innate immune function, with antimicrobial peptides contained within the lipid matrix (Baroni et al., 2012, Elias, 2007). The stratum corneum acts as a permeability barrier, allowing an appropriate amount of trans epidermal water loss (TEWL) and protecting the body from death due to excessive water loss (Rutter, 2003). Corneocytes act as a barrier to electromagnetic radiation. Both antimicrobial function and permeability of the epidermis are regulated by the slightly acidic pH of the stratum corneum 'acid mantle'. The main mechanisms used by the stratum corneum to preserve skin hydration are the intercellular lamellar lipids, the desmosome-bound hydrophobic corneocytes, and the Natural Moisturising Factor NMF (Rawlings and Matts, 2005). The role of desmosomes and tight junctions that seal cells together in the granular layer have also been described as contributors to skin barrier function. Melanosomes (contain melanin) synthesized by melanocytes protect the skin from ultraviolet radiation (Lai-Cheong and McGrath, 2017).

The fat under the skin acts as an insulator to prevent heat loss; however, the more premature an infant is born, the less fat insulation there will be. Brown adipose tissue is involved in non-shivering thermogenesis when the body temperature falls below a threshold value and serves as cushioning and protection for vital body parts such as major blood vessels and mediastinal structures (Evans et al., 2007).

These functional roles can be compromised by the external environment where the skin is exposed to a range of insults, which may be mechanical, physical, biological, and chemical in nature (Baroni et al., 2012). As an example, mechanical trauma occurs when the skin is exposed to high mechanical loads applied over a short time (< 10 s), e.g., adhesive tape removal. By contrast, the skin can be exposed to sustained mechanical loads, for example in individuals who are relatively immobile and bedridden or function in chairs for much of their waking day as adults or continuous pressure of a medical device e.g., CPAP.

Immune function of neonatal skin

At birth, infants dramatically transition from aqueous conditions to a dry gaseous environment. The epidermal barrier begins to change within hours, exhibiting decreased hydration and low stratum corneum (SC) cohesion. The SC varies by gestational age (GA), transforms over the first 2–3 months, and differs considerably from the stable adult skin, as indicated by analysis of specific protein biomarkers (Narendran et al., 2010). Regardless of gestational age, the increased infant SC proteins at 2–3 months after birth are involved in late differentiation, cornification, and filaggrin processing (Visscher et al., 2022). Additionally, the natural moisturizing factor (NMF), the product of filaggrin processing, was higher for infants than adults. This suggests that neonatal skin responds to environmental stresses and provides innate immunity and protection while promoting rapid, continued barrier development after birth.

2.4 Context: How does structure and function of skin in neonates differ?

Although the skin of healthy term neonates is structurally complete, its functional integrity remains incomplete, and the skin continues to thicken over the first years of life (Fluhr et al., 2010). Like other organs in the body, neonatal skin undergoes substantial adaptations as the infant transfers from a water-based alkaline environment (i.e., amniotic fluid) to a relatively dry gaseous environment (Fluhr et al., 2012). Preterm skin characteristics, in particular the limited barrier function, inevitably influences its ability to control body temperature and water balance (Taieb, 2018). Although skin care has been recognized as a key element of neonatal practice, limited research has been conducted, and clinical management is still commonly based on tradition, experience, and cultural factors.

Research has revealed that the skin barrier begins to function in utero, at 24 weeks gestation (Dietel, 1978), although this skin still appears red, wrinkled, shiny, and transparent (Table 2-4). It is proposed that the preterm neonate has decreased epidermal and stratum corneum thicknesses compared to an adult (Oranges et al., 2015). However, this is a point of some discussion. While some authors have observed that full term babies studied within first 3 months of age have a well-developed epidermis with epidermal and stratum corneum thickness like adult skin (Fairley and Rasmussen, 1983), others have observed that the infant epidermis is thinner compared with an adult (Stamatas et al., 2010). Children aged between 3 and 24 months of age have SC that is, on average, 30% thinner than that of adults, and the other strata of the epidermis are on average 20% thinner (Stamatas et al., 2010). More specifically, in vivo studies using confocal microscopy demonstrated that infant skin appears to have thinner epidermis and stratum corneum; and corneocytes are smaller in size (Stamatas et al., 2011). In addition, Rutter et al suggest that the newborn skin is susceptible to shear forces due to weakly undulated dermo-epidermal junction with flat rete ridges (Evans and Rutter, 1986) (Table 2-4).

An infant loses heat through its skin to the environment through radiation, conduction, convection, and evaporation. Though examination at autopsy at 29 or 30 weeks has demonstrated that eccrine sweat glands are coiled to such an extent that multiple transverse sections can be seen within the depth of the dermis (Ersch, 1999), sweating is not a thermoregulatory method in preterm neonates. In fact, when investigating thermal loss, babies born at less than 36 weeks' gestation did not sweat in similar environmental conditions where term babies did. However, postnatal existence hastens the development of sweating, so that by 13 days of age all babies studied were able to sweat (Harpin and Rutter, 1982b).

The fat under the skin acts as an insulator to prevent heat loss. However, the more premature an infant is, the less fat insulation there will be (Knobel-Dail, 2014). The incidence of hypothermia (temperatures less than 36.5°C) has significantly reduced when plastic bags were introduced at birth (McCall et al., 2010). This intervention was first published in early 1970s (Besch et al., 1971), however not brought into routine clinical practice immediately. These studies indirectly indicate the lack of thermoregulatory barrier function of skin, in absence of which the plastic bag serves the purpose. BAPM has endorsed the use of plastic bags (BAPM, 2019).

2.4.1 Structural differences

The differences in skin structure have been studied for more than 50 years and more recently new techniques like imaging have been used to investigate the structure of skin. Table 2-4 compares the characteristics of skin from previable gestational age (<20 weeks) to term infant and further into adulthood. With increasing gestational age, the acid mantle matures (Fluhr and Darlenski, 2018) and vernix starts to appear.in the perifollicular region initially before it covers the rest of the skin (Visscher et al., 2011). Stratum corneum starts to appear only when the foetus is closer to the viable age. The SC continues to become thicker into adulthood when it is 25-30 cell layer thick (Lai-Cheong and McGrath, 2017). Epidermal thickness is only measured in cell counts in newborn period and is measurable in metric units in adult skin (Sandby-Møller et al., 2003). Keratin continues to be loosely woven in early life (Goto et al., 2020). Hair follicles start to appear in the dermis in early life (Smith et al., 1982) and hair shafts appear later in life. Dermal thickness has been measured in adult skin with some certainty using ultrasonic techniques (Wang et al., 2023). However, the estimation of dermal thickness has been challenging when it was attempted histologically in the postmortem period due to distortion of tissues Similarly, there is lack of research in connective tissue content of dermis in early life.

Table 2-4 Characteristics of adult, term, and preterm skin

Skin \ GA		≤20week	21-29 week	30-40 week	Term Infant	Adult
Acid Mantle		-	Delayed	Delayed	Recovers	present
vernix			Starts to appear in perifollicular skin	Present	Present on the skin and in amniotic fluid	-
Epidermis	SC	Absent	Present	Present	Present	25–30 layers
	Keratin		Basket weave around hair follicles	Basket-weave keratin more generalized	Generalized basket-weave keratin	Compact
	Epidermal thickness	—	1–2 cell layers	2–4 cell layers	3–5 cell layers	Histology: 0.07-0.12 mm (Sandby-Møller et al., 2003)
			HFUSI, 0.172 mm (Vitral et al., 2018)			
Dermis	Rete ridges	Absent	Absent	Present	Present	Present
	Eccrine gland		Present	Present	Functional	Functional
	Hair follicle	Present	Present			
	Hair shaft	Absent	Absent		Small	Present
	Dermal thickness	Not available	Histology: ↓ collagen and elastic fibres 0.28-1.1mm (de-Souza et al., 2019, Reed et al., 2021)			Average 1 mm varies from 0.3 mm (eyelid) to 3.0 mm (back) (Wang et al., 2023)
			HFUSI: 0.68-1.24 mm (Paulina Przybysz, 2020, Vitral et al., 2018)			
Subcutaneous	Connective tissue	Research lacking	-	Present	Present	Present
	Fat	Minimal to none	Minimal; scattered fat globules	Minimal; increase in fat globules	Present	Men:1.65–18.35 mm Women: 2.7–25.2 mm (Seidenari et al., 2000, Hoffmann et al., 1994)

2.4.1.1 Measurement of dermal depth

Depth of skin in general and dermis in particular is an important measure to understand the maturity and function of skin. Histology continues to be the gold standard however imaging techniques have been extremely beneficial and demonstrate numerous advantages.

2.4.1.1.1 Histology

Historically, skin thickness was determined from biopsy or autopsy samples. Marcos-Garcés et al obtained microphotographs of abdominal skin samples at autopsy in a wide range of age groups. They reported dermal thickness at 1 month of age using software for image analysis as 1.666 mm [Papillary dermis (0.063 mm) + Reticular dermis (1.603 mm) (Marcos-Garcés et al., 2014)]. Both these measurements were statistically significantly different when compared to the corresponding measurements in adults (p value <0.05). Another post-mortem morphometric analysis of skin has been conducted by de Souza et al (de Souza et al., 2021). In this study samples were obtained from still born babies and neonatal deaths within 48 hours of death (GA 20-41 weeks) and epidermal thickness in periumbilical area was 28.1 - 99.5 µm and dermal thickness was 0.28-1.10 mm. Reed et al examined 48 skin samples postmortem, from live born preterm infants (18-36 weeks at birth) and equally divided them into 3 groups by survival to <1 day, 1-7 days and >7 days. Rete ridges were present from 30-week GA (Reed et al., 2021). Hair follicles were present in all, but hair shafts were only present from 21-22 weeks (Reed et al., 2021). The dermal thickness was reported to be 700 µm in preterm babies at corrected GA 37-43 weeks which was significantly different from dermal thickness of 1200 µm in term babies.

To date there have been no in-vivo histological studies of dermal thickness in term or preterm babies for obvious reasons. A recent systematic review also showed that there is lack of evidence on skin thickness dimensions obtained by histology in preterm babies (de-Souza et al., 2019). It has also been noted that several studies report discrepancies between the in-vivo and in-vitro measurements due to the artefacts arising from handling of the histological samples (Hoeger and Enzmann, 2002, Stamatas et al., 2010). Hence alternative methods of measuring skin thickness need to be developed.

2.4.1.1.2 Imaging

Confocal laser scanning microscopy was used by Stamatas et al to demonstrate that the border between papillary and reticular dermis observed in adult skin, is absent in 3–24-month-

old infants (Stamatas et al., 2011). Like other observations in the newborn period researchers (Holbrook, 1982, Paulina Przybysz, 2020) have revealed absence of clearly defined dermo-epidermal junction.

Ultrasound of skin is especially attractive for use in children as it is non-invasive. High-frequency ultrasound (HFUS) is a precise and validated method for measurement of skin (Tan et al., 1982, Alexander and Miller, 1979), and it has been used in adults (Alexander and Miller, 1979), and children (Seidenari et al., 1994) in various anatomical locations. It has also been used to determine the subcutaneous thickness (Fanelli and Kuczmarski, 1984) in a reproducible manner and accurately.

There are only two studies reporting dermal thickness using HFUS in newborn babies. Paulina et al used a 40MHz Derma Scan on 72 healthy 37–41-week babies, scanned at 6-24 hours of age (Paulina Przybysz, 2020). The median thickness of dermis measured on the thigh has been reported to be 0.679 mm. Vitral et al conducted HFUS using a 20MHz probe in 222 newborn babies within the first day of life, of which 68 were preterm (13 babies were 25-28 weeks). Median dermal thickness in forearm and sole was 0.97mm and 1.24 mm respectively (Vitral et al., 2018). Interestingly, the dermal thickness was not published for GA group categories, neither could they be obtained by direct communication with the authors.

Table 2-5 Dermal thickness measurement in newborn babies

Microphotographs at Autopsy	Age	Median Dermis thickness mm	References
Skin from abdomen	1 month	1.666	(Marcos-Garcés et al., 2014)
Periumbilical skin, still born and neonatal deaths	20-41 weeks	0.28-1.10	(de-Souza et al., 2019)
Live born 18-36 weeks	Corrected GA 37-43 weeks	0.7	(Reed et al., 2021)
	Term <7d	1.2	
In-vivo HFUS in well babies			
37-41 weeks, thigh	6-24h	0.679	(Paulina Przybysz, 2020)
Term and preterm Forearm	1 day	0.97	(Vitral et al., 2018)
Sole		1.24	

The interpretation of dermal thickness also varies in different studies. While some studies of children [(>2 years old) (Seidenari et al., 2000) and (0-12years)(Ni et al., 2016)] attribute the

differences in skin thickness to differences in dermal thickness and collagen content, others have attributed dermal thickness to skin oedema (Hughes-Formella et al., 2019). However, there is consensus about using B-mode scan. Seidenari et al have compared facial skin thickness by HFUS in various studies and concluded unequivocally that a cross-sectional B-scan allows better delineation of interfaces between various skin layers, allowing more reliable evaluation of skin thickness (Seidenari, 1999). The analysis in the current study will be conducted using B-mode pictures.

In summary, dermal thickness measurement using HFUS in B-scan mode will provide a unique dataset for such measurements in preterm babies. Chronological changes in dermal thickness in the neonatal period for term or preterm babies has also not been found in literature.

2.4.1.2 HFUS-Measurement of dermal echogenicity

Collagen is an echogenic marker protein, synthesized by dermal fibroblasts, which can be identified by high-frequency ultrasound (Seidenari et al., 2000). The changes in the extracellular matrix, contribute to the variations of the dermal density and echogenicity. Numerous studies have estimated the dermal water content using high frequency ultrasound scan by means of computer software that counts the number of low echogenic pixels (LEP) in the dermis (Gniadecka and Jemec, 1998, Seidenari et al., 1991).

Dermal echogenicity using High-frequency ultrasound has been considered as a non-invasive proxy tool for "histological" composition of dermis, but histology has been reported to remain the gold standard for the study of the skin (Crisan et al., 2012). An in-vivo study using skin echogenicity (Gniadecka, 2001) proposed use of ultrasound images as a non-invasive method of assessment of photo ageing and compared their own work with histological evidence of reduced collagen deposition by fibroblasts (Uitto, 1986). Amongst children decreased dermal thickness was noted on HFUS image in undernourished infants (Hughes-Formella et al., 2019). A previous study on similar population noted dermal oedema (Thavaraj and Sesikeran, 1989) on histological examination. Reduced collagen content was reported on histopathological reports by another group (Vasantha, 1970). Dermal echogenicity on imaging was unfortunately not reported by Hughes-Formella et al. Echogenicity of dermis has been studied in children in other studies as well, however there are no studies reporting echogenicity of dermis on newborn babies.

The only neonatal study of skin characteristics using HFUS, has not included dermal echogenicity measurements in the results as published to date (Paulina Przybysz, 2020). Paulina et al reported that the morphology of the dermis was found to be homogeneous

throughout the entire dermal thickness in all examined sites. Similar findings were noted in confocal laser scanning microscopy (Stamatas et al., 2011)), which is different to adults (Sandby-Møller and Wulf, 2004, Barcaui and et al., 2015) who are noted to have variable echogenicity in the dermis. Barcaui et al noted that lowest echogenicity is found in newborns, and it begins to rise in children aged a few months. Other authors have found that thickness of the epidermis and dermis, as well as the dermal density are important parameters that assess the cutaneous regeneration process (Seidenari, 1999, de Rigal et al., 1989). Gniadecka et al used pixel intensity to identify dermal collagen when quantifying cutaneous senescence (Gniadecka, 2001).

2.4.2 Functional differences

The functions of skin have been discussed in 2.3.4. The differences in skin functions in preterm and term babies in comparison to adults is valuable to consider in order to understand the gaps in knowledge.

2.4.2.1 pH

The skin pH of 6.6-7.5 on day one of life in term neonates (Yosipovitch et al., 2000, Fluhr et al., 2012) reaches a value of 5.1 by 5-6 weeks of age when it is comparable with the “acid mantle” described in adults, with a skin pH of between 4.5-6.0 (Giusti et al., 2001). Since SC pH regulates permeability, antimicrobial function, and the rate of barrier repair following injury (Mauro et al., 1998), the skin barrier cannot be said to be functionally complete in newborn babies, and more so in preterm infants (Marissen et al., 2023). Identical colonising strains of bacteria in preterm infants may show different growth rates by site e.g. in oral cavity and skin (Olm et al., 2017) and site-specific role of variable skin pH has been considered as a cause (Marissen et al., 2023).

2.4.2.2 TEWL

In addition to differences in thickness of dermis as noted in section 2.3.1 and in Table 2-4, there are some functional differences between the skin of healthy term neonates and that of adults. There are rapid changes in TEWL in the hours and days following birth (Hammarlund and Sedin, 1980, Yosipovitch et al., 2000). Immediately following birth, evaporative water loss is extremely high (Hammarlund and Sedin, 1980), but it reduces

to a more moderate level by 1 hour of life. Yosipovitch and colleagues found that TEWL was lower in healthy term neonates on day 1 of life than in adults, when measurements were taken between 5- and 10- hours post-birth (Yosipovitch et al., 2000). Other studies have reported average TEWL in healthy term neonates comparable to that of adults (Harpin and Rutter, 1983, Fluhr et al., 2012). Taken together, these data suggest a period of rapid adaptation following birth as the neonate adapts to a cold, dry, gaseous environment. The permeability barrier of the skin seems to be competent once this adaptation has occurred (Fluhr et al., 2012, Fluhr et al., 2000).

The differences in skin barrier function in preterm neonates, especially the timeline of skin maturation following extremely and very preterm birth, have yet to be fully elucidated. Neonates born extremely prematurely have impaired barrier function compared to term neonates, reflected in an inverse correlation between TEWL and GA at birth (Hammarlund and Sedin, 1979, Harpin and Rutter, 1983, Kalia et al., 1998). This is likely due to structural differences in underdeveloped skin. For example, some areas of the skin are not well-keratinised until approximately 29 weeks' gestational age (Hardman et al., 1998). It has been established histologically that the epidermis of the most immature infants resembles that of a term infant by 2 weeks of age (Evans and Rutter, 1986). Similarly, dermo-epidermal undulations are not visible until approximately 34 weeks (Evans and Rutter, 1986), suggesting that the skin of very premature neonates is also vulnerable to shearing forces.

2.4.2.3 Sweating

Sweating from eccrine glands occurs because of two mechanisms. Thermal sweating occurs due to rise in environmental temperature. Emotional sweating occurs following physiological arousal such as stress, fear or pain, and also tactile stimulation (Eriksson et al., 2008). While emotional sweating occurs mainly on the palms and soles of infants, thermally challenged activity of the sweat glands is first observable on the forehead and then at other skin sites such as the chest and upper arm (Harpin and Rutter, 1982b, Harpin and Rutter, 1982a).

Most babies greater than 36 weeks gestational age can sweat in response to raised ambient temperature from the first day after birth. Although even the most immature infants develop the ability to sweat in response to heat by 2 weeks of age, the efficiency of sweating as a thermoregulatory process is poor (Harpin and Rutter, 1982b). The inability of the newborn to regulate body temperature at the extremes of cold or heat is a cardinal feature distinguishing the immature infant from older children and adults.

Emotional sweating was initially shown to develop by 36 weeks corrected gestational age (Harpin and Rutter, 1982a). Development of a more sensitive tool (measurement of skin conductance in response to nociceptive and tactile stimuli) revealed that emotional sweating is present in preterm infants from at least 29 weeks of GA (Storm, 2000). No gender influence on the eccrine sweating was reported. Study of eccrine sweating is of importance for understanding basic skin physiology, despite lack of known relevance for significant disease process.

2.4.2.4 Inflammation

Inflammatory cytokines have been studied more recently in neonatal sepsis (Eschborn and Weitkamp, 2019). Preterm infants have a remarkably different system of immune regulation as compared with term infants and adults. There is overexpression of proinflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α) measured in blood which play an important part in Sustained Inflammation (SI) (Leviton et al., 2011). SI has been put forward as an important indicator of neonatal mortality in these babies (Humberg et al., 2020). Narendran et al have published spectrophotometric biomarker analysis (IL-1α, IL-1β, IL-6, IL-8, TNF-α, MCF) of forehead tape stripping with significantly raised IL1α, IL1β, IL6 and IL8 in <32-week preterm babies than adults (Narendran et al., 2010). Interestingly skin surface cortisol has been noted to be significantly raised in preterm babies than full term infants and adults. However, a clear understanding of the role of cytokines in skin barrier function in this age group is still unclear.

2.4.3 Adaptation of skin after birth

Processes in the immature skin that help to adapt it to the outside world include an accelerated cornification process of the SC. In term infants, the excess outermost layers of SC are shed in the first week of life (desquamation) along with a rapid epidermal turnover, reduction of water loss, alterations in skin pH, such as an increase in acidity, as well as changes in sebaceous activity. In preterm infants, however, the desquamation process is enhanced due to the lack of protease inhibitors and enzyme regulators as compared to term infants and adults (Visscher et al., 2021).

The surface pH in preterm infants is >6.0 and acidification is further delayed by the incubator humidity required to prevent water loss in the first week of life. The lack of “acid mantle” increases the risk for invasion of pathogens and may also play a role in wound healing as

recovery rates after adhesive skin injury (via tape stripping) seem to be faster with an acidic skin pH of 5.5 as compared to pH 7.4 (Mauro et al., 1998).

Filaggrin processing products which are needed to maintain skin moisture and inhibition of pathogens are less expressed in preterm infants at birth compared to term infants but levels catch up at 3 months of chronological age (Hoath, 2003). Moreover, cohesion between epidermis and dermis is reduced due to a smaller number of fibres and a wider interspace between fibres as compared to term infants. If the SC basal cell layer that generates the epidermis is damaged, healing results in wound contraction without formation of granulation tissue and significant scar formation (Hoath, 2003).

Immature skin has a higher expression of channel proteins aquaporin than mature skin in locations where they influence water transport and hydration. This might contribute to the increased TEWL (Marchini et al., 2003). Preterm infants also carry higher risk for transdermal absorption of drugs and chemicals. This has historically let to accidental poisoning resulting in methemoglobinemia after application of aniline dye to the diaper (Kagan et al., 1949), hypothyroidism from use of iodine containing antiseptics (Sammons and Choonara, 2016) and high systemic levels of theophylline after transdermal application (Cartwright et al., 1990).

A period of environmental adaptation to a terrestrial as opposed to the wet intrauterine environment is required in newborn babies (Fluhr et al., 2012). During this period of maturation, the dysfunctional skin physiology, including increased trans epidermal water loss (TEWL) (Sedin et al., 1985), alkaline pH (Yosipovitch et al., 2000, Fluhr et al., 2012), invasion of micro-organisms and absorption of potential toxins from topically applied products increase the vulnerability of these fragile babies. However, barrier development increases with gestational age, and the epidermal maturation is reported to be complete by 34 weeks of age (Hammarlund et al., 1982).

2.5 Biophysical measurement of neonatal skin structure

TEWL (Transepidermal water loss) and pH are the most common measurements to assess skin integrity. Skin structure has been assessed histologically in the past however a variety of imaging techniques are used lately. High frequency ultrasound is a relatively new technique for assessment of skin structure.

2.5.1 TEWL

Trans epidermal water loss (TEWL) is the loss of water from inside the body through the epidermal layers to the surrounding atmosphere. It is also called insensible water loss (IWL) as it is a process over which the organism has very little physiologic control. TEWL is regarded as one of the most important and most widely used objective parameter used for measurement of the integrity of the skin barrier function (Fluhr et al., 2006). Historically, the first approach to measurement of TEWL was described in 1911 (Kottner J, 2017). Rutter and Hammarlund used this tool extensively to describe the characteristics of neonatal and preterm skin over 3 decades ago (Harpin and Rutter, 1983, Hammarlund et al., 1977, Hammarlund et al., 1979, Hammarlund and Sedin, 1979, Hammarlund and Sedin, 1980, Hammarlund et al., 1980). And more recently, this measure has been regarded as the standard in variety of dermatological and skin research contexts (Steiner et al., 2011). There are three primary physiological processes involved in the measurement of TEWL:

1. Diffusion: Diffusion is a passive mode of transport of particles, because of their random thermal motions. There are more molecules in the region of high concentration, therefore there will be a net migration of molecules from high to low concentration until the concentration in both regions are the same. There are no driving forces in pure diffusion.
2. Evaporation: Evaporation is the process of turning from liquid into vapour. Some degree of evaporation is necessary for the measurement of TEWL, because the measurement takes place in the air above the skin. However, TEWL does not stop when the skin is occluded, in fact occlusion of skin is known to damage its barrier properties (Zhai and Maibach, 2002).
3. Osmosis: Osmosis can be a special case of diffusion in which diffusion occurs across a semipermeable membrane and only the water or other solvent moves. The mechanisms involved in trans-epidermal water loss are traditionally called diffusion.

TEWL provides a measure of the skin barrier property because the TEWL water must diffuse through this barrier, from the high concentration region inside the body to the low concentration region at the surface. TEWL does not just measure the volume of water. TEWL is a flux density, i.e., a quantity of water per unit area per unit time. Flux density is a vector quantity that has both magnitude and direction. The magnitude *J* is defined as below.

$$J = \frac{Mass\ of\ Water}{Area \times Time}$$

and its direction is perpendicular to the area under consideration. The *SI* units of *J* are kg/m^2/s, i.e., kilograms of water per square meter of skin per second. These units are useful for mathematical modelling, but they give numbers that are awkwardly small for practical use. For this reason, the practical units of g/m^2/h, i.e., grams of water per square meter per hour are widely used.

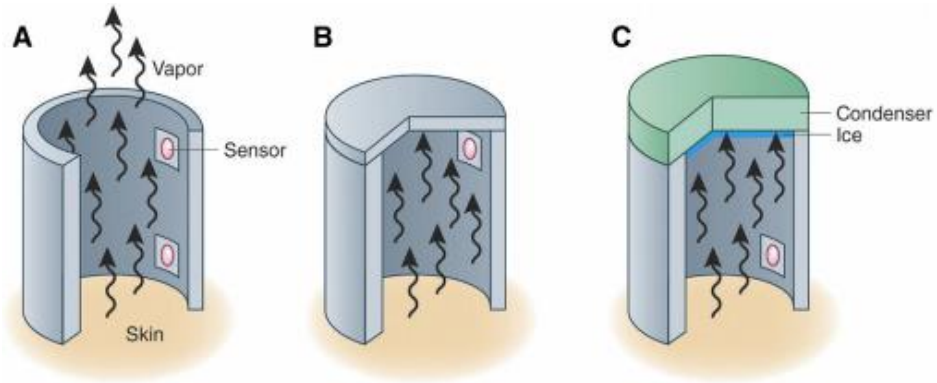


Figure 2-3 TEWL devices: Open Chamber (A), Closed chamber (B, C)

The vapour flux over a defined area of the skin surface can be measured using a variety of instruments which broadly use the Open chamber and the Closed chamber methods. They have been described further in Table 2-5.

While some studies report good correlation between different open- and closed-chamber TEWL devices in both in vivo and in vitro studies (Fluhr et al., 2006) and others report good reproducibility over a wide range of evaporation rates (Levin and Maibach, 2005). However, there is disagreement in sensitivity of the open chamber and closed chamber devices.

Both the open and closed chamber devices have their strengths for different applications, but their results cannot be directly compared (Steiner et al., 2011). Also, it was not possible to find a statistical model that would allow to transform the measurements made on one device for a comparison with the results generated by the other device (Steiner et al., 2011). The “open chamber” measurement method is the only method to assess the TEWL continuously without

influencing its micro-environment. The criticisms on the traditional open system are related to effects of ambient and body-induced airflows near the probe, probe size, the limitation in measurement sites and probe angles during application.

With careful consideration to ambient conditions (avoiding air flow in the close vicinity of the open chamber, holding the open chamber perpendicular to the skin) over and above the controlled ambient conditions needed for TEWL measurement anyway (stable ambient temperature and humidity, stable body temperature), open chamber system has been widely used in numerous studies including those on newborn babies (Sedin et al., 1985, Kelleher et al., 2013). In addition, two commercially available open chamber devices, Tewa meter and Evaporimeter have been compared in vivo and in vitro and demonstrated to have a high correlation (correlation coefficient r= 0.97) across wide range of TEWL values (Barel and Clarys, 1995) demonstrating between device reliability of measurements when using these devices.

Table 2-6 Comparative table of Open and Closed chamber method of TEWL measurement

Characteristic	Open chamber	Closed chamber
Physical principle	The evaporation flux passes through the chamber, into the ambient air. TEWL is determined from evaporation flux in relation to time measured within 10mm thick physiologic water vapour mantel.	The evaporation flux is trapped in a closed chamber. TEWL is determined from timed rate of humidity increase in immediate vicinity of skin surface.
Instrument	Hollow cylinder containing two pairs of sensors for relative humidity and temperature placed 3mm and 6 mm above the skin and a microprocessor	Closed chamber with a humidity sensor and a microprocessor
Measuring time	Stabilisation time followed by continuous measurements	Single measurement after 7-12s
Advanta-ges	Only method to provide continuous measurements	Varied measurement sites and probe angles possible
Disadvanta-ges	Strict control of ambient conditions is required to avoid airflow near the probe. Limitation in measurement sites and probe angles during application	Instrument needs rest for at least 20s between the measurements to allow the elevated RH and temperature inside the closed chamber to equilibrate with the ambient conditions. Accumulation of water vapour inside the chamber
Refere-nces	(Levin and Maibach, 2005, Steiner et al., 2011, Rogiers, 2001, Rogiers, 1995, De Paepe et al., 2005, Pinnagoda et al., 1990)	(Levin and Maibach, 2005)
Good correlation	(Taylor, 1953, Rogiers, 2001, Alanen et al., 2003, Barel and Clarys, 1995)	(Barel and Clarys, 1995, Taylor, 1953)

2.5.2 Skin pH

pH is a quantitative measure of the acidity or basicity of aqueous or other liquid solutions. The concept of pH was introduced in late 19th century by Heuss (Sorensen, 1909). Most of the initial interest was in the agriculture. Though the first scientific study relating to skin surface pH appears to have been carried out by Schade and Marchionini in 1928, who called it the "acid mantle" (Chikakane and Takahashi, 1995), the earliest studies on neonatal skin pH have only been published in 1950s (Behrendt and Green, 1958). More extensive research studies of neonatal skin pH have been published (Lund et al., 1999, Behrendt and Green, 1958, Yosipovitch et al., 2000, Hoeger and Enzmann, 2002) including some others for preterm babies (Fox and Rutter, 1998, Kanti et al., 2014, Visscher et al., 2020). A more recent review suggested that both the vernix caseosa and the amniotic fluid impart a neutral pH (6.6–7.5) to the newborn skin surface (Rahma and Lane, 2022). Review articles on skin pH (Oranges et al., 2015, Fluhr and Darlenski, 2018) have discussed the barrier properties with development of the acid mantle. The lack of acid mantle increases the risk of invasion by pathogens and may also play a role in delayed wound healing.

In water, molecules (H_2O) are in equilibrium with hydrogen ions (H^+) and hydroxide ions

(OH^-) $H2O \rightleftharpoons H^+ OH^-$.

Applying the law of mass action

$[H^+][OH^-]/ [H2O]$ = constant, where [ ] indicates the concentration in units of moles per cubic decimetre (mol/dm^3). Sörensen in 1909 proposed the use of the H^+ ion and pH be defined by the relationship $pH = -\log_{10}[H+]$. As a logarithm must be dimensionless, pH does not have units.

Table 2-7 Nomenclature of pH in relation to molar concentration of [H+]

At 25°C	mol/dm^3	pH
Neutral solution	$[H+] = 10^{-7}$	7
Strong Acidic solution	$[H+] = 10^0 = 1$	0
Strong Alkaline solution	$[H+] = 10^{-14}$	14

Using the pH scale, a change of 1 corresponds to a 10-fold change in the H^+ ion concentration and a change of 2 corresponds to a 100-fold change, etc. The pH scale has the advantage that all solutions from 1 mol dm^{-3} acid to 1 mol dm^{-3} alkali can be expressed by positive numbers from 0 to 14.

Calorimetric method

Determination of pH using the colour of acid–base indicators is a very simple technique that can be carried out rapidly and reproducibly. Indicator papers, comparative solutions, and coloured glasses (Lovibond comparator) can be used as the calorimeter and provide accurate results but are reported to potentially have numerous sources of error due to e.g., chemical reaction between indicator and particle, the presence of colloids/salts/proteins in the solution may give erroneous results (Webster, 2003). Calorimetric method is still used with visible spectroscopy (Chiba, 2003) but is not ideal for bedside measurements.

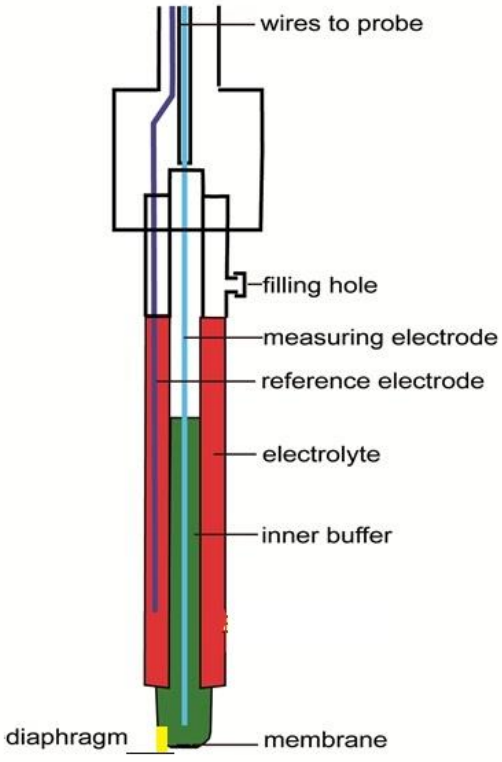


Figure 2-4 Schematic diagram of a glass pH meter

Electrochemical method

To measure pH by the electrochemical method, the electromotive force (which is related to the hydrogen ion concentration) of a reference cell is determined. In this cell, the potential of one electrode changes with pH, and the potential of the second, reference, electrode does not (Webster, 2003). Historically, the hydrogen gas electrode was used as the pH electrode, but the discovery that the potential difference across a glass membrane depends on the H^+ ion concentration on either side of it led to the development of the glass electrode. The development of the glass electrode in the pH meter has enabled the widespread application of pH measurement (Webster, 2003). Portable, cheap, and easy-to-use electrochemical pH meters are available. The studies on newborn babies including preterm babies have used this tool safely (Yosipovitch et al., 2000, Visscher et al., 2020, Kanti et al., 2014).

2.5.3 B mode Ultrasound Imaging

Ultrasound is based on the use of high-frequency sound to aid in the diagnosis and treatment of patients. The ultrasound beam originates from mechanical oscillations of numerous crystals placed in a transducer which is excited by electrical pulses. This is known as piezoelectric effect. The transducer converts the electric energy into mechanical energy which produces sound waves. The ultrasound waves are sent from the transducer, propagate through different tissues, and then return to the transducer as reflected echoes. The returned echoes are converted back into electrical impulses by the transducer crystals and are further processed to form the ultrasound image presented on the screen.

The ultrasound image is produced due to reflection of the ultrasound waves at the surfaces between the tissues of different density, the reflection being proportional to the difference in impedance. If the difference in density is increased, the proportion of reflected sound is increased, and the proportion of transmitted sound is proportionately decreased. If the difference in tissue density is very different, then the sound is completely reflected, resulting in total acoustic shadowing as is present behind bones, calculi and air. Echoes are not produced if there is no difference in a tissue or between tissues. Homogenous fluids like blood, urine, ascites, and pleural effusion are seen as echo-free structures. Ultrasound frequencies range from 2 to >15 MHz, although even higher frequencies may be used in some situations as detailed in the table.

Table 2-8 Depth of measurements with various frequencies

Transducer Frequency	Appropriate for the organ
2.5 MHz	deep abdomen, obstetric and gynaecological imaging
3.5 MHz	general abdomen, obstetric and gynaecological imaging
5.0 MHz	vascular, breast, pelvic imaging
7.5 MHz	breast, thyroid
10.0 MHz	breast, thyroid, superficial veins, superficial masses, musculoskeletal imaging
>15.0 MHz	superficial structures, musculoskeletal imaging

Attenuation in ultrasound is a phenomenon that explains the decrease the amplitude and intensity of ultrasound waves as they travel through tissue. Attenuation affects the higher frequency ultrasound waves much more than lower frequency waves. Hence lower frequency transducer is used for deeper areas of interest, though at the expense of resolution. Attenuation occurs due to interaction of the sound waves with tissue and tissue boundaries. Several phenomena occur leading to attenuation. Absorption and scattering in the tissues are considered the most significant though there is reflection, divergence, diffraction, and interference. Aerated lung and cortical bone are virtually impermeable while water and blood cause the least attenuation. Time gain compensation is used to overcome attenuation. The signal gain is increased as time passes from the emitted wave pulse. This correction enables equally echogenic tissues look the same even if they are in different depths. In absence of time gain compensation ultrasound image will appear brighter in superficial layers and darker in deeper layers.

Axial resolution in ultrasound is the ability to differentiate two separate objects that are present adjacent to each other longitudinally in an ultrasound image. Axial resolution is measured as 1/2 x spatial pulse length (speed at which a wave travels). Spatial pulse length is measured as number of cycles in a pulse x wavelength. Shorter the spatial pulse length higher the Axial

resolution. Spatial pulse length can be reduced by using higher frequency transducer. In turn, Axial resolution can be increased by using a higher frequency transducer. However shorter spatial pulse length affects the penetration of the beam. Lateral resolution in ultrasound refers to ability to discern two separate objects that are adjacent to each other. Axial resolution is roughly 4 times better than lateral resolution. Temporal resolution represents the extent to which an ultrasound system can distinguish changes over time. Improving temporal resolution increases the likelihood of discerning valve movements in echocardiography.

Ultrasound of skin

Ultrasound imaging of the skin is becoming more popular. Skin ultrasound examinations are used both to assess healthy skin and to evaluate pathological lesions. They are mainly performed in dermatology as well as in broadly understood aesthetic medicine and cosmetology.

Irrespective of the ultrasound scanner, three layers may be distinguished in the image of the healthy skin: epidermal echo, dermis, and subcutaneous tissue. High-frequency equipment allows for detailed imaging of the epidermal echo, dermis, and upper part of the subcutaneous tissue. It is also possible to visualize the skin appendages (hair with follicles and nails) as well as blood vessels that run in the dermis and upper subcutaneous tissue. Contrary to high-frequency equipment, conventional scanners do not allow for a detailed assessment of the epidermal and dermal echoes because the high frequency transducers provide high resolution. On the other hand, the conventional ultrasound scanners enable the visualization of the entire subcutaneous tissue due to lesser attenuation of low frequency ultrasound transducers. The parameters used for the assessment of skin ultrasound images are thickness of individual skin layers, caliber of blood vessels, echogenicity of the dermis or its individual layers, echogenicity of the subcutaneous tissue as well as the presence or absence of flow in the venous vessels.

The first layer visible from the head of the transducer is a hyperechoic line that corresponds to the epidermis. This line is created by the reflections between the ultrasound gel and the surface of the skin as well as the reflections from the epidermis and air bubbles located between the callused epidermal cells. This has been confirmed now using HFUSI and is called ‘epidermal echo’ (Altmeyer, 1991)

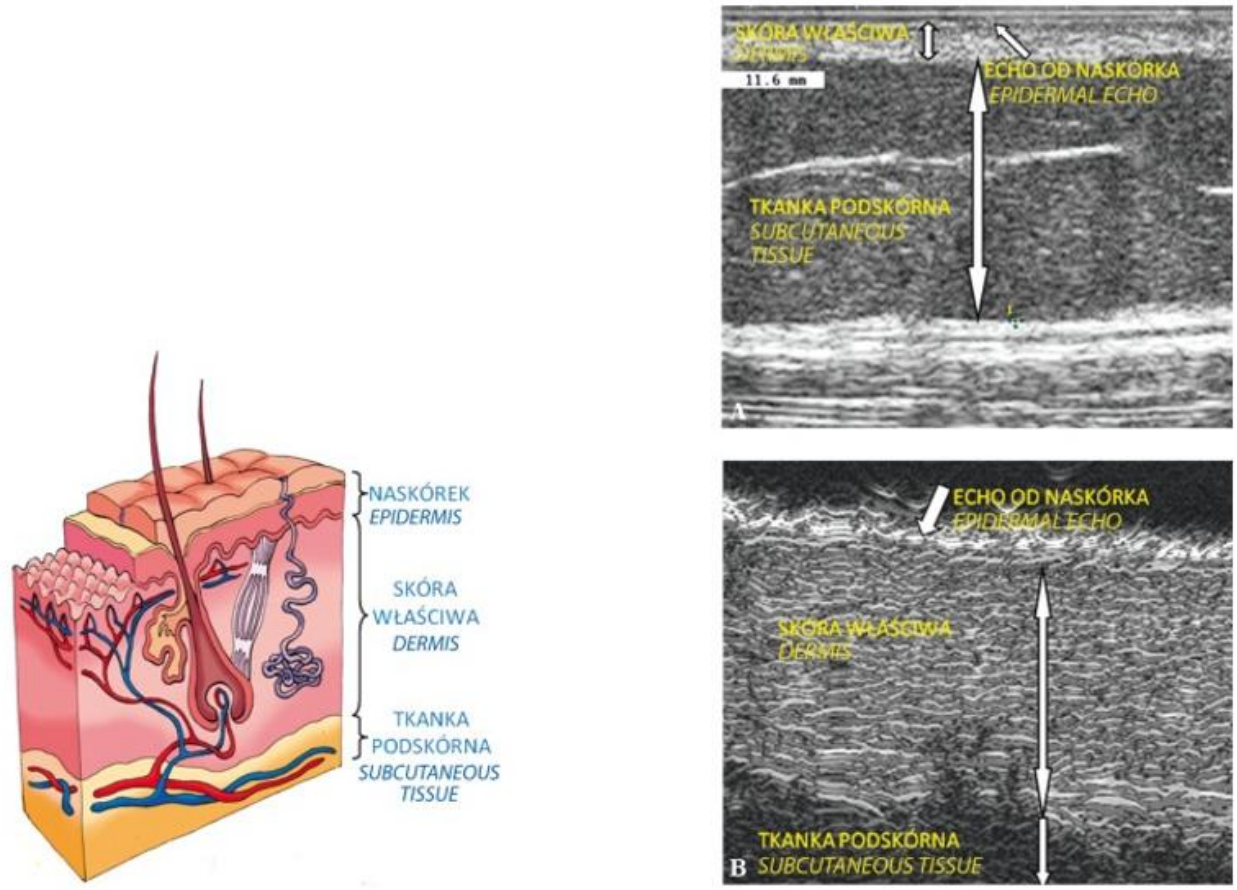


Figure 2-5 Ultrasound image of the skin

Three visible three layers: epidermis, dermis and subcutaneous tissue: **A**. image obtained by means of a classical scanner Philips HD11 XE with a linear-array transducer; **B**. scan obtained by means of a high-frequency ultrasound machine EPISCAN with a mechanical transducer of 50 MHz

Beneath the epidermis, lies the dermis. It may be anatomically divided into the papillary dermis (uppermost part of the dermis lying beneath the epidermis) and reticular dermis (a layer located under the papillary dermis). The papillary dermis constitutes 20% of the dermis) and contains blood vessels and irregularly arranged thin collagen and elastin fibres. On the other hand, in the reticular dermis, constitutes approximately 80% of the dermis, and consists of the collagen, elastin and reticular fibres arranged regularly. Hence in ultrasound scanning, the dermis is a heterogeneous layer with hyperechoic reflections of the collagen fibres and hypoechoic ones originating from the extracellular matrix that lies between the collagen fibres. Furthermore, by means of the ultrasound machines supporting the transducers of 30 MHz and more, two layers may be distinguished in the dermis that differ in terms of their echogenicity

The upper layer is usually thinner and presents decreased echogenicity in comparison to the lower one. This diversification of the dermal image represents the anatomical structure of the skin. As was shown in Figure 2-6, the papillary dermis includes a lower number of the collagen fibres, and they are much thinner. This results in lower echogenicity as compared to the echoes of the collagen fibres situated in the lower layer where they are thicker and thus, produce stronger echoes. Unfortunately, the division of the skin into the upper and lower layers as seen in the ultrasound image, may not be strictly understood as the division into the papillary and reticular dermis.

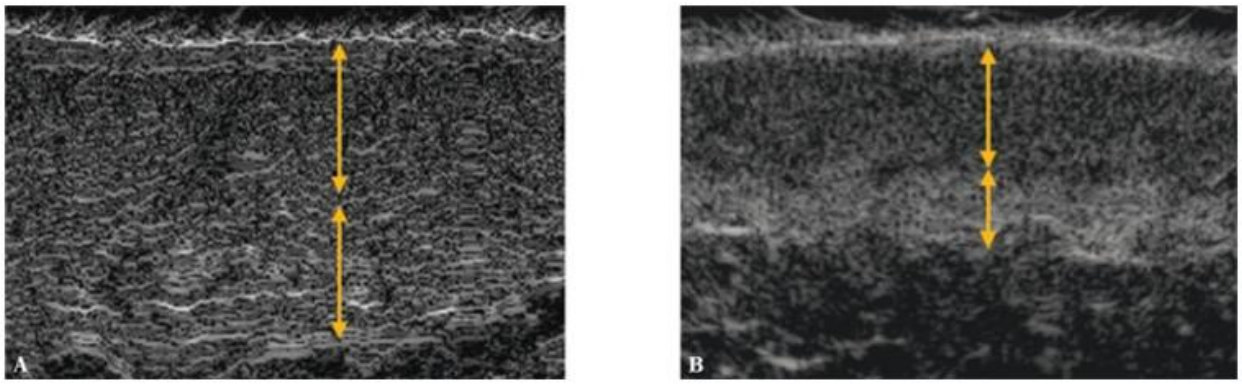


Figure 2-6 Ultrasound image of the dermis with visible division into upper and lower layers

A. image obtained by means of a high frequency scanner Episcan with a mechanical transducer of 50 MHz; B. scan obtained by means of a high-frequency ultrasound machine Derma View with a mechanical transducer of 48 MHz.

In some ultrasound images, a thin anechoic band may be observed between the epidermal echo and the dermis so-called SLEB (subepidermal low-echogenic band) or SENEB (subepidermal non-echogenic band). SLEB appears in the aged skin or skin subjected to excessive exposition to ultraviolet radiation Moreover, the increase in the thickness of SLEB is related to water retention in the papillary dermis (Richard et al., 1993).

In the case of the healthy skin, the researchers have attempted to describe the changes caused by natural ageing processes and photo ageing connected with excessive exposure to ultraviolet radiation, based on its thickness and echogenicity. They also try to determine sex-related differences (Waller and Maibach, 2005, Seidenari et al., 1994). As Waller and Maibach indicate, there is no agreement among researchers concerning the changes of the skin's thickness occurring with age. Several papers demonstrated that the dermis is the thickest in young persons and subsequently, it becomes thinner with age. Other authors claim that it is not the age, but external factors, such as UV radiation, that play a crucial role in the change of the thickness of the dermis. The changes in the echogenicity of the skin are also

interpreted in various ways. Nonetheless, most of the researchers confirm that in the ageing skin, echogenicity undergoes changes and a SLEB (subepidermal low-echogenic band). Such divergent results obtained by different researchers are due to studies conducted with the use of different scanners, and the examination of skin in different locations. Similar attempts to describe skin structure using ultrasound has been made in children. More recently researchers are looking at neonatal skin using high frequency ultrasound scanning however similar issues of using different machines, different frequencies, different locations on skin and subjects of varying gestations make comparisons of these research results challenging.(Vitral et al., 2018, Paulina Przybysz, 2020). There is also different nomenclature used for skin thickness e.g., skin thickness, dermal thickness are used interchangeably.

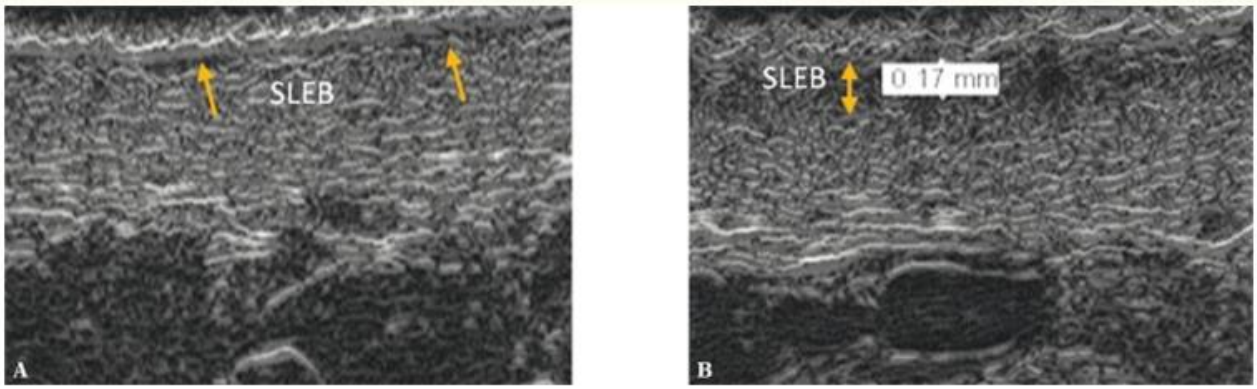


Figure 2-7 Imaging of the anechoic band lying beneath the epidermis (SLEB) by means of a high-frequency ultrasound scanner.

Episcan with a mechanical transducer of 50 MHz: **A**. SLEB of slight thickness on the cheek; **B**. well visible SLEB on the dorsal side of the hand in the region of the dermis, one may also visualize skin appendages such as nails or hair follicles, small blood vessels and openings of the sweat glands.

2.6 Gaps in knowledge about neonatal skin

With limits of viability changing and improving survival of extreme preterm babies, the quality of life of these babies while in the neonatal unit and in the long term can be influenced by tailoring the intensive care strategies to the requirements of these babies according to their physiological needs. The lack of studies exploring the physiological characteristics of skin in preterm babies and how it differs from term babies' skin leaves a gap in knowledge which in turn hampers development of evidence based intensive care strategies for these vulnerable babies.

2.6.1 Chronological changes to Skin Structure and Function

Much of the evidence available to inform prevention of hospital-acquired skin breakdown in neonates is adapted from research in adults or older children. The studies exploring the physiological characteristics in newborn babies, do not establish the chronology of these changes in sufficient detail to translate them into clinical practice. For instance, Hammarlund et al have published a series of articles about changes in TEWL in extreme preterm babies (Hammarlund et al., 1982). However, the publications only include serial TEWL measurements for >25-week babies. Other studies detailing TEWL by serial measurements over time (Rutter and Hull, 1979, Hammarlund et al., 1982, Sedin et al., 1985, Kalia et al., 1998) or reviews of published data (Visscher and Taylor, 2014, Oranges et al., 2015) focus on knowledge of neonatal skin, mainly its function. Visscher has included histological features of neonatal skin discussed previously by other authors (Holbrook, 1982, Maibach, 2003). A recent systematic review noted that despite understanding the importance of studying the human skin barrier, there is limited evidence on skin thickness dimensions obtained by histology (de-Souza et al., 2019). Amount of collagen in dermis increases with age and gestation (Stamatas et al., 2011, Marcos-Garcés et al., 2014, Barcaui and et al., 2015, Haydont et al., 2019). However, none of these studies give adequate details of structural changes imaged in preterm or term neonates e.g., epidermal thickness, dermal thickness, dermal echogenicity.

There is no chronological data depicting changes in either skin thickness in preterm infants at various gestational ages or the change in echogenicity of dermis. The two studies of skin characteristics in newborn babies mentioned in the Table 2-9 have measured epidermal and dermal thickness soon after birth. 52% of babies included by Vitral et al are preterm but the data is presented by birth weight categories only. The distribution of data by gestational age could not be obtained from the authors on direct contact. All the babies included by Przybysz et al were born at term (Paulina Przybysz, 2020) . There are no studies presenting data on echogenicity of dermis in newborn babies. The echogenicity presented for adults and children are useful in making qualitative comparisons but cannot be compared objectively due to variation in study protocols in terms of area within the dermis used to assess echogenic pixels. The number of pixels will be comparable if counted within same size of region of interest. Hence while studies have published echogenicity data it is neither replicable nor can be used to compare with another data as the area of interest is not defined.

In essence, there is no comprehensive chronological evaluation of skin structure and function in both preterm and term infants in the published literature. The early work in the second half of 20th century by Hammarlund et al and Rutter et al was before modern advances in neonatal care more recently summarised by Costeloe et al (Costeloe et al., 2012), when neonatal survival in extreme preterm babies was a less common event. Also, while these studies address functional properties of skin, they leave a gap in knowledge about structural properties of skin. There is gap in knowledge of dermal characteristics and change in the characteristics of the dermis chronologically.

Table 2-9 Comparison of Skin parameters (µm) on HFUSI in different age groups

Skin parameter	Epidermis (µm)	Dermis (µm)	Skin thickness (µm)	references
24-41 weeks	171-177	948-1053		(Vitral et al., 2018)
37-41 weeks	81-84	679-722		(Paulina Przybysz, 2020)
2-3 years 4-10 years 11-13 years			860-1490 890-1720 950-1970	(Seidenari et al., 2000)
Adult	115-149	999-2840		(Wang et al., 2023)
			980-2340	(Seidenari et al., 2000)

2.6.2 Biomarkers produced by skin

Skin under pressure releases inflammatory cytokines, prior to damage or redness becoming visible. The research into pressure wounds in adults has found biomarkers from the skin surface which have been believed to provide a means to examine the epidermal and dermal tissues (Bronneberg D, 2007, Cornelissen et al., 2009, Bader and Worsley, 2018). IL-1α, IL-1RA and TNF-α have been identified in various studies, as parameters for measuring epidermal reactivity (Lee et al., 1997, Uchi et al., 2000, Worsley et al., 2016). This has never been studied in newborn babies, let alone preterm, who are frequently noted to have device related skin injury (Ligi et al., 2008, August et al., 2018), especially at lower gestational ages.

2.6.3 Impact of nutrition on neonatal skin

The lipids are essential components of the protective skin barrier. Essential fatty acids have documented roles in both the dermal and epidermal layers of the skin (Hughes-Formella et al., 2019). Linoleic acid plays a central role in maintaining skin barrier integrity whereas α-linoleic acid serves primarily as immune modulator (McCusker and Grant-Kels, 2010). Plasma levels can be a good indicator of availability of fatty acids to the skin as shown in paediatric population (Hughes-Formella et al., 2019).

Experimental models to study the influence of dietary macronutrient balance on skin structures using geometric framework for nutrition showed that high protein intake increased dermis and epidermis thickness in male mice, and high carbohydrate intake in females resulted in thinner dermis and increased subcutaneous fat (Hew et al., 2016). Thinner dermis when subcutaneous tissue was increased was due to decreased fibroblast proliferation, decreased collagen precursors and suppression of genes for collagen synthesis (Yamane et al., 2010, Ezure and Amano, 2010).(Yamane et al., 2010, Ezure and Amano, 2010). In trying to understand age related changes in dermis leading to decreased skin elasticity and delayed wound healing Ezure et al established that subcutaneous fat physiologically controls the dermal condition via secretory factors (Ezure et al., 2022). This has been reported as the explanation for the previous finding that high protein intake causes adipogenesis and high fat intake induces adipocyte hypertrophy (Ezure and Amano, 2010).

However, there is lack of studies exploring the impact of nutritional status of the mother/baby or the impact of the antenatal/postnatal environment on development of the skin barrier. A recent study of 320 infants and toddlers compared well-nourished and moderately nourished infants and found little difference in TEWL and pH between the groups but noted interesting findings on skin thickness measurements (Hughes-Formella et al., 2019). Total skin thickness (proxy for subcutaneous fat thickness) increased from 1 week of age till 4 weeks and the measurements were smaller for moderately nourished infants. Counterintuitively, dermal thickness was maximum at 1 week and decreased till they plateaued at 6 months of age in well-nourished infants. Dermal thickness was also higher in undernourished than in well-nourished till 6 months of age. It was suggested that these findings were due to delayed adaptation from intra uterine environment to dry environment in moderately nourished children. Histopathological studies in skin of children with clinical protein energy malnutrition have shown dermal oedema and decreased collagen content. (Thavaraj and Sesikeran, 1989). Vasantha et al demonstrated

decreased soluble collagen and delayed collagen maturation in dermis of rats exposed to protein energy malnutrition (Vasantha, 1970).

Amongst adult research there is moderate statistical association between nutritional status and developing a pressure injury (Munoz et al., 2020). It is suggested that age related dermal thinning, decreased elasticity, and delayed wound healing is due to the physiological effect of secretory factors from subcutaneous fat tissue and can be influenced by paying attention to nutrition (Ezure et al., 2022). While impaired nutrition and its relationship to pressure injury development has not been as rigorously studied in children, a systematic review recently was able to highlight that early nutritional intervention is an effective strategy to prevent pressure injury in neonates and children who have been identified as high risk (Setchell et al., 2023).

2.6.4 Questions arising from gaps in knowledge.

As a result of the unknowns in the current literature listed in section 2.6 the following research questions were proposed:

A. What are the chronological changes in barrier properties of skin in extreme preterm, very preterm and term babies?

B. What are the structural differences in the skin of extreme preterm babies and very preterm babies compared to the term babies?

C. How does the ambient environment and inherent individual factors influence the structural and functional changes in preterm skin?

D. How does nutrition effect the structure and function of skin?

2.7 Aims and Objectives

Current practice of delivering and caring for preterm infants (23-37 weeks gestation) is associated with physical consequences, which can be long term, especially in extreme preterm babies (Humberg et al., 2020). The skin of these neonates has not fully developed with associated limitations in its biomechanical and physiological functional capacity, influencing its natural barrier role towards injury and disease. Consequently, skin tissues, particularly when medical devices challenge them, are vulnerable to damage due to mechanical loading and microenvironment conditions.

The aims of this research were derived from the questions raised at the end of the literature review (Section 2.6.4) and are as follows-

- Establish the relationship between gestational age and the development of skin structure and function including temporal pattern with postnatal age.
- Evaluate the interaction between the environment, antenatal/postnatal factors, and skin development in preterm infants.
- Explore if evidence can be created as a basis to promote best practice for skin assessment and management.

To achieve these aims, the following specific objectives have been set in this study:

1. Measure trans-epidermal water loss and skin pH in neonates of varying gestational age.
2. Measure skin structure objectively using high frequency ultrasound.
    a. thickness of dermis
    b. density (echogenicity) of dermis
    c. observation of functional skin layers
3. Evaluate factors that might impact development of skin barrier.
    a. Effect of maternal nutrition
    b. Effect of neonatal nutrition
    c. Effect of intensive care interventions –antenatal and postnatal

Chapter 3 Methods

This is a single centre longitudinal observational cohort study over 18 months to explore the structural and functional development of skin of preterm neonates. Comparison will be made to a cohort of babies born at term. This study incorporates a series of non-invasive measurements of skin function and structure. Institutional ethics, IRAS and HRA approval was sought for the study (Ref ERGO 31460, REC approval 248650). This was part of an NIHR funded programme of research investigating the risk of skin damage in neonates (Principal Investigator, Prof Peter Worsley).

3.1 Recruitment

Patients were recruited through the NICU at the Princess Anne Hospital (PAH) Southampton, under the clinical guidance of Professor Howard Clark (Head of Academic Department of Child Health). Posters were presented on the unit and information sheets were provided to the parents in advance (at least 4 hrs) of seeking consent. Informed written consent was obtained from each legal guardian/parent of the infant prior to data collection in line with the NIHR GCP principles. It was made clear that participation is optional, and that care will not be affected by agreeing or declining to participate in the study. Due to the exploratory nature of this research the plan was to collect data as a convenient sample (20 preterm and 20 term babies). However, the recruitment went very well, and both the parents of the babies and nursing team were very supportive of the study protocol, so decision was taken (with Research and ethics committee approval) to move to purposeful sampling stratified by gestational age groups with the aim to conduct a prospective study on term and preterm babies with adequate numbers to enable categorisation of skin changes with gestational age. The following criteria were used to include or exclude babies from the study.

3.1.1 Inclusion criteria

All infants born in PAH over the course of the study were eligible for inclusion.

3.1.2 Exclusion criteria

- Infants with dermatological conditions (e.g., collodion skin, harlequin baby)
- Infants receiving therapeutic hypothermia.
- Infants with medical condition prohibiting skin data collection.

3.2 Equipment

To understand the structure and function of skin in preterm and term neonates three methods of measurement were used as depicted in the Schematic diagram below.

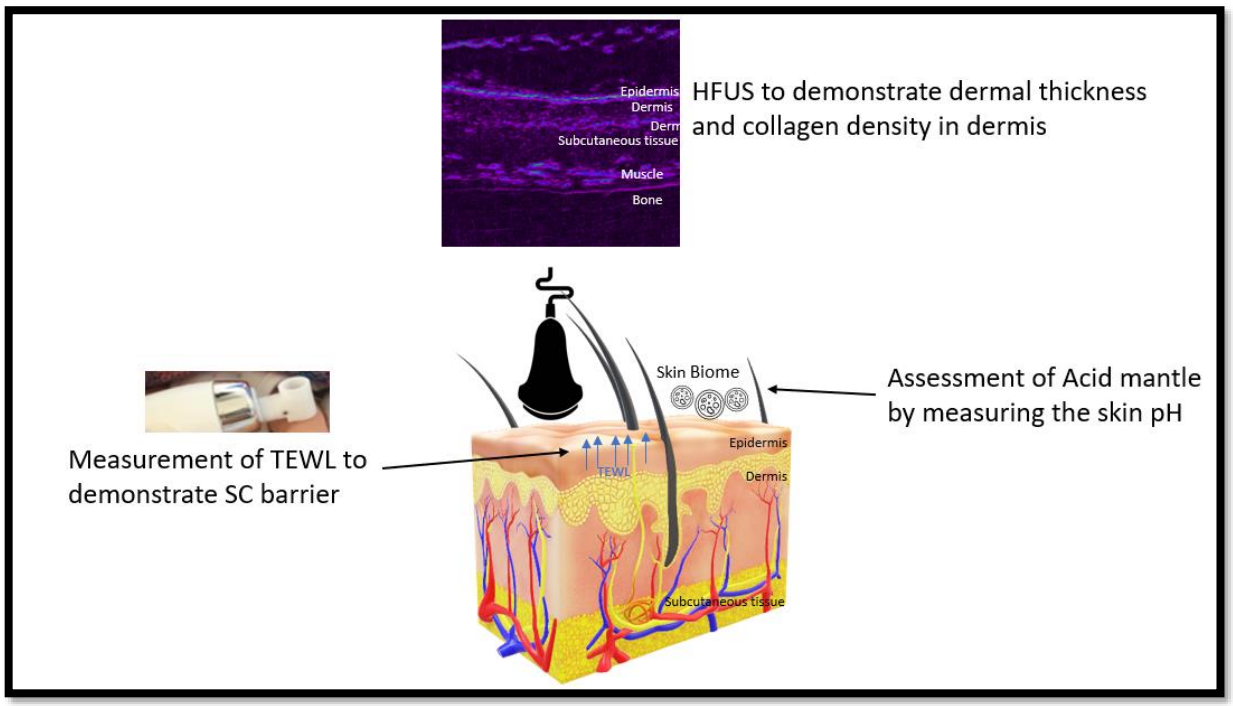


Figure 3-1 Schematic diagram showing assessment of skin structure and function.

Techniques were employed to determine the integrity and the physiological status of skin of infants at specific gestational ages and birth weights. Specific measurements included biophysical skin assessment (Trans epidermal water loss and pH) and imaging (high frequency ultrasound imaging (HFUSI). Devices were CE marked and appropriate for clinical studies.

3.2.1 TEWL and pH

TEWL measurements were made using widely validated open-chamber system (Tewameter® TM 300, Courage+khazaka Electronic, Cologne, Germany, Figure 3-2 B) (Barel and Clarys, 1995, Fluhr et al., 2006). Tewameter allows continuous measurement over a period, allowing it to recognise changes to flow (Darlenski et al., 2009). In a comparison against the closed-chamber device, it was more sensitive in detecting changes in TEWL values at lower level

(<45g/m^2/h) and the closed-chamber was more sensitive in detecting measurements in the high value range (>80g/m^2/h). Therefore, the data presented in this research paper is reliable only as a reference for open systems (Steiner et al., 2011). TEWL is measured in g/m^2/h. (Figure 3-1 A and B). The Tewa meter device operated through a blue tooth connection to a laptop and sampled water loss at 1Hz.

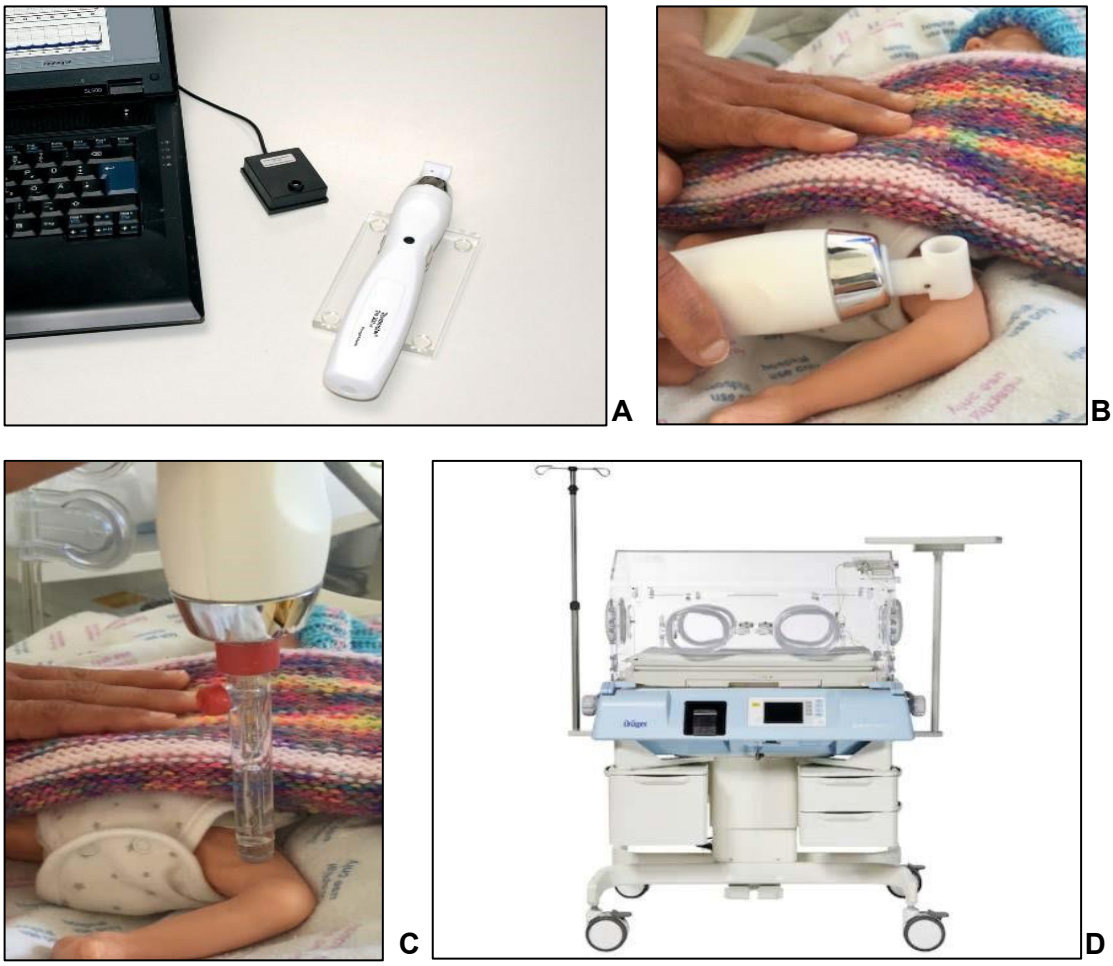


Figure 3-2 Images of the test equipment

(A) Picture showing equipment used for measuring TEWL.

(B) Picture showing TEWL being measured inside the incubator.

(C) Picture showing pH being measured inside the incubator.

(D) Incubator used at PAH, Southampton

pH was measured using a separate non-invasive probe (Skin pH meter pH-905, Courage+khazaka Electronic, Cologne, Germany, Figure 3-2 C) which has been used in neonates in the past with no adverse effects (Sedin et al., 1985, Hammarlund et al., 1977). pH is measured as negative logarithm of H^+ ions. The pH probe was calibrated daily and held in a buffer fluid between assessments. (Figure 3-2 C).

3.2.2 High Frequency Ultrasound

High frequency ultrasound images of the skin, including epidermal and dermal layers were recorded using a portable scanner specifically developed to examine the skin and underlying soft tissue (EPISCAN I-200. Longport, Inc., Glen Mills, PA). Measurements are made with high frequency probe (35 MHz) to obtain better resolution at superficial depths, where the focus of interest of this study lies (Figure 3-1 and 3-3). The system displays the information obtained in the form of a B-scan as either a colour or grey-scale image. The EPISCAN is a high-resolution ultrasound imaging tool (resolution 40 microns in vertical dimension, less than the diameter of human hair 50 microns). The EPISCAN cannot image as deep as conventional ultrasound and is designed to image the first 15mm of soft tissue. In cross-section the probe interrogates 15mm length, as shown in Figure 3-4.

A   B

Figure 3-3 (A) High frequency ultrasound scan Episcan I-200 system (B) Ultrasound probe

The ultrasound probe is filled with pH neutral buffer solution and an impermeable membrane is placed over the tip of the probe. This provides a medium for the ultrasound wave to travel prior to reaching the skin surface. The surrounding casing of the transducer provides the optimal distance to the skin, where the operator is required to hold the device at a perpendicular angle for the best attenuation with the underlying tissues. Structures under the skin with higher stiffness values e.g., stratum corneum, fascia, provides the highest levels of attenuation and are highly viable on the B-mode image (Figure 3-4).

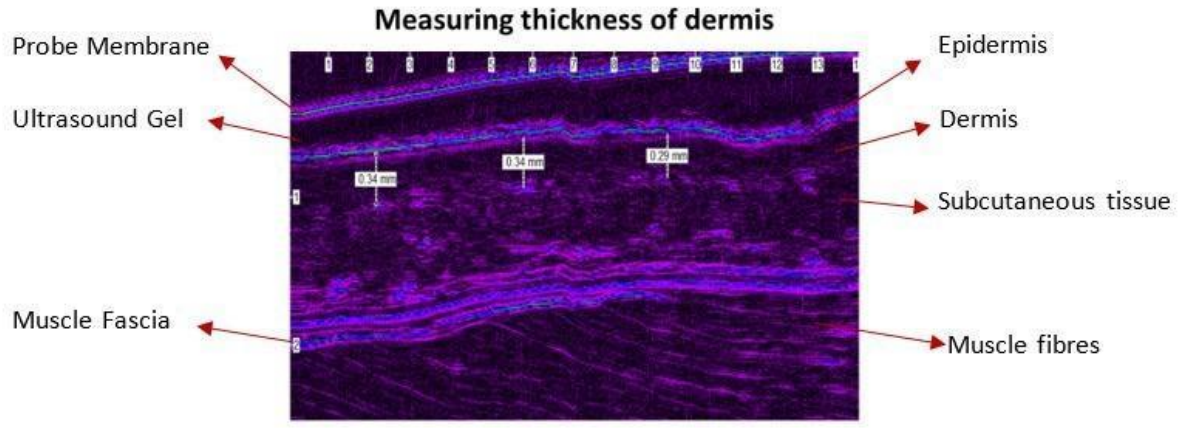


Figure 3-4 HFUS picture (depth 10.8 mm) of measurement of dermal thickness in EPISCAN

3.3 Measurement protocol

3.3.1 Protocol for study subjects

The measurement time points (Table 3-1), and protocols were different for preterm and term babies as term measurements were used as standard for comparison while preterm measurements were studied to explore time dependent changes following birth during their stay in neonatal ICU. The demographic and other clinical information were collected from both groups including sex, gestational age, ethnicity, medications (including antenatal steroids), daily nutritional intake, nutritional status (weight, length, and head circumference), incubator temperature and humidity. Data was collated from the medical notes as part of routine assessment.

3.3.1.1 Preterm infants

While conducting the proposed study, routine care of babies was not disrupted and convenience of babies, parents and clinicians was considered while also adhering to the measurement protocols. This included detailed discussion with the nursing and the medical team before the measurements were undertaken, regarding the suitability of the assessment in a neonate needing intensive care. The measurements were only undertaken if it was felt that the baby is likely to tolerate the handling for up to 25-30 minutes. During the first week, all babies born before 27+6 weeks were nursed in incubator humidity of 80% as per NICU

protocol. It was weaned by 5% each day from the eight day of admission and ceased when incubator humidity of 40% was achieved. For babies born at 28-29+6 weeks, incubator humidity was commenced at 80% and weaned as the other group from day two. Humidity levels were tailored to individual needs of each baby, depending upon their thermoregulation and hyperosmolarity. Opportunistically, TEWL was also measured during Kangaroo care which is part of daily routine of in the neonatal unit.

Table 3-1 Table of data collection time points

GA ↓ /Time→ Investigations ↓		Birth/ Consent	<48hours	3-4 days	6-7 days	9-11 days	13-15 days	28-34 days	50-56 days or before discharge
Preterm		√							
	pH		√		√	√	√	√	√
	TEWL		√	√	√	√	√	√	√
	USS skin		√	√	√	√	√	√	√
Term		√							
	pH		√		√				
	TEWL		√		√				
	USS skin		√		√				

* Where the infant has been exposed to a medical device

Where appropriate, all measurements were taken from right thigh in all the infants. If it was not appropriate, due to its inaccessibility or past exposure to interventions, for example, intramuscular injections, tapes or ECG leads, the left thigh was used (Figure 3.2 B and C). The position of the baby was not changed, especially in ventilated babies if they had been settled in a particular position and felt to be comfortable by the clinical staff. This strategy allowed measurements to be obtained from the same site (medial, lateral, or anterior thigh) in all babies to provide data for longitudinal comparison and avoid any interference with routine

monitoring and cares. To ensure the research process caused minimum disruption, all data collection was performed whilst the babies were in the incubators (Figure 3.2 D). The protocol also accommodated open cots or during skin-to-skin or cuddles with parents. Room temperature, incubator/heated mattress temperature and incubator humidity were recorded three times during the measurement (before, midway and at the end) to ensure standard ambient conditions were maintained during the measurements. The time needed for data collection was kept to a minimum to avoid disruption of care with a total target period of 25-30 minutes. If the baby became unstable during measurements, the data collection was stopped. Initial screening was robust, hence, only one term baby missed the first measurements completely, as he became very unstable after consent.

3.3.1.2 Term infants

The term infants not receiving intensive care provided normative data for a comparison with pre-term infants. All measures were taken using the same measurement protocol as for preterm babies (Section 3.3.1.1). Skin was assessed on two occasions (<48 hours and approximately 6 days after birth) to obtain values of pH, TEWL, and HFUS skin thickness. More babies were recruited from NICU to facilitate second set of measurements at 6-7 days, as most term babies are generally discharged by 24-48 hours of age (Table 4-1). All the first set of measurements were taken while babies were in hospital, at the local maternity unit. However, in 4 cases the second sets were measured when the infants were brought back to an organised clinic setting from home. All these visits were organised to coincide with other clinical care for mothers or babies. The only incentive offered to these parents was a parking permit for the hospital visit.

3.3.2 Protocol for measuring TEWL and pH

TEWL was the first measurement made on the patient, to ensure the least disturbance to the ambient environment prior to the recording. The TEWL probe was placed gently and perpendicularly in contact with the skin (Figure 3-2 B) for approximately 60 seconds, sampling at 1Hz, to allow stabilisation of the measurement. Three recordings were taken, with the average of last 5 stable measurements from each used for further analysis, as shown below. This was conducted according to international standards for its use (Plessis et al., 2013).

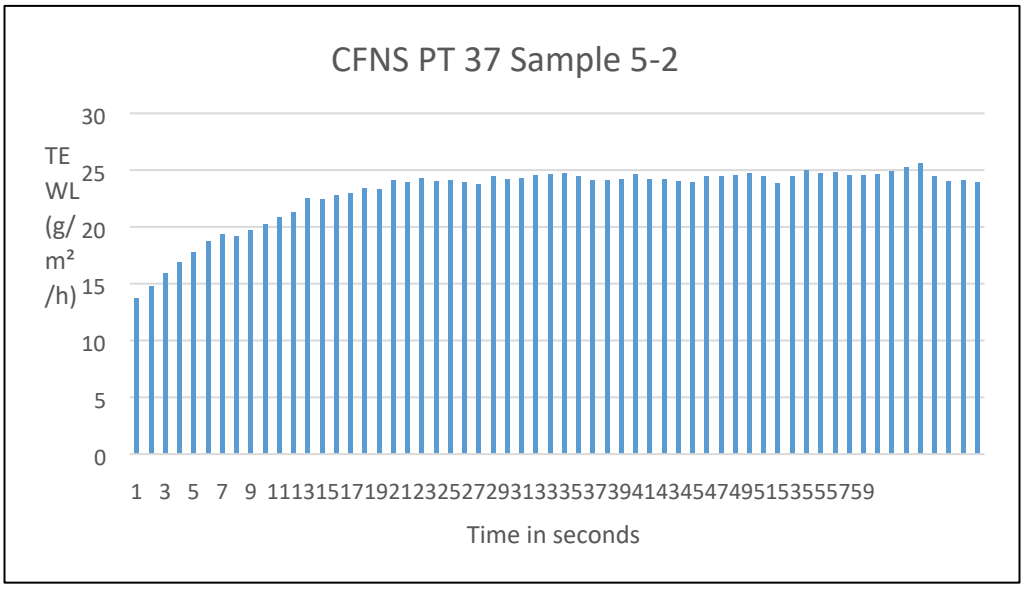


Figure 3-5 Example of a TEWL measurement.

This figure shows one of the three measurements taken from a patient at one time point, which were used to calculate average TEWL for that time point.

Skin pH was also measured similarly, by holding the probe perpendicularly in contact with the skin for 7-8 seconds (Figure 3.2 C). This was followed by three ultrasound images of the skin, recorded at each contact, immediately after the TEWL and pH measurement.

3.3.3 Protocol for High frequency ultrasound scan

The scan probe was filled with ultrapure water and sealed with a latex membrane. The interface for high frequency ultrasound scan (the ultrasound gel) was same as conventional ultrasound which is regularly used in neonates. The ultrasound transducer was placed perpendicular and in light contact with the skin via copious warmed aqueous-based gel to avoid compression of skin layers. The leg was held steady by the nurse looking after the baby allowing fine, controlled movements of the probe. Three images are taken (to ensure repeatability of measures) with each probe at 2 depths (Table 3.3). The layers of skin could be identified from the scan pictures as in Figure 3.6. Protocols for scanning were established to ensure consistent images at two depths. All examinations followed the same imaging protocol regarding ultrasound probe type, frequency, and image settings as shown in Table 3-2.

Table 3-2 Protocol for HFUS at two depths

	Probe 35 MHz	**Probe 35 MHz**
Size	512x1024 pixels	512x1024 pixels
Position	7.5 mm	7.5 mm
Depth	5.4 mm	10.8 mm
Time Gain Compensation	28%	28%
Gain	31%	31%

The exact anatomic site, date and time of the scan, and any untoward clinical signs were recorded for each contact. I was designated to perform the scanning of all the babies. As part of evaluation of the images a Quality Control (QC) process was undertaken. The methodology for the QC process is detailed in Section 6.2. From each QC image linear measurements of dermis, total skin thickness and echogenicity were taken using the Episcan software.

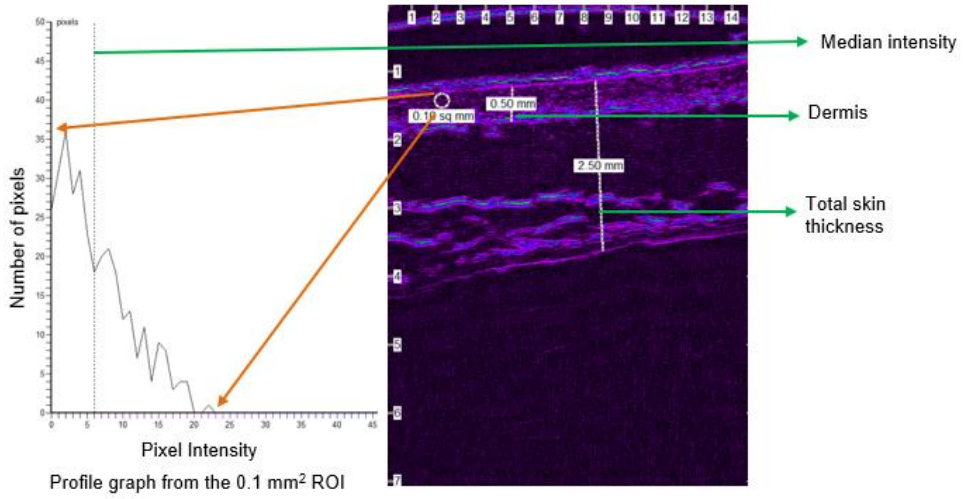


Figure 3-6 Example of a HFUSI measurement.

Dermal depth (vertical distance between lowest edge of the most well-defined epidermis and upper most layer of the most well-defined lower dermis) was taken from the image best delineating the layers of skin for each patient contact. Total skin thickness was similarly measured from the lowest edge of epidermis to the upper most layer of the fascia as shown in the Figure 3-6. A mean of each of these measurements was calculated. In addition, three areas were selected in the dermis to measure pixel density. In the interest of harmonising the measurements in various scans, a protocol was designed for measuring pixel intensity. The measurements were taken from three regions of interest (ROI) (0.1sqmm area) in each chosen image. Each ROI was 0.1 mm from the lower margin of epidermis (Figure 3-6). An average pixel intensity was calculated for that image by the image software. Three profile charts of pixel intensity were obtained from each image (to be used as surrogate for collagen content of dermis) for further analysis. Percent pixels ≥ intensity 10, median intensity and relative dispersion of intensities were also calculated from them and a mean of each of the three measurements from each scan image was used. In summary each QC image provided five measurements, dermal thickness, total skin thickness, percent pixels ≥ intensity 10, median intensity and relative dispersion of intensities.

3.3.4 Protocol for correcting TEWL values according to incubator settings

Because open chamber TEWL values are strongly related to ambient RH, any comparison of TEWL data obtained from measurements at different relative humidity levels requires correction of TEWL values to a standard RH. Accordingly, the TEWL values obtained were corrected to an RH of 50% (cTEWLH50) for all the measurements taken in RH >50% with the following equation using a previously published formula (Agren et al., 1998, Jain et al., 2000):

$$cTEWLH50 = 50 \times TEWL / (100 - RH)$$

The absolute and corrected TEWL was then used to judge the difference in time to achieve functional maturity. The environmental temperature, individual baby temperatures and room humidity were consistently in normal ranges for each individual measurement but were not comparable between measurements for the same baby and between babies across different gestational ages, due to use of variable incubator humidity settings (Section 3.3.1.1) hence by using cTEWLH50 the TEWL was comparable across all the babies in the study.

3.3.5 Protocol for nutritional data

SENNAT Southampton Electronic Neonatal Nutrition Assessment tool (Johnson et al., 2015) was used to assess the nutritional status of each preterm baby <30 weeks. Data was collected for each patient on the data collection forms (Appendix 4) as well as the electronic tool (Figure 3.7). This data has been extracted into excel sheets for computation.

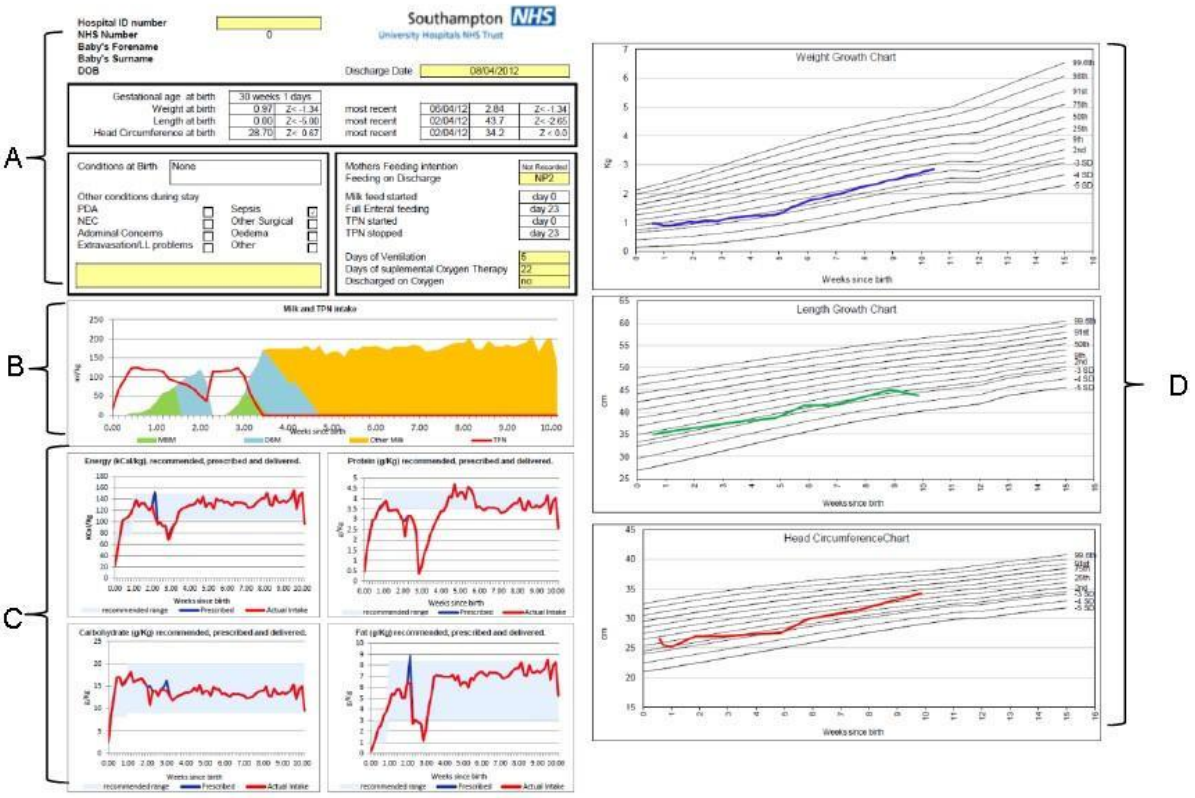


Figure 3-7 Example of SENNAT data

A: Patient details, B: Contributions of parenteral and enteral nutrition (PN ■, MBM■, DBM■, Formula■) C: Intakes of (clockwise from top left) Energy, Protein, Fat and Carbohydrate, (shaded area represents Recommended Intake range) and D: Growth charts for weight, length and head circumference.

3.3.6 Protocol for infection prevention

Infection prevention was ensured when moving measurement probes between infants. This followed the hospital and the local NICU infection prevention policy, in which all equipment contacting infants was thoroughly cleaned with Actichlor and dried before and after use. Where possible, single use attachments on the probes (ultrasound probe membrane) was utilised to minimise the risk of spreading infection. Similar infection control measures were used throughout the study.

3.4 Statistics

Quantitative variables were summarised as frequencies, range, means and qualitative variables as frequencies and percentages in each category. Normality assessment revealed that the data set is not normally distributed hence non-parametric descriptor, and tests were applied using SPSS statistics v29. Due to the exploratory nature of the research a focus on exploring trends in time dependent and group dependent differences between babies was undertaken. Time series analysis was performed for all the measurements against the post menstrual age. Correlations were established for single measurements e.g., TEWL and gestational age and gender. Similarly dermal thickness and echogenicity were subjected to the statistical tests. Mann Whitney U test was used to test the null hypothesis for comparison between groups. Other non-parametric tests used were Wilcoxon rank-order test and Spearson's rank-order test.

Like the TEWL analysis descriptive exploratory approach was used for analysis of HFUSI analysis. The same statistical tests using the SPSS statistics v29 were applied for analysis of the dermal thickness, total skin thickness and pixels in regions of interest (ROI).

Research questions

Specific statistics were used to answer the research questions as noted in the Table 3-3.

Table 3-3 Research questions linked to null hypothesis and statistical tests

No.	Research question	Null hypothesis	Statistic test
A	Does TEWL differ between gestational age groups <27 weeks and >27 weeks and preterm/term infants?	TEWL is not affected by gestational age at birth	Mann Whitney U test, significance at p-value <0.05
B	Does TEWL differ by gender, mode of delivery, maternal BMI or antenatal steroids?	TEWL is not affected by gender, mode of delivery or maternal factors	Mann Whitney U test
C	Does TEWL have any correlation with birth weight	There is no correlation of TEWL and birth weight	Spearman's order correlation test, significance at p-value <0.05
D	Does skin pH differ between gestational age groups <27 weeks and >27 weeks and between preterm and term infants?	pH is not affected by gestational age at birth	Mann Whitney U test, significance at p-value <0.05
E	Does GA or incubator humidity affect the time it takes for the SC to reach functional maturity (i.e., TEWL≤10 g/m²/h?	GA and incubator humidity do not affect the time it takes to reach TEWL ≤ 10 g/m²/h	Wilcoxon signed rank test, significance at p value ≤0.05

F	Qualitative analysis of HFUSI to study the delineation of skin layers, both in first scans and the scans obtained temporally as described in ‘protocol for high frequency ultrasound scan in Section 3.3.3.	The delineation of skin layers is well established within 48 hours in term and late preterm babies (≥28w GA), but does not show clear differentiation for numerous weeks in extreme preterm babies (<28 weeks GA)	Focus on description of the ultrasound images. Descriptive statistics for term and preterm infants
G	Comparing preterm and term scans on the images that satisfied the QC	The dermal thickness and echogenicity measurement is only possible in scans with clear delineation of skin layers	Presentation of ultrasound scans from infants with varied gestation for visual appreciation
H	Do skin characteristics differ in preterm <28 weeks and term infants- Dermal thickness, total skin thickness and echogenicity?	There is no difference in skin characteristics of all newborn babies	Mann Whitney U test, Significance at p value ≤ 0.05

Chapter 4 Results for Demographic data

Princess Anne Hospital has approximately 8000 deliveries every year and about 700 are admitted to the neonatal unit. Most of the 8000 babies born are well enough to stay with their mothers immediately after birth while they learn to feed before going home. Most babies admitted to the neonatal unit are those born before the expected date for delivery. The further a baby is born from their due date, higher the likelihood of the need for specialist care after birth. Some babies born with abnormalities in the structure and function of various organs in their body also need to be cared for in the neonatal unit. For this study babies were recruited through convenience sampling. Following the first 20 recruited pre-term babies, further recruitment purposefully targeting specific gestational age groups was undertaken.

4.1 Demographic data

In total 75 patients were considered eligible for this study, but not all could be approached in a timely manner due to various reasons. These included mothers and/or baby being too unwell after birth 2 mothers and 4 babies). The burden of consenting process was too overwhelming for the parents of 2 sets of twins. Parents so could not provide consent. I one instance, enough time was not left to recruit before 48 hours of age as needed for satisfying the eligibility criteria. 3 mothers wished to consult with partners before consenting and got delayed in providing consent. Subsequently, 65 parents were approached for consent and 59 consented to participate in the study.

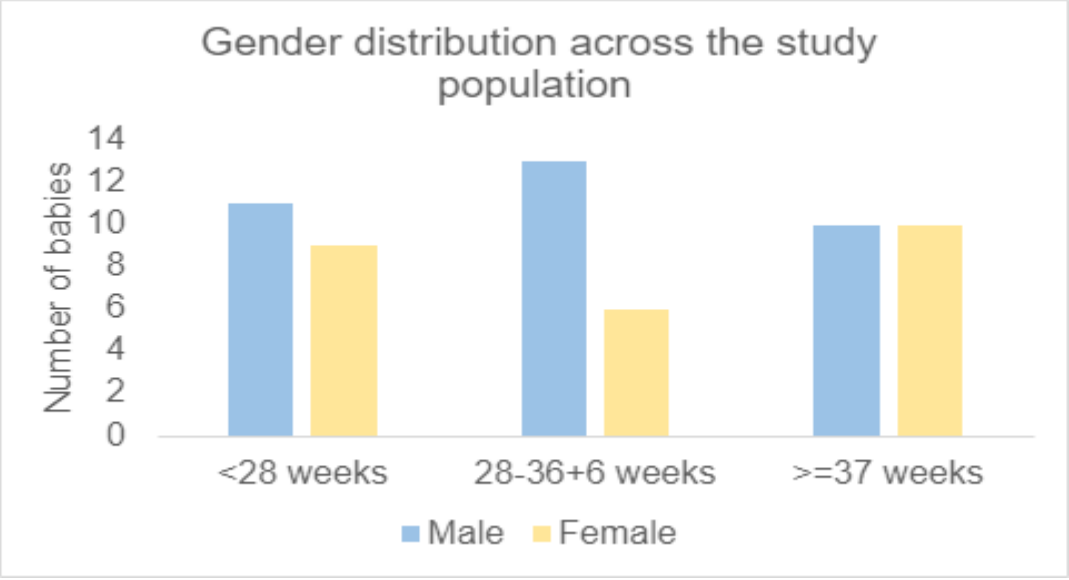
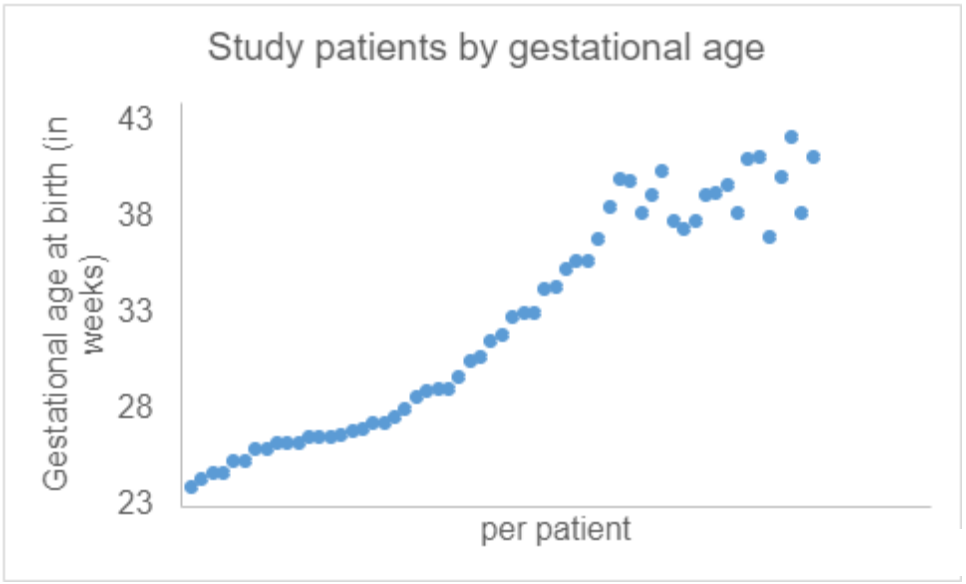
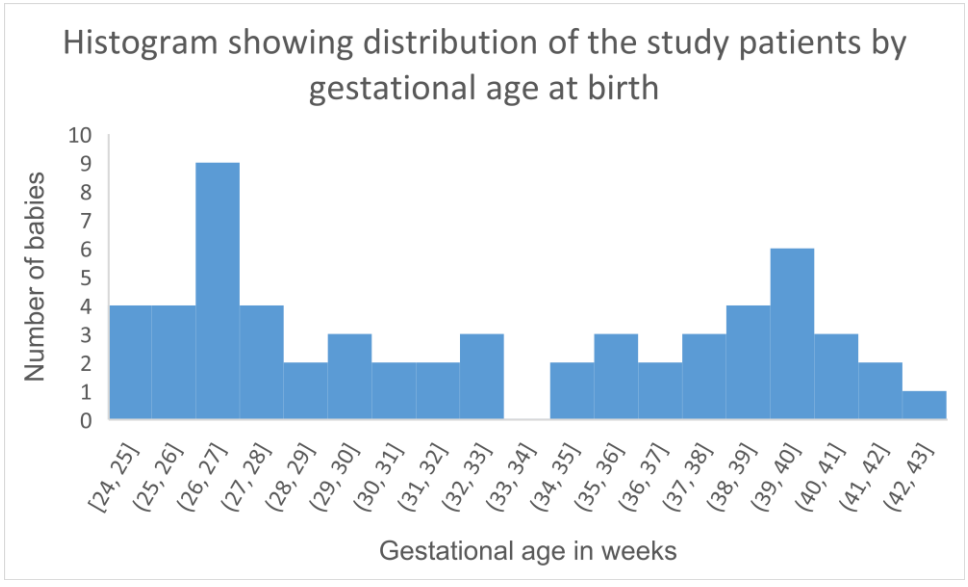


Figure 4-1 Gender distribution across the study population

Babies were recruited from diverse gestational ages which ranged between 24-42+1 weeks. The median for gestational age was 31+6. The median among babies born at term (>36+6 weeks at birth) was 39+1 and for preterm babies (<37 weeks at birth) was 27+4. The recruitment was purposeful with the aim to include patients from varying gestations, to have the opportunity to explore data for each gestational age group.



A



B

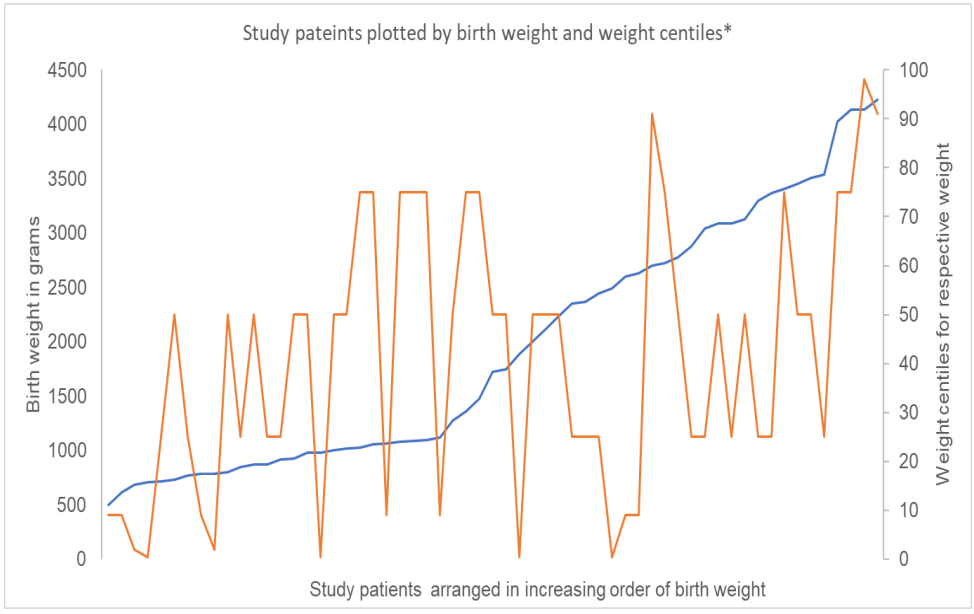
Figure 4-2 Study patients by gestational age

A: Scatter plot of all study patients by gestational age

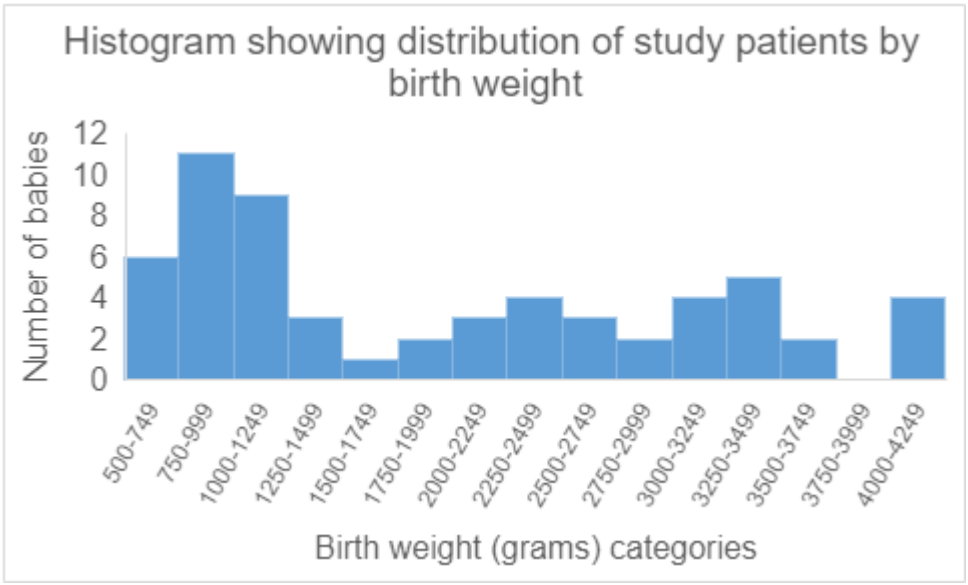
B: Frequency distribution of all study patients by gestational age groups

The purposeful recruitment strategy was as follows:

1) 20 babies in the study were born at term.
2) 19 babies were those born at gestational age of 28-36+6 weeks.
3) 20 babies were those born at gestational age less than 28 weeks.



A



B

Figure 4-3 Study patients by birth weight

A: Scatter plot of all study patients by birth weight

B: Frequency distribution of all study patients by birth weight groups

As shown in Figure 4-1 the distribution of male and female babies is comparable in two groups (<28 weeks and 28-36+6 weeks) but more male babies were present in 28-36+6 week group. The graph in Figure 4-2A shows that distribution of the study population was balanced across the gestational age. However, when looking at the histogram (Figure 4-2B) data by classifying babies by weeks of gestational age at birth bimodal distribution was observed.

The graph in Figure 4-3A shows that distribution of the study population by birth weight was balanced across the spectrum of birth weights. However, when looking at the histogram (Figure 4-3B), by classifying babies by birth weight groups a bimodal distribution of patients emerges, like the histogram for gestational age in Figure 4-2B.

The bar chart in Figure 4-4 shows that more preterm babies were born by caesarean section than by normal vaginal delivery. On the contrary more term babies were born by normal vaginal delivery.

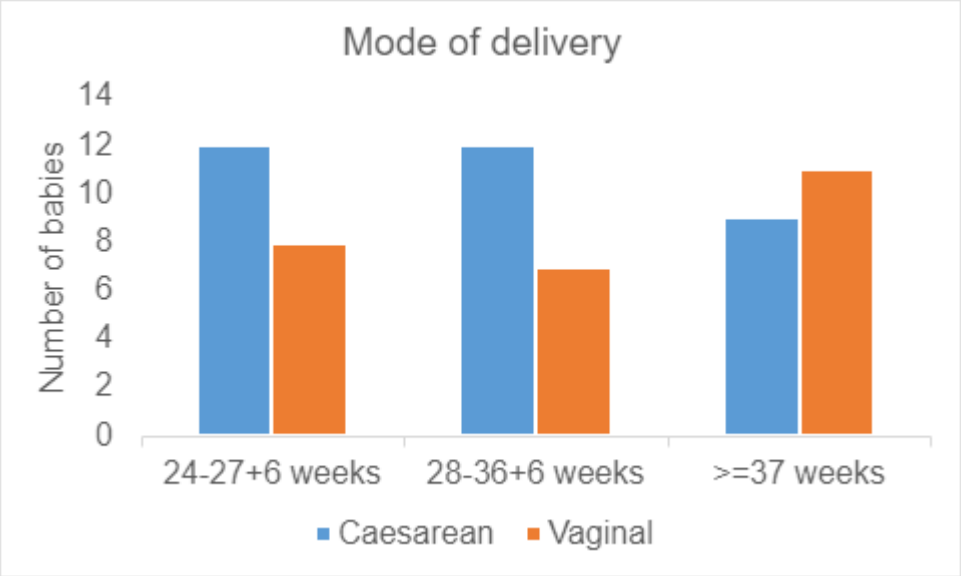


Figure 4-4 Study patients by mode of delivery

Data was collected on 59 patients between August 2017 and August 2018. Babies were recruited from diverse gestational ages with a mean gestational age 33+5(24-42+1 weeks) and mean birth weight 2253g (500-4230g). One baby was born to parents of Indian origin (CFNST16), another one to Caribbean ethnicity (CFNSPT18), rest were all born to white European parents. In total 752 measurements were analysed across the study. Individual demographics of these babies are shown in Tables 4-1 and 4-2.

Table 4-1 Recruitment of term babies

ID	Sex	Birth weight (g) and centile		GA (weeks)	Mode of delivery	Place of care	No. of IC days	Days of stay	Discharge location	No. of Measurements
CFNS T1	F	3090	50	38+4	Vaginal	NICU	0	3	Home	1
CFNS T2	M	3370	25	40+0	Vaginal	NICU	0	3	Home	1
CFNS T3	F	3505	50	39+6	Vaginal	NICU	0	4	Home	2
CFNS T4	F	2600	9	38+2	Vaginal	NICU	41	72	Home	1
CFNS T5	M	3450	50	39+1	LSCS	NICU	31	57	Home	2
CFNS T6	M	3300	25	40+3	LSCS	NICU	0	3	Home	2
CFNS T7	M	3405	75	37+6	LSCS	NICU	4	31	Home	2
CFNS T8	F	2780	50	37+3	Vaginal	NICU	2	3	Home	2
CFNS T9	M	2630	9	37+6	Vaginal	NICU	3	39	Home	2
CFNS T10	M	3040	25	39+1	LSCS	NICU	37	37	Died	2
CFNS T11	F	4135	98	39+2	LSCS	NICU	6	11	Home	2

CFNS T12	F	3090	25	39+5	Vaginal	PNW	0	2	Home	2
CFNS T13	F	2875	25	38+2	Vaginal	PNW	0	2	Home	2
CFNS T14	M	4025	75	41+0	LSCS	PNW	0	2	Home	2
CFNS T15	M	4130	75	41+1	Vaginal	PNW	0	2	Home	2
CFNS T16	F	1888	0.4	37+0	LSCS	NICU	6	15	Home	2
CFNS T17	M	2490	0.4	40+1	LSCS	PNW	0	3	Home	2
CFNS T18	F	4230	91	42+1	Vaginal	PNW	0	2	Home	2
CFNS T19	F	3130	50	38+2	Vaginal	PNW	0	2	Home	2
CFNS T20	M	3540	25	41+1	LSCS	PNW	0	2	Home	2

Table 4-2 Recruitment of preterm babies

ID	Sex	Birth weight (g) and centile		GA in weeks	Mode of delivery	Place of care	No. of IC days	Days of stay	Discharge location	No. Measurements
CFNS PT1	F	2700	91	34+2	Vaginal	PNW	0	0	Home	2
CFNS PT2^	M	2370	25	35+5	LSCS	PNW	0	0	Home	2
CFNS PT3^	M	2355	25	35+5	LSCS	PNW	0	0	Home	2
CFNS PT4	M	1750	50	31+6	LSCS	NICU	0	23	Home	5
CFNS PT5	M	2000	50	32+6	Vaginal	NICU	0	21	Home	4
CFNS PT6	F	980	0.4	33+0	LSCS	NICU	5	47	Home	7
CFNS PT7^	M	790	2	29+1	LSCS	NICU	6	73	Home	7
CFNS PT8^	M	1275	50	29+1	LSCS	NICU	6	45	Home	8
CFNS PT9	F	2443	25	36+6	Vaginal	NICU	7	142	Home	2
CFNS PT10	F	690	2	28+0	LSCS	NICU	21	100	Home	7
CFNS PT11	F	2725	75	35+2	Vaginal	NICU	0	5	Home	2

CFNS PT12	M	2235	50	34+3	Vaginal	NICU	0	6	Home	2
CFNS PT13	M	1725	50	31+4	LSCS	NICU	1	24	Home	6
CFNS PT14^	F	730	50	24+5	LSCS	NICU	41	115	Home	7
CFNS PT15^	M	800	50	24+5	LSCS	NICU	39	115	Home	6
CFNS PT16	M	1475	75	29+0	Vaginal	NICU	12	51	Home	7
CFNS PT17	F	790	9	27+4	LSCS	NICU	41	92	Home	7
CFNS PT18	F	1080	75	27+0	LSCS	NICU	20	68	Home	6
CFNS PT19	M	2120	50	33+0	Vaginal	NICU	0	11	Home	4
CFNS PT20	F	870	25	26+6	LSCS	NICU	13	106	Home	4
CFNS PT21	M	1360	75	28+5	LSCS	NICU	7	42	Home	7
CFNS PT22	F	1120	9	30+5	LSCS	NICU	2	37	Home	7
CFNS PT23^	F	870	50	26+2	LSCS	NICU	21	78	Home	6
CFNS PT24^	M	1060	75	26+2	LSCS	NICU	34	97	Home	6
CFNS PT25^	F	1090	75	27+2	Vaginal	NICU	12	73	Home	5
CFNS PT26^	F	850	25	27+2	Vaginal	NICU	12	72	Home	5

CFNS PT27	M	1065	9	30+4	LSCS	NICU	2	40	Home	7
CFNS PT28	M	710	0.4	29+5	LSCS	NICU	7	66	Home	7
CFNS PT29[$]	F	926	50	26+4	Vaginal	NICU	17	98	Home	6
CFNS PT30[$]	M	1004	50	26+4	LSCS	NICU	92	23	Home	6
CFNS PT31[$]	M	1016	50	26+4	LSCS	NICU	23	92	Home	6
CFNS PT32	M	1026	75	26+0	Vaginal	NICU	20	20	Died	4
CFNS PT33[^]	M	770	25	25+2	Vaginal	NICU	85	141	Ward	7
CFNS PT34[^]	M	715	25	25+2	Vaginal	NICU	103	135	Home	7
CFNS PT35	M	980	50	26+0	LSCS	NICU	49	77	Home	7
CFNS PT36	M	920	25	26+5	LSCS	NICU	20	64	Home	7
CFNS PT37	F	500	9	24+0	LSCS	NICU	66	117	Died	7
CFNS PT38	M	1100	75	26+2	Vaginal	NICU	22	96	Home	7
CFNS PT39	M	620	9	24+3	Vaginal	NICU	46	46	Died	5

NB ([$] = triplets, [^]=Twins)

4.2 Preterm demographic results

39/59 (66%) recruited babies were <37 weeks at birth, their mean gestational age was 29±3.6 weeks (range 24+1-36+6). To enable measurement of physiological characteristics in extreme preterm babies, purposeful recruitment from lower gestational ages was conducted (41% were <27 weeks and 67% were <30 weeks). The mean birth weight among preterm babies was 1272 ± 630g (500-2725g). 21% of the preterm babies were small-for-gestational age (weight <10th centile), corresponding to the recent population statistics published by INTERGROWTH 21 (Lee et al., 2017)

Table 4-3 Measurements in incubator humidity of infants born at <30 weeks.

GA group (n)	Days of life when measured in humidity	Incubator humidity	Total number of measurements in humidity (number of measurements for each infant)	Total measurements
24-24+6 (4)	2-16	90-55%	16(3-5)	21
25-25+6 (2)	2-14	80-55%	7(3-4)	12
26-26+6 (10)	1-16	80-40%	41(2-5)	57
27-27+6 (4)	1-14	80-45%	20(5)	21
28-28+6 (2)	1-13	80-45%	7(4-5)	12
29-29+6 (4)	2-10	75-40%	12(3-4)	23

Most of the preterm babies (92%) were admitted to NICU, and amongst the <30-week babies more than 80% spent 38 days in intensive care for every 100 hospital admission days. In the ≤26 week's cohort, more than 75% spent at least 50 intensive care days per 100 hospital admission days. Three preterm babies died before discharge; data was retained for analysis (as per the ethics statement).

The measurement schedule (Table 3-1) was followed closely, and more measurements were completed in the first 2 weeks, as it is the period of rapid skin changes and when the effect of incubator humidity on TEWL could be assessed (Table 4-3 and 4-4).

Table 4-4 Amount of humidity by gestation and age

GA	Days of life	Number of babies in ambient humidity						
		90%	80%	70%	60%	50%	40%	nil
24 weeks (n=4)	48h	2	2					
	7d	2	1	1				
	14d		1	1	2			
	21d				1	1	1	1
25 weeks (n=2)	48h		2					
	7d		2					
	14d		1			1		
	21d				1			1
26 weeks (n=10)	48h		10					
	7d		6	4				
	14d				3	1	2	3
	21d						1	8
27 weeks (n=4)	48h	4						
	7d	4						
	14d					3	1	
	21d							2
28 weeks (n=4)	48h		2					
	7d		1		1			
	14d				1			1
	21d							2
29 weeks (n=4)	48h		1	3				
	7d				3	1		
	14d						1	3
	21d							4

4.3 Term demographic results

Of the 59 babies recruited 20 (34%) were born at term with the mean birth weight 3235 ± 395g (1888-4230g). A quarter of the term babies were small-for-gestational age (birth weight <10th centile) and half were girls, representative of the general population. 60% of the babies born at term were recruited from NICU. Six babies born at term needed surgical input [congenital diaphragmatic hernia (two), gastroschisis (two), omphalocele (one) and meningomyelocele (one)] and another 6 needed a short admission to the neonatal unit for observations. 17/20 term babies were able to have two sets of measurements (TEWL, pH, USS), as per protocol. All measurements were performed on the front or lateral aspect of either thigh, depending upon the resting position of the baby at the time of measurements.

4.4 SENNAT data

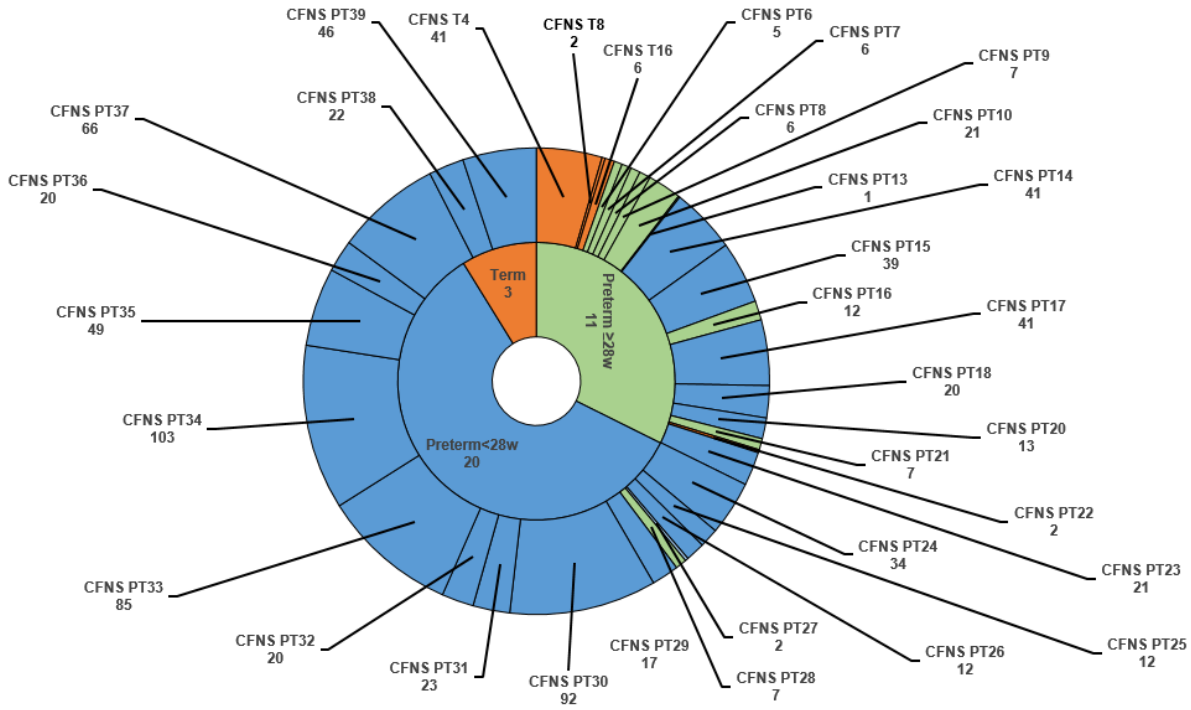


Figure 4-5 Nutrition data

The doughnut chart shows the nutrition data for the term and preterm infants. The innermost tier of the doughnut shows that nutrition data was available for 20 preterm infants <28 weeks at birth (blue), 11 preterm infants ≥28 weeks (green) and 3 term infants (orange). The outermost tier is labelled with the patient identification and the number of days of nutrition data available for each infant with same colour coding in all the tiers.

Of the 59 patients, detailed nutrition and anthropometric information was collected for 44 patients in total, 35 preterm and 9 term infants. Data was also collected for weight, head circumference, length as well as nutritional intake and blood test results for each of these patients (Figure 4-5).

The doughnut chart in Figure 4-5 shows that all the infants <28 weeks at birth had nutrition data available. Only 11/19 preterm infants ≥28 weeks at birth and 3/20 term infants had nutrition data. This is because the babies with lowest gestational age stayed the longest in the neonatal unit and needed assisted feeding, parenteral or enteral, till they could maintain their nutrition without support in the neonatal unit. This gave an opportunity to measure the nutrient intake along with their growth (weight, head circumference and length) and study the effect of the nutrients on the measured features in the skin.

4.5 Exclusions

The study case numbers CFNS PT1, 2, 3 (GA 34, 35, 35 weeks respectively) had unusually large TEWL values (41, 30 and 37g/m^2/h). These babies were the first three TEWL measurements by the operator in preterm babies, hence the unusual values could be attributed to operator use. These measurements were also taken outside the NICU (CFNS PT 1 in adult ITU, CFNS PT 2 and 3 in postnatal ward) where the ambient temperature is not as well maintained as the NICU. Due to these reasons these 3 patients were removed from all the statistical analyses.

Chapter 5 Results for Biophysical analysis

Results from TEWL and pH data were analysed for term and preterm babies separately and comparison was made between the term and preterm TEWL and well as pH. The first TEWL measurements decreased as gestational age at birth increased. TEWL at birth is less than 10g/m^2/h in late preterm babies suggesting that the stratum corneum is more mature in them than extreme preterm babies (Figure 5-1).

5.1 Results from TEWL data

The first TEWL measurements (<48 hours of age) decreased as gestational age at birth increased (Figure 5-1). The TEWL at <48 hours of age in <28-week babies ranged from 14-31 g/m^2/h and was less than 10 g/m^2/h in >34 weeks preterm babies, suggesting that when measured within 48h of age, stratum corneum was functionally more mature in late preterm babies than in extreme preterm babies (Figure 5-1).

5.1.1 Gestational age and weight

Table 5-1 Statistical significance GA categories and TEWL at <48h of age

GA groups(n)	Mann Whitney U	p-value	Median difference	95% Confidence interval
24-26+6 (16) vs 27-29+6 (10)	40	**0.034**	4.8	0.79-0.89
24-26+6 (16) vs 30-33+6 (7)	20	**0.016**	6.2	0.2-12.09
24-26+6 (16) vs 34-36+6 (3)	0	**0.007**	12.1	9.45-14.77
24-26+6 (16) vs 37-42 (19)	23	**0.001**	10.6	7.52-13.78

TEWL changed within the first week of life (Figure 5-1). Generally, the trend was towards normative values of TEWL <10g/m^2/h. While babies born 28-37 weeks had TEWL close to or less than 10, the more preterm babies (<28 weeks) were all born with TEWL >>10g/m^2/h with the second measure decreasing in most cases. The correlation of TEWL values in the first week with GA showed that TEWL was significantly higher at lower gestational age (r_s= 0.620,

p= 0.001, n=36) (Table 5-1). Further statistical tests revealed significance of TEWL with GA groups and birth weight categories, summarised in Table 5-1 and 5-2. Indeed, the difference in TEWL was significant across all GA groups, but the significance in terms of birth weight groups was not seen consistently.

Table 5-2 Statistical significance of weight categories and TEWL at <48h of age

Weight categories (n)	Mann Whitney U	p-value	Median difference	95% confidence intervals
500-800 (9) vs 800-1200(17)	64	0.49	0.62	-4.08-5.32
500-800 (9) vs 1200-1500 (3)	3	**0.049**	8.44	3.69-13.20
500-800 (9) vs1500-2000 (3)	8.5	0.35	6.11	-4.26-16.48
500-800 (9) vs 2000-2500 (5)	3	**0.009**	10.04	5.40-14.69
500-800 (9) vs 2500-4500 (18)	18	**0.001**	9.50	4.74-14.26

5.1.2 Maternal indices

Data from 51 mothers of 59 babies was analysed. The median maternal age was 30 years (range 18-43 years). Amongst the mothers of preterm babies BMI was available for 17/31, median BMI was 25.5 (range 19-40). The TEWL at <48hours of age was not statistically significantly influenced by maternal age and BMI. 36/39 babies (92%) and 28/31 (90%) mothers received antenatal steroid. Though 24/36 (67%) babies were born after 2 doses of antenatal steroids as per NICE guidelines (Wapner et al., 2016, NICE, 2010) only 13/31 (46%) mothers and 17/36 (47%) babies received the adequate dose of antenatal steroids (2 doses of 12mg Betamethasone 24 hours apart, within 1-7 days of birth) for optimum clinical benefit. A Mann Whitney U test was used to demonstrate the difference between early TEWL (<48h of age) in babies with and without adequate antenatal steroid. No statistical significance was found in the TEWL ($p>0.05$). The TEWL in >27 weeks babies born to mothers without adequate/no antenatal steroid cover was not statistically different ($p >0.05$) to those with adequate steroid cover. There were no significant differences in <48hr TEWL with all other maternal indices.

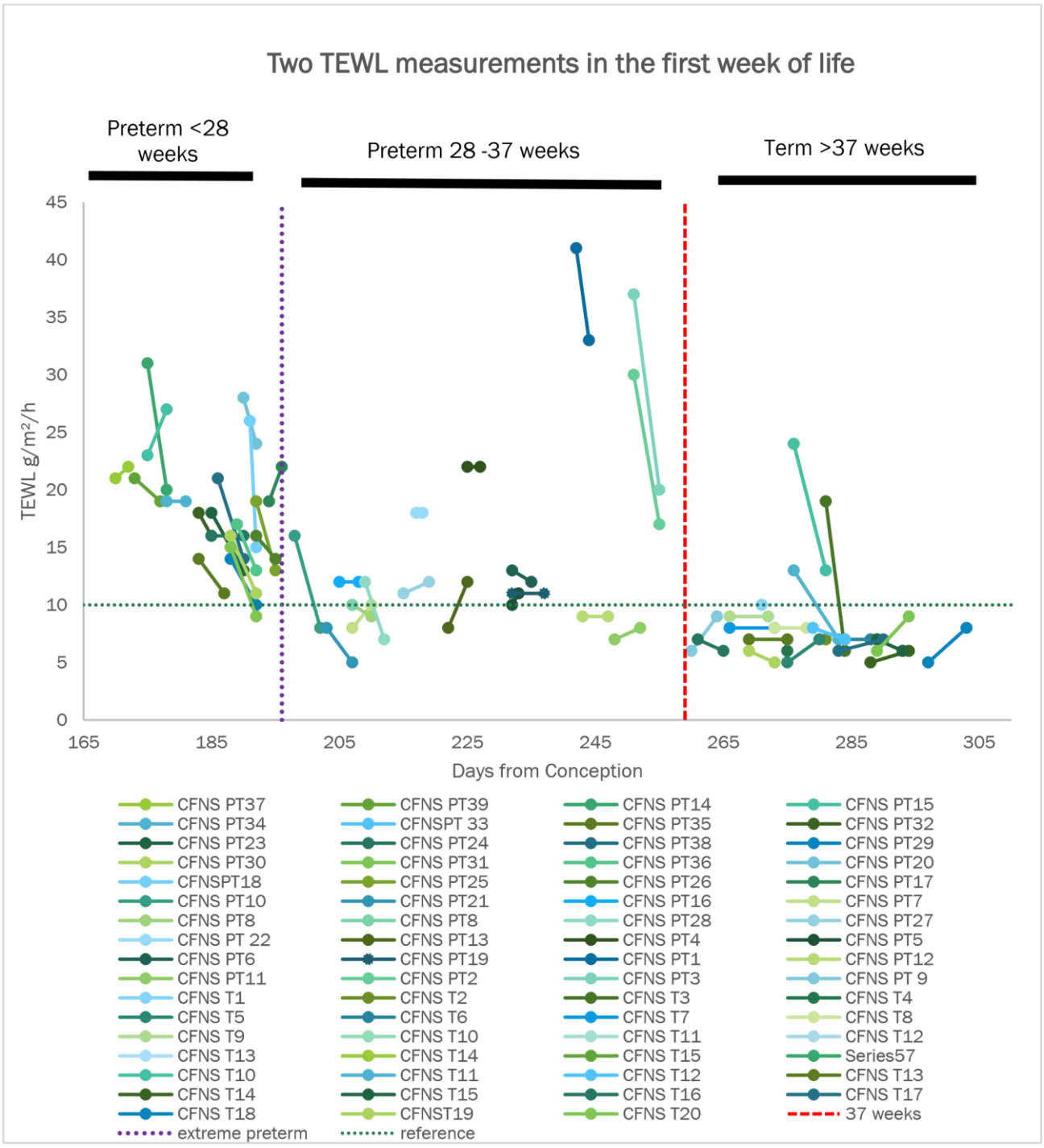


Figure 5-1 Two TEWL measurements in the first week plotted against post-menstrual age.

5.1.3 Chronological changes

As detailed in the methodology (Table 3-1) TEWL was measured serially in all preterm babies, during their stay in the hospital. Time to reach TEWL ≤10g/m^2/h was clearly dependent on GA at birth (Figure 5-2). The median time to reach TEWL ≤10g/m^2/h for GA 24-26+6 weeks was 11.5 days (62 days in one baby to achieve TEWL ≤10g/m^2/h), while it was 6.5, 4.5 and 2 days for GA groups 27-29+6, 30-33+6 and 34-36+6 weeks respectively. It is noted that TEWL measurements for <30-week babies were made in incubator humidity (as described in section 3.3.1.1), and hence the initial decrease in TEWL is not completely attributed to full maturation of epidermal barrier (Figure 5-3 and 5-4).

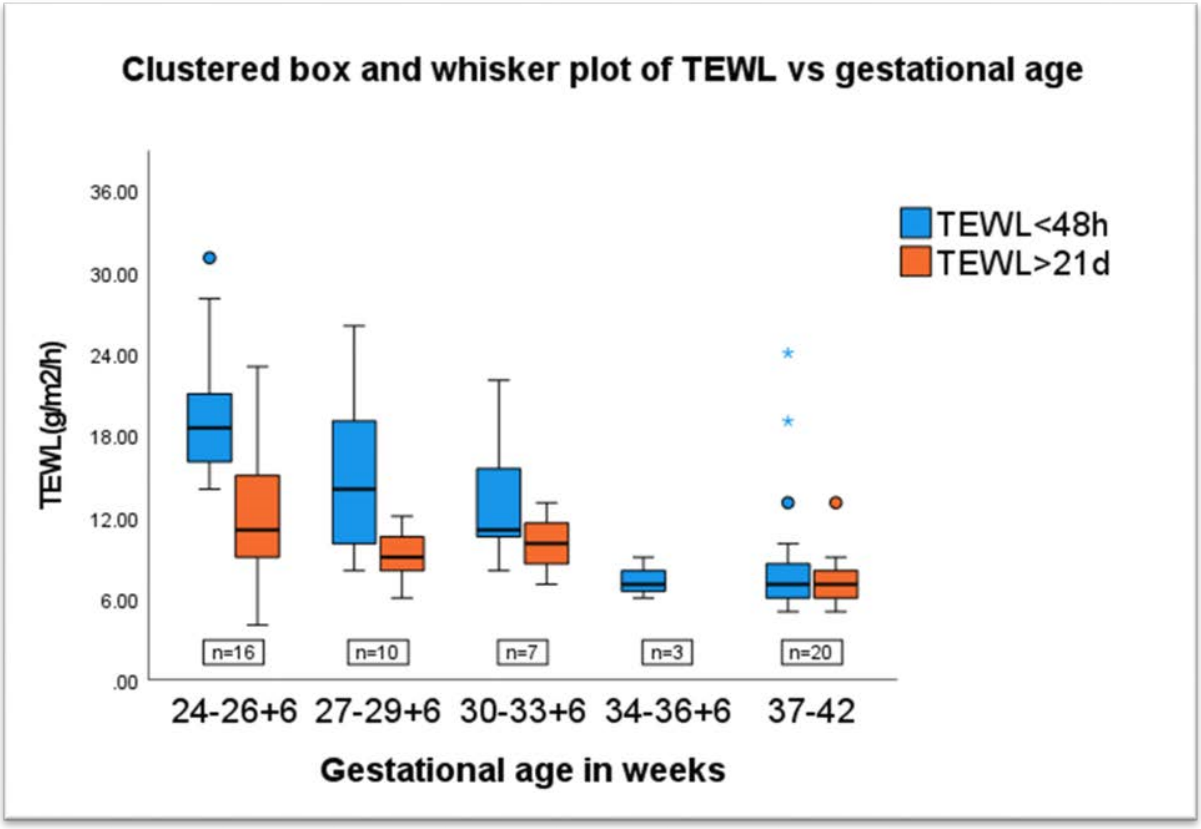


Figure 5-2 Variation in TEWL with gestational age categories and chronological age

TEWL decreases with increasing GA as well as with advancing postnatal age in all categories. The outliers in GA 24-26+6- and 37-42-weeks categories were included in all the statistical tests applied.

The first decrease in TEWL did not persist, and it only decreased to <10 g/m²/h between 3-4 weeks of age in the extremely preterm babies (Figure 5-4). A Spearman's rank-order

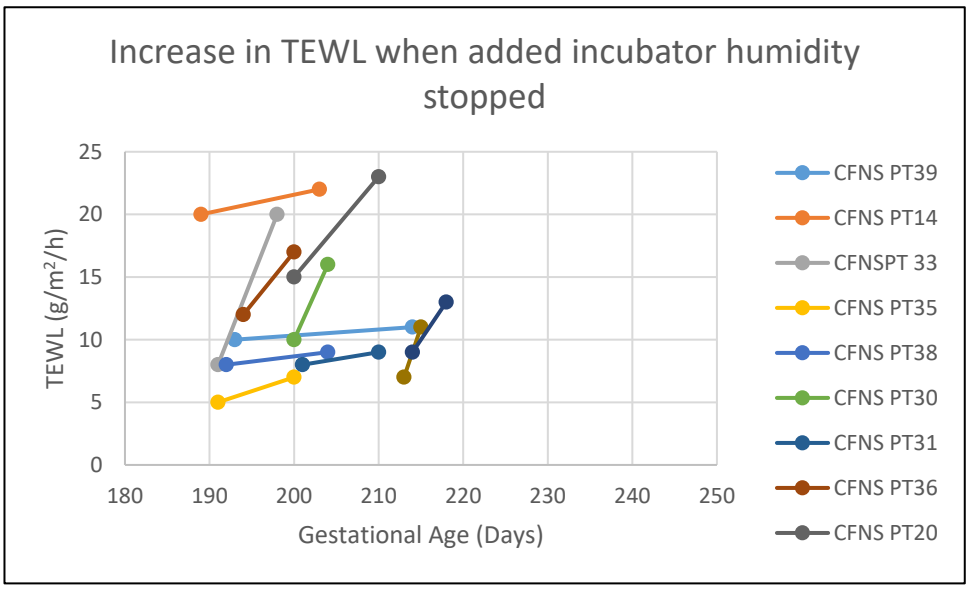
correlation was used to determine the relationship between GA at birth and time to reach TEWL ≤10g/m^2/h. There was a strong, negative correlation between GA at birth and days to reach TEWL≤10g/m^2/h, which was statistically significant (r_s = - 0.633, p = 0.001). There was a statistically significant difference (p<0.05) between TEWL at <48h and >21 days in all preterm babies (Z = -4.08, p = 0.001). Indeed, TEWL was significantly lower at >21 days of age in preterm babies taken as a group. The same comparison was made by GA groups (Table 5-3). The median TEWL decreased from 19.4 to 12.3 g/m²/h (<27week GA group) and 14.6 to 9.5 g/m²/h (27-29+9week GA group) between the two measurements at <48hours and >21 days (Figure 5-2). The statistical significance of difference in TEWL at <48h and >21 days ranged from highly significant in GA 24-26+6 weeks (p=0.001) to not significant in GA 30-33+6 weeks (p=0.59) (Table 5-3).

Table 5-3 Wilcoxon signed-rank test results for TEWL at <48 hours and >21 days.

GA groups	n	Z	p-value
24-26+6 weeks	15	-3.4	**0.001**
27-29+6 weeks	8	-2.03	**0.04**
30-33+6weeks	4	-0.53	0.59

5.1.4 Effect of Incubator Humidity on TEWL

As mentioned in the previous section 5.1.3 the drop in TEWL to more mature levels (≤10 g/m²/h) within first 2 weeks in ≤27-week babies and within one week in >27week babies (Figure 5.4) can be the influence of incubator humidity rather than maturation of SC barrier. The data was also analysed to assess effect of humidity on TEWL by charting the change in TEWL when humidity was ceased (Figure 5-3). Out of 24 patients with TEWL measured before and after stopping humidity, four had same TEWL value following the change in humidity. 12 patients had increased TEWL upon stopping incubator humidity and 8 had decreased TEWL in similar circumstances. On comparison the increased TEWL after stopping humidity was significantly higher than the decreased TEWL after stopping humidity. (Mann Whitney U test U=36, p-value 0.039).



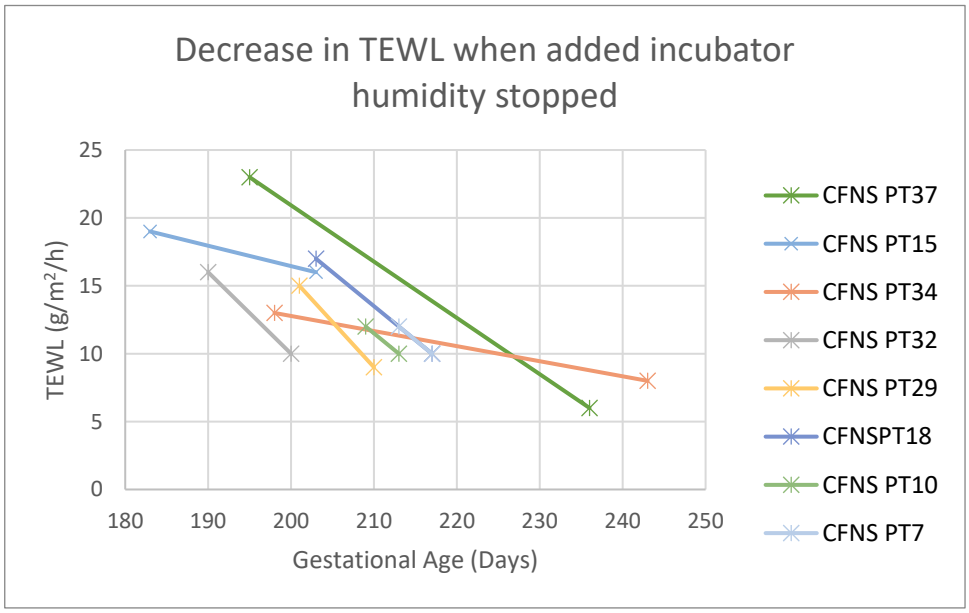


Figure 5-3 Change in TEWL with cessation of incubator humidity (Increase in TEWL n=8, Decrease in TEWL n=12)

The first measurement depicted in these charts is in humidity and the second measurement is the one taken without added incubator humidity.

As described earlier, TEWL decreases in presence of humidity in all babies but rises again when humidity is stopped (also shown in Figure 5-4). The amount of decrease and increase in TEWL is dependent on multiple factors and not explored fully in this study. As shown in Figure 5-4 while TEWL decreases to ≤10 g/m²/h within 2 weeks of age in 24, 25 and 26weeks GA, it increases again as the incubator humidity is removed indicating that the SC barrier is not fully matured, until TEWL drops again at later age. Same is true for babies with GA 28

and 29 weeks, but in this group the initial drop in TEWL occurs by 1 week of age, coinciding with the application of incubator humidity as per protocol.

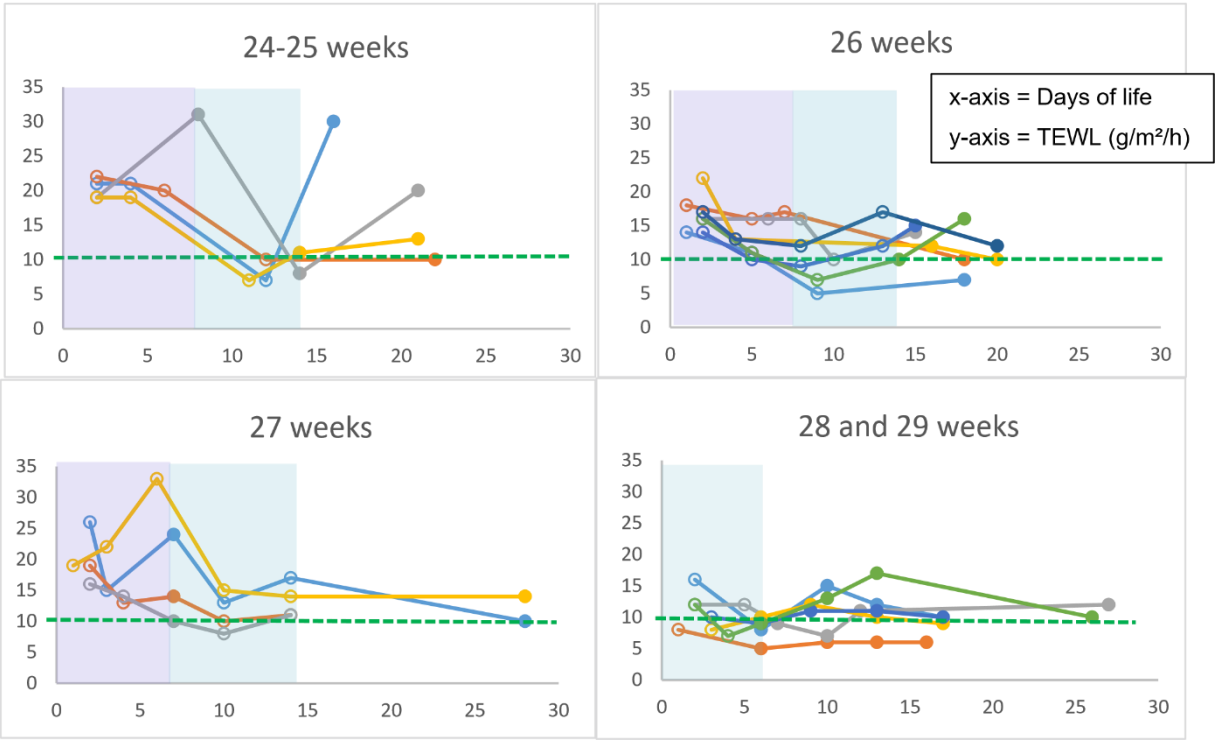


Figure 5-4 Representation of time (in days) to reach TEWL≤10 g/m²/h in individual babies at GA 24-25, 26, 27 and 28-29-week babies in relation to incubator humidity.

(□ = 80-90% Incubator humidity and □ = 40-80% Incubator humidity).

The graphs depict rise in TEWL with decrease and cessation of humidity. The y-axis from 0-35 is allowing clear appreciation of change in TEWL, being more in 24-25, 26 and 27 week charts.

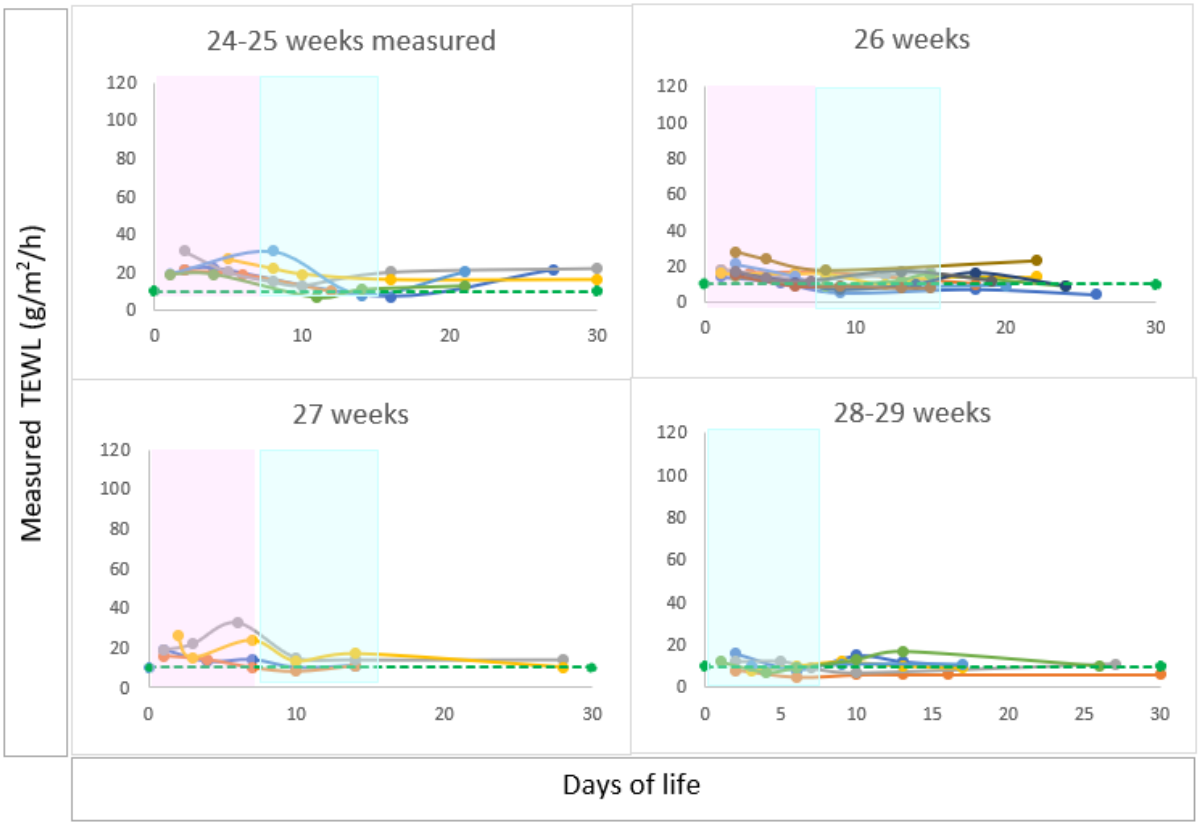


Figure 5-4 Representation of time (in days) to reach TEWL≤10 g/m²/h in individual babies at GA 24-25, 26, 27 and 28-29-week babies in relation to incubator humidity.

(□ = 80-90% Incubator humidity and □ = 40-80% Incubator humidity).

Same graph with a different y-axis (0-120), to allow comparison with Figure 5-5.

TEWL was also calculated using the correction factor detailed in Section 3.3.4. Here values were adjusted for the RH setting in the incubator by calculating cTEWLH50. The results revealed a substantial increase in cTEWL50 values (Figure 5-5), compared to the absolute values (Figure 5-4). These values were more comparable as RH in the incubator was reduced to no added incubator humidity (thus making the correction factor redundant for TEWL values measured without incubator humidity).

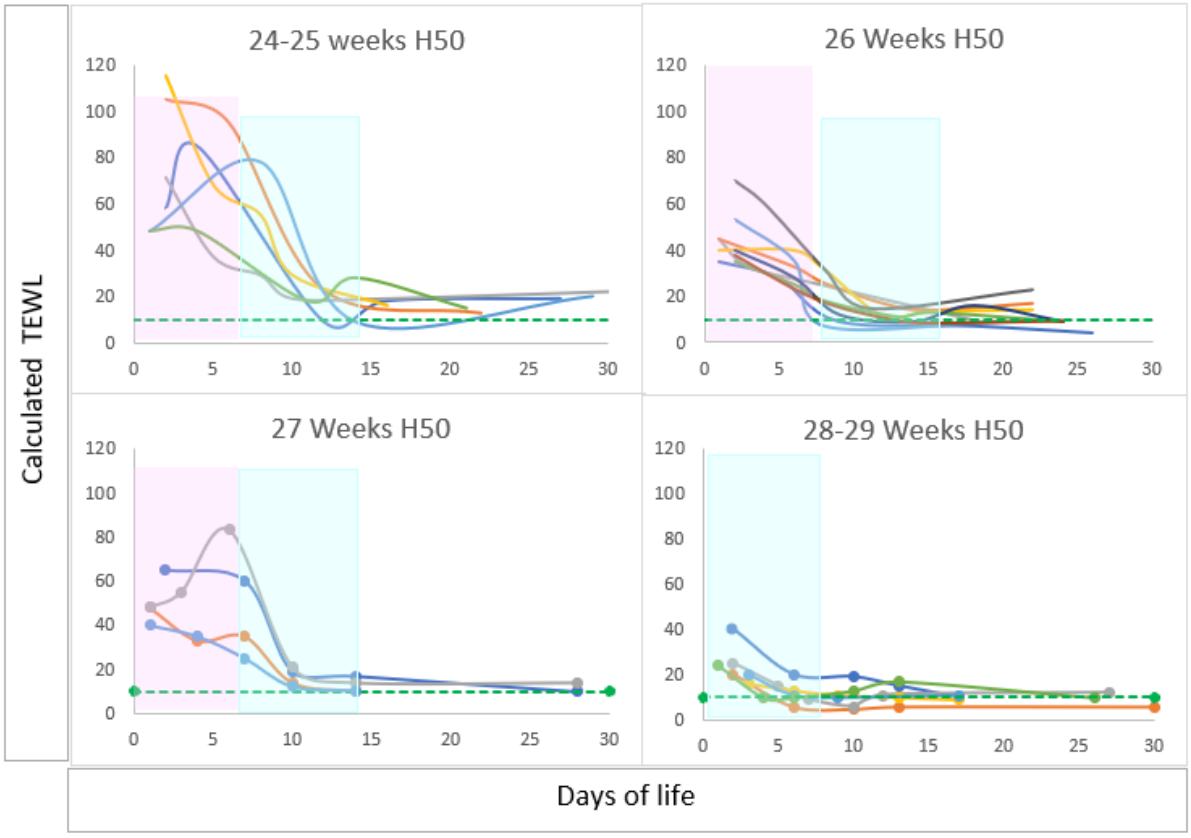


Figure 5-5 Representation of time (in days) to reach calculated TEWL≤10 g/m²/h in individual babies at GA 24-25, 26, 27 and 28-29 week babies in relation to incubator humidity of 50% rather than actual incubator humidity

(□ = 80-90% Incubator humidity and □ = 40-80% Incubator humidity).

The shaded areas representing incubator humidity during measurement remain on the chart for reference only. The graph however is drawn to depict the calculated TEWL if incubator humidity was 50% (cTEWL50)

The Figure 5-5 showing calculated TEWL at 50% humidity demonstrates that TEWL would be much higher if incubator humidity was decreased to 50% (from 80%), reinforcing the need for incubator humidity to keep the transepidermal loss in clinically manageable range so that haemostasis is easier to maintain while the epidermal barrier is maturing. The use of incubator humidity makes the use of TEWL measurements as proxy for SC maturity impossible as the influence of ambient humidity on TEWL measurements change these values significantly (decreasing them to nearly mature SC barrier). This is the reason for using cTEWLH50 values as they reflect the SC maturation truly, as described in Section 3.3.4.

cTEWLH50 is much higher than measured TEWL indicating that the ambient humidity is artificially decreasing the TEWL. Hence by correcting TEWL using the formula a better idea of transepidermal water loss and immaturity of the skin barrier is obtained.

5.2 pH Results

The mean pH in <48 hours in preterm babies was 6.4 (range 5.1-7.1) and in term babies was 6.7 (range 5.9-8.9). There were three term babies with pH 7.9, 8 and 8.9 at <48 hours which decreased on second measurement before 7 days of age in two of them (no second measurement in one baby) (pH 8→5.8 and pH 8.9→6.4). The mean pH in term babies at 1 week of age was 6.2 (range 5.4-6.9) (not shown in Figure 5-6).

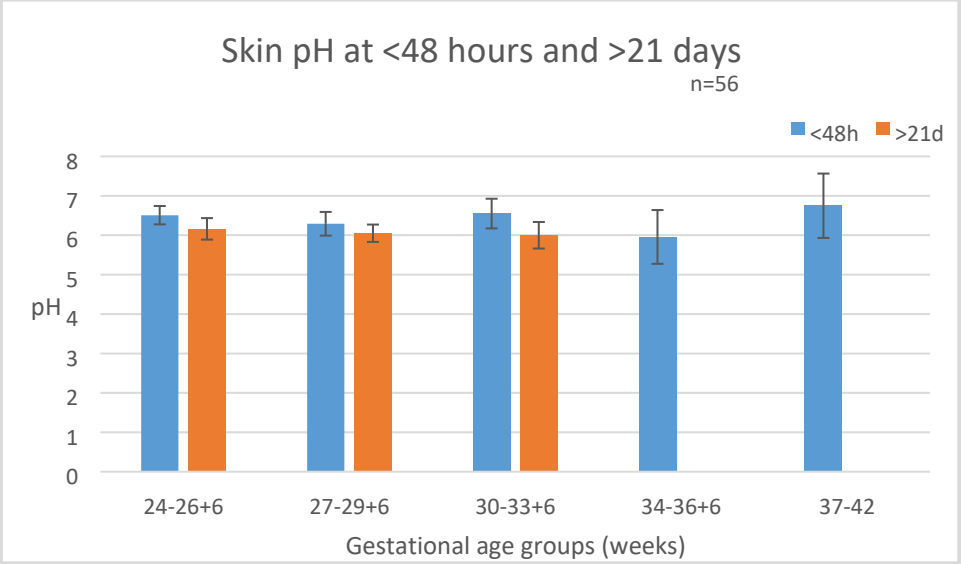


Figure 5-6 Skin pH with gestation and age

As shown in Figure 5-6, pH is elevated at <48hours, with the maximum value of 6.8 (mean 6.5) in the most premature group. This declined chronologically to mean pH 6, closer to the more mature acid mantle by 21 days post-birth especially in the most mature group. A dependent paired t-test has revealed a significant difference between pH at <48h and >21 days. This comparison could be made in 27 preterm babies (Table 5-4).

Table 5-4 Dependent paired t-test results for pH at <48 hours and >21 days

GA groups	n	Difference of means	t(d*f*)	p-value
24-26.9 weeks	15	0.45	t (14) = 4.3	**0.001**
27-29.9 weeks	8	0.2	t (7) =1.3	0.23
30-33.9 weeks	4	0.42	t (3) =5.1	**0.014**

The decrease in pH from <48h to >21 days in GA group 24-26+6 weeks (n=15) was statistically significant, decreasing from 6.5±0.1 to 6.2±0.3 ($p<0.05$), a decrease of 0.3±0.0. And the decrease in pH at the two time points in GA group 30-33+6 weeks (n=4) was 6.7±0.3 to 6±0.3, a decrease of 0.6±0.05 ($p<0.05$). The decrease in pH in the other GA groups was either not statistically significant or not possible to compare due to unavailability of values at >21 days as shown in the bar chart (Figure 5-6). The changes indicate decrease in skin pH to develop the acid mantle, which is not complete at 3 weeks of age.

5.3 Comparisons between term and preterm babies

As shown in the box and whisker plot in Figure 5-7, the TEWL is elevated in the premature babies in comparison to the term infants, with median value of 19 g/m2/h in the most premature group. This declined for each increasing gestational age group at <48 hours and had started to mature in all the groups by 21 days of age (median TEWL is 11 g/m²/h in 24-26+6 weeks, 9 g/m²/h in 27-29+6 weeks and 10 g/m²/h in 30-33+6 weeks, 7 g/m²/h in 37-42 weeks).

Basal TEWL values revealed distinct differences between different categories of birth weight and gestational age. Indeed, those with the lower birth weight (<1200g) had significantly ($p<0.05$) higher TEWL values than those with birth weight normative values (2500-4500g) (Figure 5-7A). The median difference in TEWL between the lowest birth (500-800g) and highest birth weight (2500-4500g) was 11.5 g/m2/hr. There was also an increasing trend in high TEWL values with lower gestational age (Figure 5-7B). The only significant difference was observed between the most premature (24–27week GA) group and term infants (38-42 weeks GA), with a median difference of 11.1 g/m2/hr ($p<0.01$).

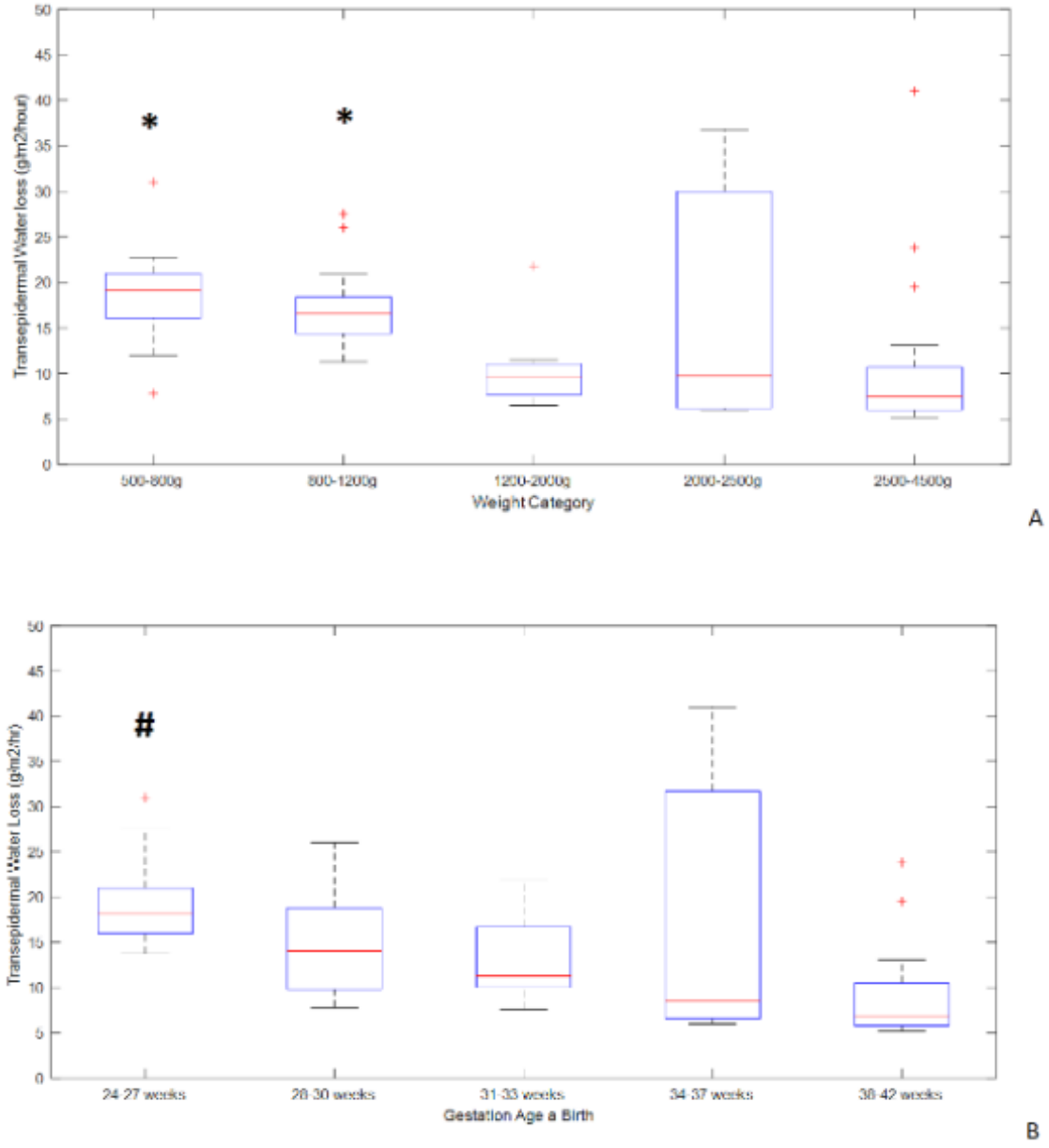


Figure 5-7 Baseline TEWL values according to (A) Birth weight and (B) GA

Statistically significant comparisons to normative birth weight (2500-4500g) denoted with a '*'. Statistically significant comparison to term infants (38-42 weeks gestation) using a Mann Whitney U test denoted with '#'.

The temporal evaluation of TEWL was used to assess the time to achieve functional barrier in the skin defined as values <10 g/m^2/hr. The corresponding analysis revealed that most term infants had a functional barrier within 48 hours birth (74%), which increased to 89% within one week of being born (Table 5-5). By contrast, in infants born extremely premature (24-26+6

weeks GA) a functional barrier was not observed at birth and only 33% of these infants had TEWL<10 g/m^2/hr two weeks post-birth. The Table 5-5 shows that functional barrier increased with increasing gestation at birth and with increasing time post-birth. In the 24-26+6-week GA group, only 33% of patients presented with a functional barrier three weeks post-birth. In the babies in 27-29+6 weeks group 60% had achieved functional barrier at 3 weeks post-birth. On the other hand, similar proportion of babies (57%) in the 30-33+6 had already achieved functional barrier by 1-week post-birth.

On the other hand, while 5/10 (50%) babies in 27-29+6 weeks GA category had achieved functional barrier at 7 days, only 1/10(10%) showed the same at 14 days and only 2/8 (25%) at 21 days. This is because of the increased incubator humidity in the first week (80%) which decreased gradually over the second week as per protocol (section 3.3.1.1) hence raising the TEWL in several babies who had decreased their TEWL by coming in equilibrium with the ambient incubator humidity during week 1.

A similar trend was also observed for weight categories, with the lowest birth weight group (500-800g) having the smallest proportion of infants with a functional barrier at birth (11%), which increased with time. 33% babies in 500-800g group demonstrated functional barrier at 14 days post birth, but this decreased to 11% at 3 weeks post birth. This is due to cessation of the ambient humidity in the incubator after the first 2 weeks of life as per protocol (Section 3.3.1.1). Half of the of babies with birth weight 1200-2000gm achieved functional barrier within 48h after birth, while none of the 17 babies with birth weight 800-1200gm achieved this within the same timeframe. 50% of babies with birth weight 800-1200gm achieved functional barrier at 3 weeks. In the weight category 1200-2000g, the number of babies with functional maturity if skin barrier halved (5/6 to 2/5) from 7 days to 14 days post birth. While this is contrary to the observation that SC barrier improves with increasing age, it can be attributed to change in incubator humidity as described in section 3.3.1.1.

TEWL measured in increased incubator humidity does not truly represent the status of SC maturation as the TEWL measurement merely represents the water vapour equilibrium across the stratum corneum barrier. However, the practice of increased incubator humidity is firmly established in clinical practice and has demonstrated its advantages, hence it will be unethical to compare TEWL in differing incubator humidities. Hence, as described in Section 3.3.4 cTEWLH50 was calculated and the time to achieve functional SC maturity (TEWL <10g/m^2/h) using these calculated TEWL values is presented in Table 5-6. Less babies achieved functional SC maturity when cTEWLH50 was used compared to measured TEWL (5 vs16 in 24-29+6-week babies) in the first 2 weeks, further confirming the advantages of increased incubator humidity for lower gestational ages.

Table 5-5 Percentage of infants with functional barrier (<10 g/m^2/hr) measured.

GA Category	Days of life			
	48hrs	7 days	14 days	21 days
37-42 weeks n=20	14/19 74%	16/18 89%		
24-26+6 weeks n=16	**0/16 0%**	**2/16 12%**	**5/15 33%**	**5/15 33%**
27-29+6 weeks n=10	**2/10 20%**	**5/10 50%**	**1/10 10%**	**2/8 25%**
30-33+6 weeks n=7	1/7 14%	3/7 43%	2/7 29%	2/5 40%
34-36+6 weeks n= 6	3/6 50%	3/6 50%		
Weight Categories				
2500-4500g, N=20	13/19 68%	14/18 78%		
500-800g, N=9	1/9 11%	2/9 22%	3/9 33%	1/9 11%
800-1200g, N=17	0/17 0%	3/17 18%	3/16 19%	7/14 50%
1200-2000g, N=6	3/6 50%	5/6 83%	2/5 40%	1/4 25%
2000-2500g, N=7	3/7 43%	4/7 57%	0/2 0%	

GA Categories with raised incubator humidity are in bold.

Table 5-6 Percentage of infants with functional barrier (<10/g/m^2/hr) as calculated in 50% humidity (cTEWLH50)

GA Category	Days of life			
	48hrs	7 days	14 days	21 days
37-42 weeks n=20	14/19 74%	16/18 89%		
24-26+6 weeks n=16	**0/16 0%**	**1/16 6%**	**4/15 27%**	**5/15 33%**
27-29+6 weeks n=10	**0/10 0%**	**2/10 20%**	**1/10 10%**	**2/8 25%**
30-33+6 weeks n=7	1/7 14%	3/7 43%	2/7 29%	2/5 40%
34-36+6 weeks n= 6	3/6 50%	3/6 50%		
Weight Categories				
2500-4500g, N=20	13/19 68%	14/18 78%		
500-800g, N=9	0/9 **0%**	0/9 **0%**	2/9 **22%**	1/9 11%
800-1200g, N=17	0/17 0%	2/17 **12%**	3/16 19%	7/14 50%
1200-2000g, N=6	2/6 **33%**	4/6 **67%**	2/5 40%	1/4 25%
2000-2500g, N=7	3/7 43%	4/7 57%	0/2 0%	

GA Categories with raised incubator humidity are in bold and percentages that are affected due to cTEWLH60 are underlined.

The post-birth age at the last measurement was analysed and presented in Table 5-7. Median time of last TEWL measurement in preterm babies with TEWL never recorded <10g/m^2/h was 11 days (range 1-90 days). This is a limitation of the study due to inability to continue to have the baby at the research site till TEWL had reached structural maturity. Such study design will not be possible with the centralisation of neonatal and maternal services needing continuous flow of patients to and from the Tertiary neonatal units (NICUs). The CFNS PT18 with TEWL 15g/m^2/h at 13 weeks was an outlier amongst this group.

Table 5-7 The maximum TEWL in babies who never demonstrated functional SC maturity.

Gestation (Weeks)		Max TEWL g/m^2/h	Min TEWL g/m^2/h	Humidity at min TEWL %	Days to reach last measurement	TEWL at last measurement g/m^2/h
24-26+6	CFNS PT20	28	15	55	2	23
	CFNS PT24	16	10	55	22	14
27-29+6	CFNS PT18	26	10	nil	90	15
	CFNS PT 25	19	10	65	14	10
	CFNS PT 26	16	8	67	14	11
30-33+6	CFNS PT 19	13	11	nil	11	11
34-36+6	CFNS PT 1	41	33	nil	4	33
	CFNS PT 2	30	17	nil	5	17
	CFNS PT 3	37	20	nil	5	20

In the Table 5-7 CFNS PT26 achieved TEWL 8g/m^2/h while in humidity however at the last measurement TEWL was 11g/m^2/h. 3 babies (CFNS PT 24, 18, 24) had achieved TEWL 10g/m^2/h before their last measurements at 22, 90 and 14 days respectively. CFNS PT 18 was an outlier, as this last measurement was done at 90 days post birth rather than the median time 11 days in the table above. Both CFNS PT 24 and 25 achieved TEWL 10g/m^2/h in humidity however TEWL increased when measurements were taken without added ambient humidity. The ambient humidity clearly artificially decreased TEWL and TEWL measured in added humidity cannot be taken to be indication of functional maturity of skin.

The babies born at >30 weeks did not have their minimum TEWL measurement in humidity, however TEWL increased when measured at day 4-5 for CFNS PT 1,2, and 3 and remained the same for CFNS PT 19 till day11, indicating that the SC needed longer time before function maturity of skin was achieved. As mentioned in Section 4.5 TEWL in CFNS PT 1, 2 and 3 were also not measured inside the NICU environment (adult ICU and postnatal ward), so the raised TEWL could be due to difference in ambient environment for these preterm babies.

Table 5-8 Dependent paired t-test comparing measurements in term babies.

	n	t(d*f*)	p value
TEWL <48h vs 2-7d	17	1.3(16)	0.21
pH <48h vs 2-7d	17	2.1(16)	0.051

The comparison of TEWL and pH at two time points within the first week of life in term babies was also made. Both TEWL and pH appeared to change over this time. The dependent paired t-test to determine the significance of difference in means of TEWL and pH at <48 hours and 2-7 days was completed and is detailed in Table 5-8. There was no statistically significant difference between the two measurements for TEWL and pH made within 1 week of birth in babies born at full term.

Chapter 6 Results for analysis of structure and morphology of skin

The knowledge of structure and morphology of skin especially in extreme preterm infants is important especially as the survival of these babies has improved in recent times. We observed functional immaturity in extreme preterm infants in Chapter 5; therefore, it would be good to see whether there are morphological features that are also different at this gestational age. Ultrasound is a non-invasive bedside tool and the intention to use this was driven by the ability to conduct serial examinations to generate temporal changes in skin morphology.

6.1 Inter-rater reliability

Inter-rater reliability for image interpretation was established by 2 individual independent interpretations for the same 20 images. Image interpretation focused on the dermal depth. The scans were reviewed and interpreted by researcher (AS) and the principal investigator (PW) in a blinded fashion. Each independent interpreter selected 20 best images from preselected 5 patients and measured dermal depth using the Episcan software. Inter-rater reliability was determined with Intraclass correlation coefficient (ICC) using SPSS v27, based on absolute agreement two-way mixed effects model. The ICC is a value between 0 and 1, where values below 0.5 indicate poor reliability, between 0.5 and 0.75 moderate reliability, between 0.75 and 0.9 good reliability, and any value above 0.9 indicates excellent reliability (Koo and Li, 2016). The correlation between the two raters was 0.99 p=0.001.

The equipment and step wise method used for HFUSI have been discussed in detail in Section 3.2.2 and 3.3.3 respectively. Several processes were required to verify the scan quality before analysing the images. The steps included are shown in the flow chart (Figure 6-1).

6.2 Quality checks on ultrasound scans

The ultrasound scan pictures taken from the study subjects were subjected to rigorous quality checks before specific measurements for thickness of dermis or echogenicity could be measured as per the objectives of this study. As described in the protocol in Section 3.3.3 up to two and seven scans were planned for each term and preterm patient, respectively. However, the preterm term babies born at later gestational ages had only 2 scans as they were discharged home within a few days after birth. Also, some babies born at term were too unwell to have both their scans (Table 4-1). 253 scans were available in total for quality check (QC). The steps in the QC are described in the flow chart in Figure 6-1.

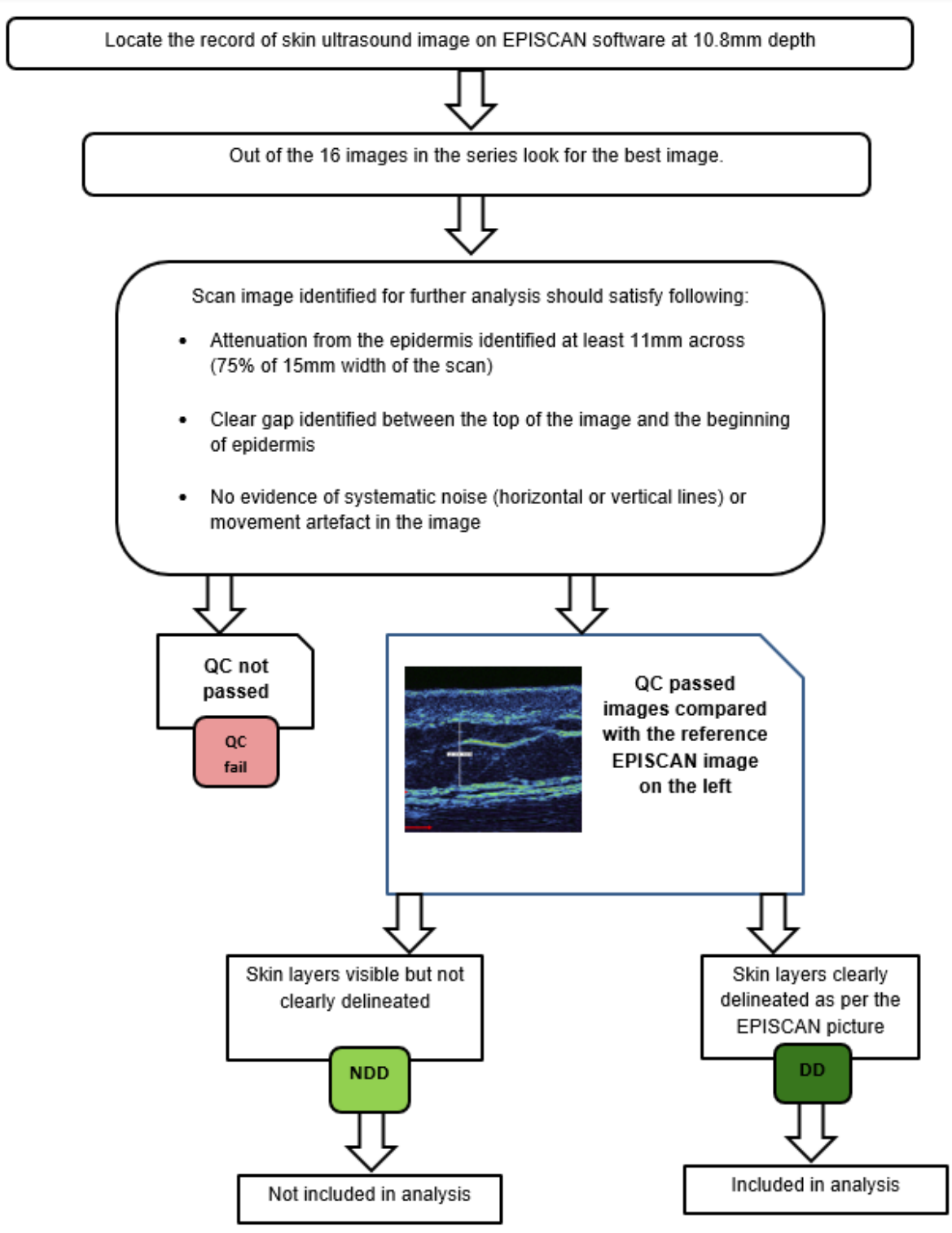


Figure 6-1 Flow chart showing the decision matrix for the Quality Control process for each finalised image included in analysis. NDD = No definite delineation, DD = Definite delineation

All the scans were stored in the EPISCAN software, which was accessed from a secure, password protected device. Each scan was stored as a series of images of the layers of skin. Each series contained 16 snapshots, as set up by EPISCAN, providing scope for selecting the best quality images from a series. The best snapshot at each contact was identified by the candidate. Each scan image was put through the Quality check process before it was used for analysis. The scans recorded on EPISCAN for each patient were obtained using different depths 5.4mm and 10.8mm. However, the scans obtained at the depth of 10.8mm were used for analysis here to capture the maximum thickness of the skin layers.
The quality check process used to identify and finalise each scan was as follows:

i. The scan should have at least 11mm of epidermis across (75% of total 15mm width of the scan. The examples of acceptable and unacceptable images are shown in Figure 6-2.
ii. Clear gap should be visible between the top of the image and beginning of the epidermis.
iii. No evidence of systematic noise (horizontal and vertical lines) should be visible in the image.
iv. No evidence of movement artefact in the image.

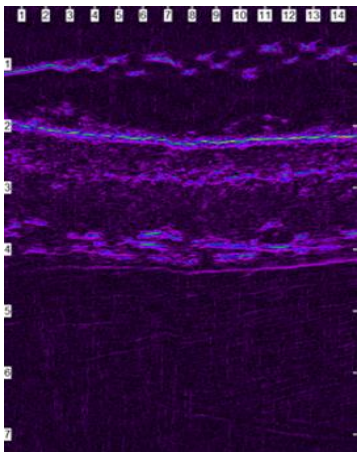
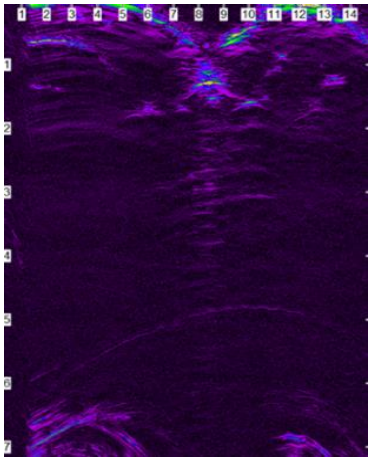
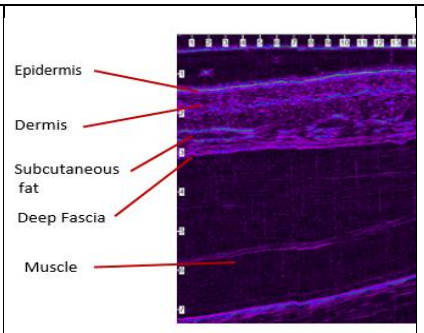
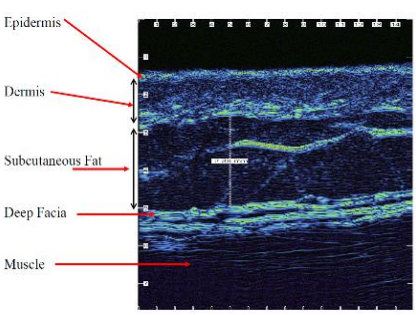
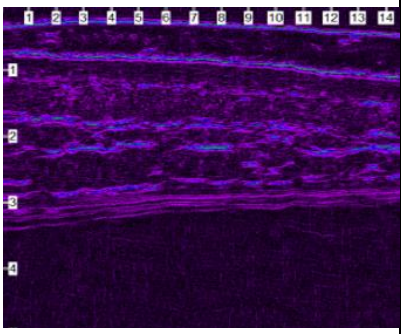
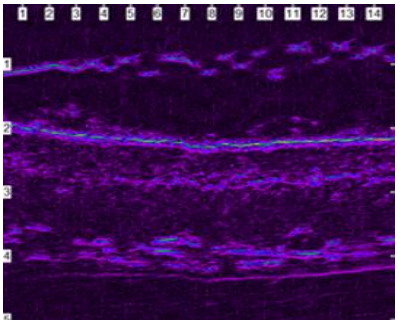
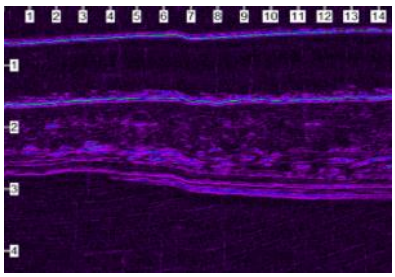
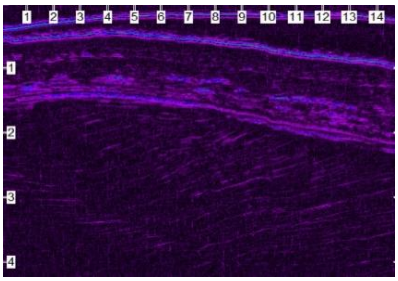
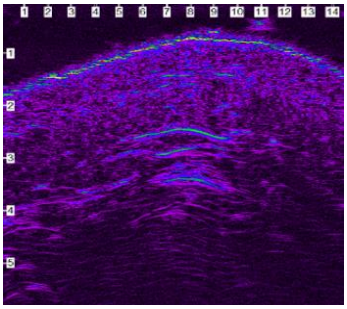
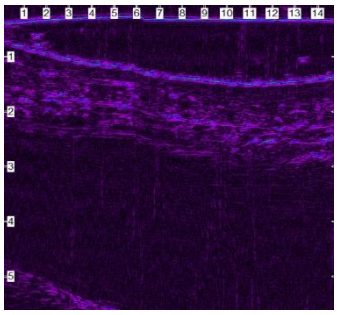
QC passed and acceptable	QC not passed and not acceptable
	
CFNSP PT 21 Sample 6	CFNS PT 4 sample 2

Figure 6-2 Examples of acceptable and not acceptable scans

Table 6-1 Overview of the process (with examples) used for categorisation of the QC passed images.

Standard used for categorisation of images	 Image from a study subject delineating all the skin layers like that in images from adult	 Adult skin ultrasound image as described by EPISCAN software in the product manual
Examples of images that passed QC and showed delineated dermis, epidermis, and subcutaneous layer. Such images were included in the analysis.	 CFNS PT 31 sample 4	 CFNS PT 21 sample 6
Examples of images that passed QC but not all layers of skin are clearly defined. The epidermis and subcutaneous tissue layers are clearly defined while the dermis was not delineated in both these images. Such images were excluded for analysis of the dermis.	 CFNS PT 31 Sample 1	 CFNS PT 35 Sample 1

Examples of images that passed QC but not all layers of skin were clearly defined. The epidermis was clearly defined while the dermis and the subcutaneous layers were not delineated in both these images. Such images were excluded for analysis.	 CFNS T2 Sample 1	 CFNS PT 29 Sample 1

The images that did not pass the QC and rendered unacceptable were labelled 'QC fail' as shown in the flow chart in Figure 6.1. The QC passed scans were then categorised using the published EPISCAN ultrasound pictures of skin layers on adult patients as comparator for identifying the epidermis, dermis and subcutaneous tissue as described in the Table 6-1.
All the scan images were subjected to a rigorous QC process as shown in Figure 6-1 followed by categorisation as shown in Table 6.1 to pick out the images with clearly defined layers of skin. Only the images with all clearly defined layers of skin were included for analysis. The results of the QC process and categorisation are tabulated in the Table 6.2 for term and preterm infants.

All the scans labelled as QC fail (**Quality control failed**) were excluded from the analysis.

All the scans labelled as NDD (**No dermal delineation**) were also excluded from the analysis and though the dermis was visible it was difficult to be confident about the margins of the dermis.

All scans labelled as DD (**Dermal delineation**) were included in the analysis.

Table 6-2 Results of the Quality Check process for all study subjects

A: Preterm infants B: Term infants C: Summary of quality check process

A: Preterm infants

ID	GA	Sample						
N=39	(weeks)	1	2	3	4	5	6	7
CFNS PT 37	24+1	NDD	NDD	NDD	NDD	NDD	DD	DD
CFNS PT 39	24+3	NDD	NDD	NDD	NDD	NDD		
CFNS PT 14	24+5	NDD	NDD	NDD	NDD	NDD	DD	DD
CFNS PT 15	24+5	QC fail	DD	NDD	NDD	NDD	DD	
CFNS PT 34	25+2	NDD	NDD	NDD	NDD	DD	DD	DD
CFNS PT 33	25+2	NDD	NDD	NDD	DD	DD	DD	DD
CFNS PT 32	26+0	NDD	NDD	NDD	NDD			
CFNS PT 23	26+2	NDD	NDD	NDD	NDD	DD	DD	
CFNS PT 24	26+2	NDD	NDD	NDD	NDD	NDD	DD	
CFNS PT 38	26+2	NDD	NDD	NDD	DD	DD	DD	DD
CFNS PT 29	26+4	QC fail	NDD	NDD	NDD	DD	DD	
CFNS PT 30	26+4	NDD	NDD	QC fail	NDD	DD	DD	
CFNS PT 31	26+4	NDD	NDD	NDD	NDD	NDD	DD	
CFNS PT 35	26+0	NDD	NDD	NDD	NDD	DD	DD	DD
CFNS PT 36	26+5	NDD	NDD	NDD	NDD	NDD	DD	DD
CFNS PT 20	26+6	NDD	NDD	NDD	DD	DD		
CFNS PT 18	27+0	NDD	NDD	NDD	NDD	NDD	NDD	DD
CFNS PT 26	27+2	NDD	NDD	NDD	NDD	NDD		
CFNS PT 25	27+2	NDD	NDD	NDD	NDD	DD		
CFNS PT 17	27+4	NDD	NDD	NDD	NDD	NDD	NDD	DD
CFNS PT 10	28+0	NDD	QC fail	NDD	NDD	QC fail	NDD	DD
CFNS PT 21	28+5	NDD	NDD	NDD	NDD	NDD	DD	DD
CFNS PT 16	29+0	NDD	NDD	NDD	NDD	NDD	NDD	DD
CFNS PT 7	29+1	NDD	QC fail	NDD	NDD	NDD	NDD	DD
CFNS PT 8	29+1	NDD	NDD	NDD	NDD	NDD	DD	DD
CFNS PT 28	29+5	NDD	NDD	NDD	NDD	NDD	NDD	DD
CFNS PT 27	30+4	NDD	NDD	NDD	NDD	DD	DD	DD
CFNS PT 22	30+5	NDD	NDD	NDD	NDD	DD	DD	DD
CFNS PT 13	31+4	NDD	NDD	NDD	NDD	DD	DD	
CFNS PT 4	31+6	QC fail	QC fail	NDD	NDD	NDD		
CFNS PT 5	32+6	QC fail	NDD	NDD	NDD			
CFNS PT 6	33+0	NDD	NDD	NDD	QC fail	NDD	NDD	NDD
CFNS PT 19	33+0	NDD	NDD	NDD	DD			
CFNS PT 1	34+2	QC fail	QC fail					
CFNS PT 12	34+3	DD	DD					
CFNS PT 11	35+2	DD	DD					
CFNS PT 3	35+5	QC fail	NDD					
CFNS PT 2	35+5	DD	QC fail					
CFNS PT 9	36+6	DD	DD					

B: Term infants

ID	GA	Sample	
n=20	(weeks)	1	2
CFNS T1	38+4	QC fail	
CFNS T2	40+0	QC fail	
CFNS T3	39+6	QC fail	QC fail
CFNS T4	38+2		DD
CFNS T5	39+1	QC fail	QC fail
CFNS T6	40+3	QC fail	DD
CFNS T7	37+6	DD	DD
CFNS T8	37+3	QC fail	QC fail
CFNS T9	37+6	QC fail	DD
CFNS T10	39+1	QC fail	QC fail
CFNS T11	39+2	QC fail	QC fail
CFNS T12	39+5	DD	DD
CFNS T13	38+2	DD	DD
CFNS T14	41+0	DD	DD
CFNS T15	41+1	DD	DD
CFNS T16	37+0	DD	DD
CFNS T17	40+1	DD	DD
CFNS T18	42+1	DD	DD
CFNS T19	38+2	DD	DD
CFNS T20	41+1	DD	DD

C: Summary of Quality check

Term	Number of QC passed scans =29		Number of scans with dermis delineated clearly=29	
Time of scan	<48h	2-7 days	<48h	2-7 days
	13	16	13	16

Preterm ≥28 weeks (n=19)	Number of QC passed scans=83		Number of scans with dermis delineated clearly=35	
Time of scan	<48h	After 48h	<48h	After 48h
	15	68	6	41

Preterm <28 weeks (n=20)	Number of QC passed scans=120		Number of scans with dermis delineated clearly=35	
Time of scan	<48h	After 48h	<48h	>48h
	18	102	0	35

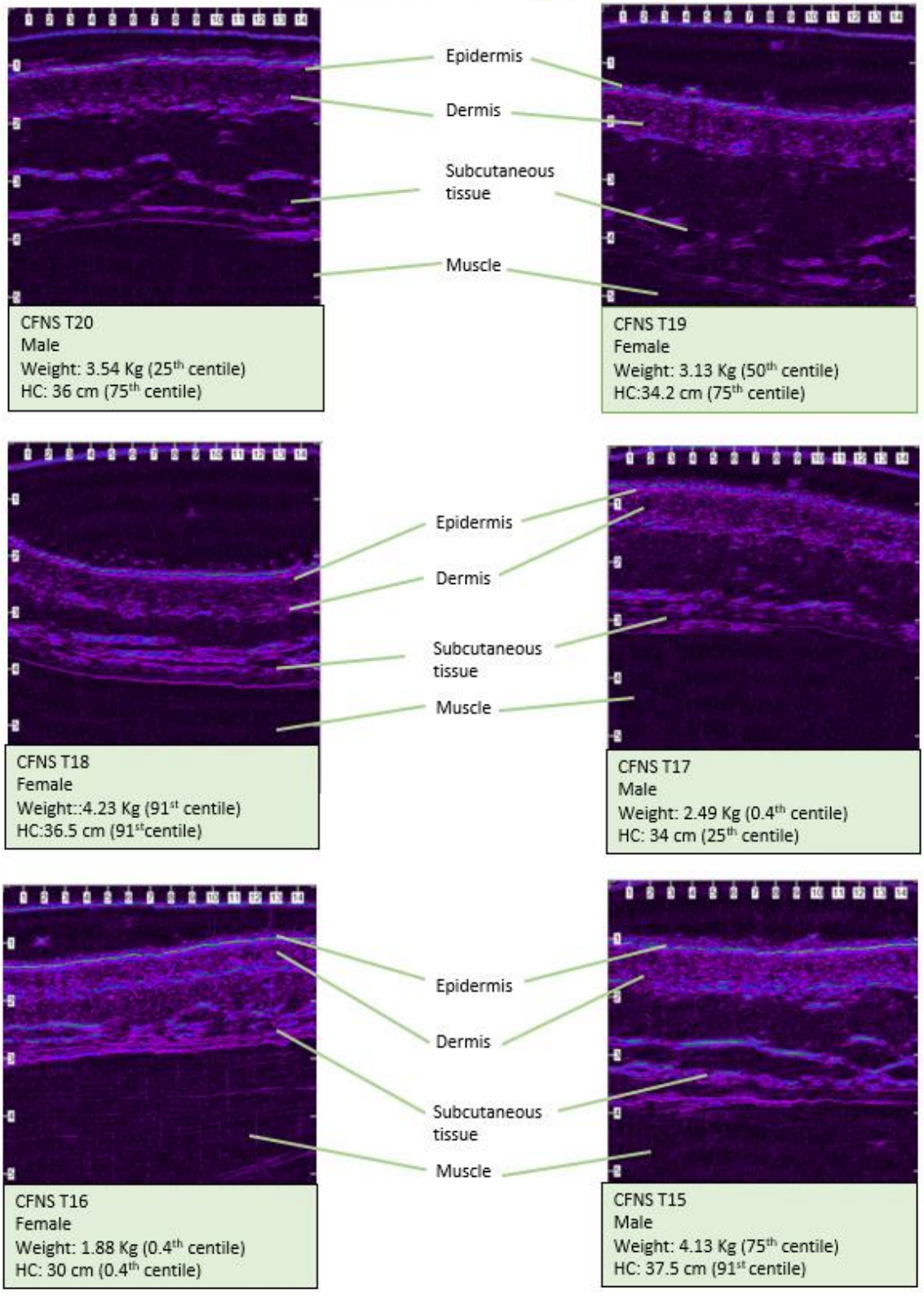
The Table 6.2 shows that most of the scans on term infants passed the QC and were satisfactory for inclusion in the analysis. The scans in term infants that did not pass QC were the scans done in the earliest babies recruited to the study as familiarity with the equipment was being established during those scans. Similarly early recruits in the preterm babies also had images that did not pass QC for the same reason. There were only 6 images that did not pass QC after the 5th recruit amongst preterm babies, and this demonstrates the that once familiarity is gained with the equipment and procedure, the yield from the scans improves significantly.

29 images from the term infants passed QC and all those images demonstrated dermal delineation. On the other hand, 163 images from the preterm infants passed QC but only 70 demonstrated dermal delineation. Also, the table demonstrates that while the babies born ≥ 33 weeks did show dermal delineation from the first sample taken within 48 hours after birth, most preterm infants did not demonstrate dermal delineation for several weeks after birth indicating a change in the structural composition of the dermis with increasing gestational age, which produces the echoes recognised by HFUS image.

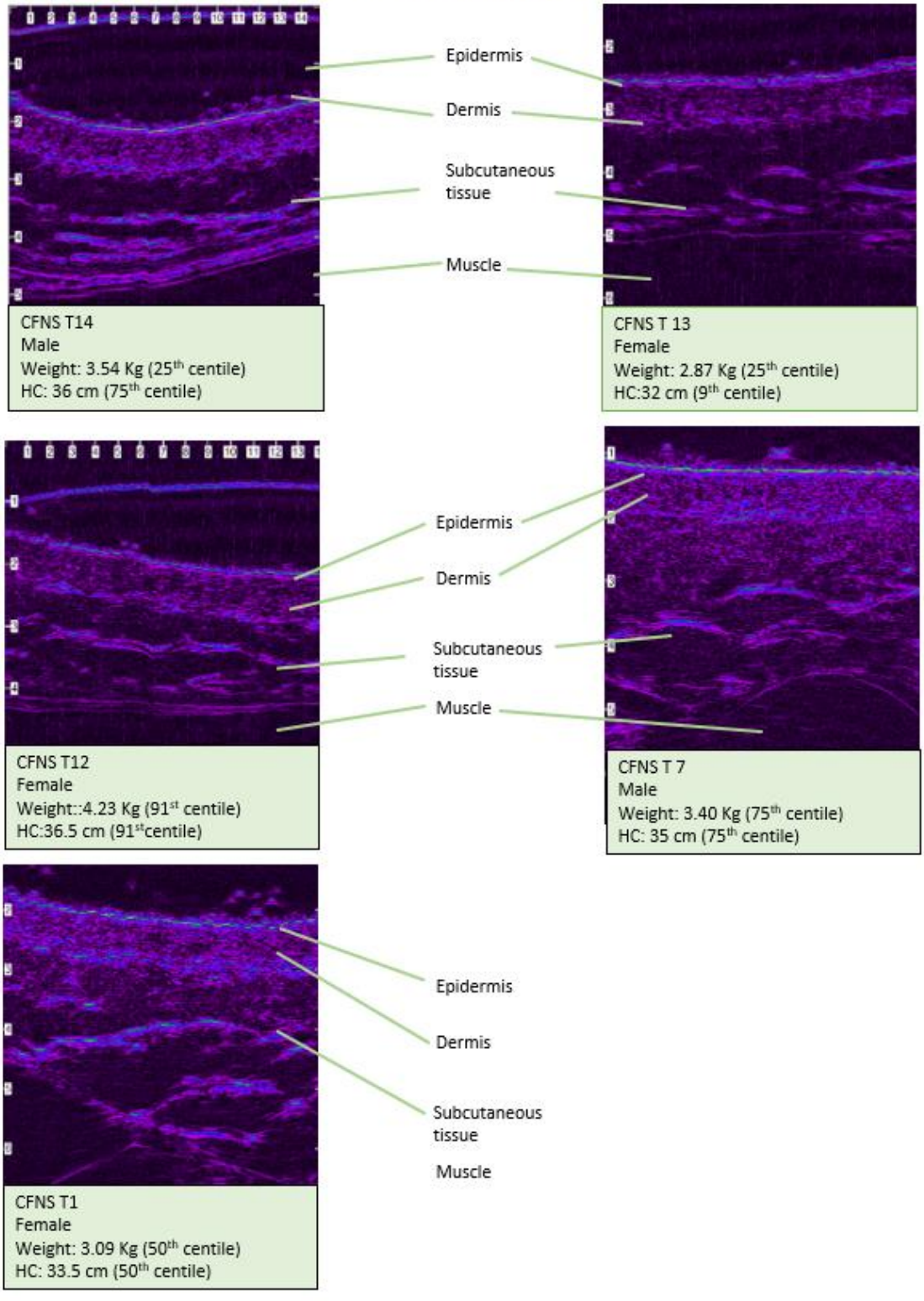
6.3 Examples of scans

Examples of satisfactory and unsatisfactory scans which have gone through the QC are on the following pages in Figure 6-3.

Ultrasound scans of study subjects done within 48 hours after birth deemed satisfactory for analysis



Ultrasound scans of study subjects done within 48 hours after birth deemed satisfactory for analysis



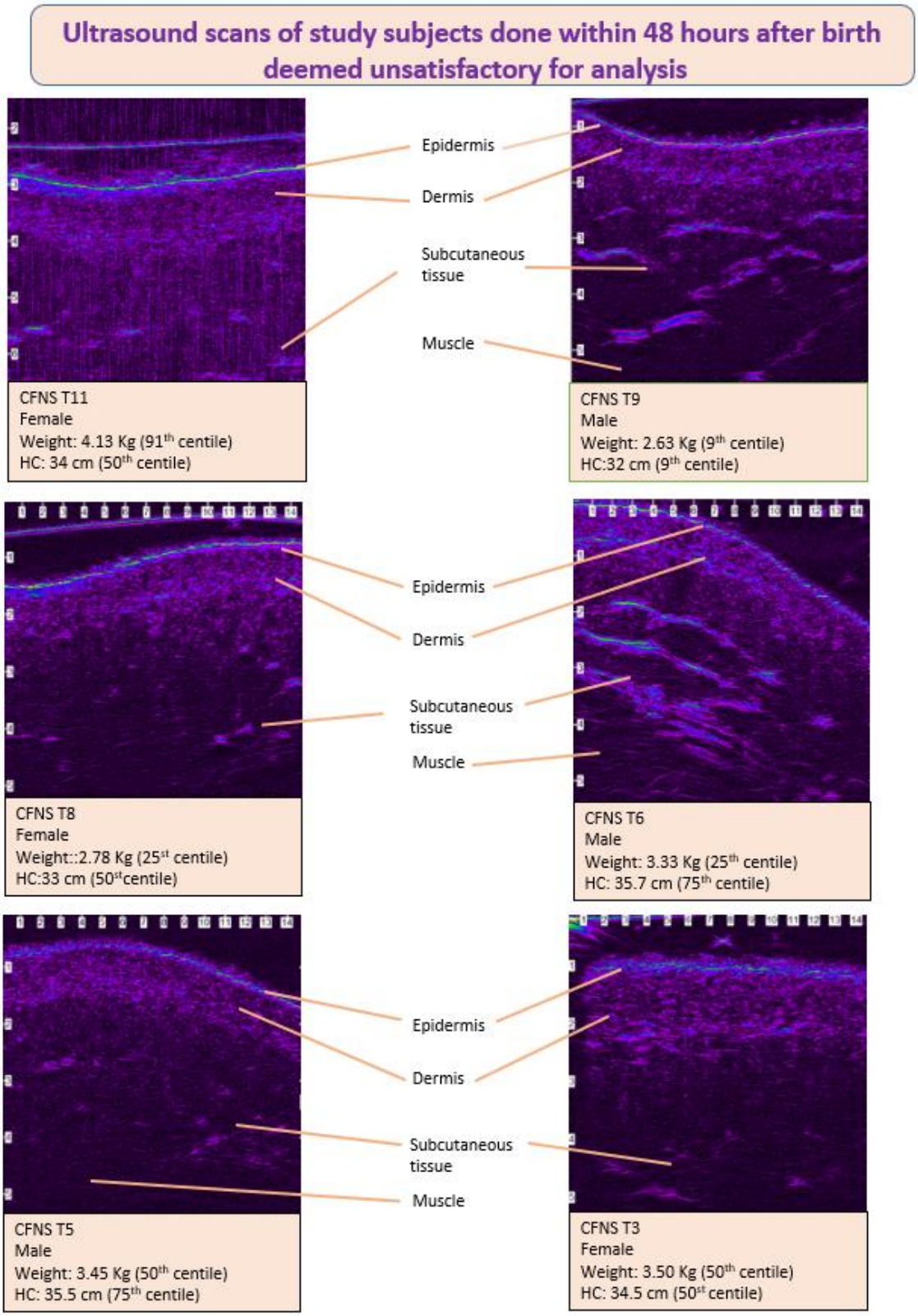


Figure 6-3 Examples of satisfactory and unsatisfactory scans following the Quality control process

6.4 Analysis of HFUS images

The analysis of ultrasound images was conducted separately for term and preterm infants. The relation of dermal thickness, TST (total skin thickness) and echogenicity of the dermal layer with other demographic characteristics were explored. In addition, the variation in the skin characteristics with increasing age and gestation at birth in preterm infants were analysed. The difference between the term and preterm babies were also considered. The epidermal thickness was not measured on these images because the resolution of EPISCAN I-200 is 0.04 mm (Section 3.2.2), which is not enough to measure the reported epidermal thickness (adult=0.056-0.084mm (Sandby-Møller et al., 2003) and newborn=0.081mm (Paulina Przybysz, 2020). Two primary linear measurements were taken, which included the total skin thickness (TST), and where delineated the dermal thickness (Figure 6-4).

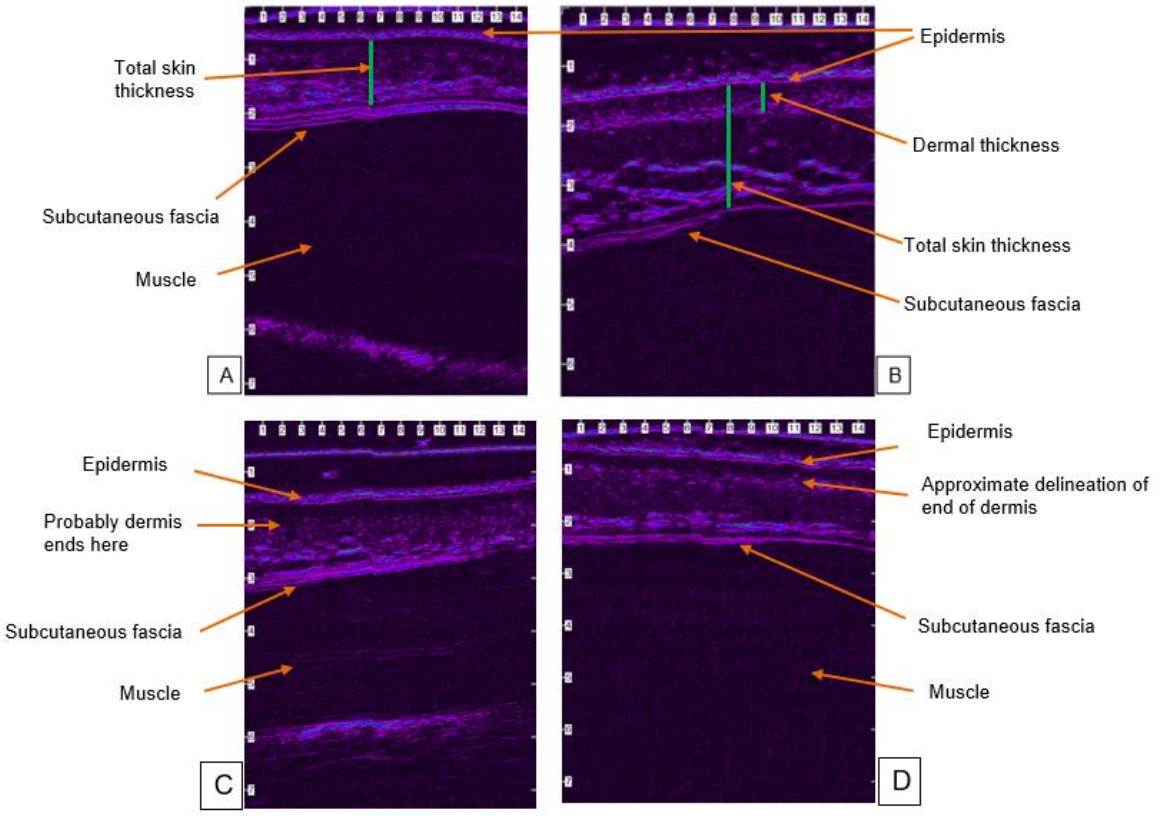


Figure 6-4 US images showing difference in delineation of skin layers.

A Epidermis and subcutaneous layer are clearly delineated however dermis is not differentiated. B The epidermis, dermis and subcutaneous layer are clearly delineated. C dermis is not visible. D Dermis is starting to delineate but not clearly demarcated. Dermal thickness was measured in B but not in any other images due to difficulty in being confident about the dermal boundaries.

The dermis appeared heterogeneously echogenic. For standardisation of data collection, the region of interest (ROI) was selected in the area just below the epidermis. This was selected to represent the upper dermis, where functional development of microvascular structures, hair follicles and sebaceous glands occurs over time (Section 2.3.2.2). Importantly, during functional development there will be collagen deposited forming the structure of the papillary dermis. For this study, other regions of interest were not analysed. The echogenicity profile of the ROI produced by the EPISCAN software provided the distribution of the pixels across 128 intensities. All the images had pixels in the 0-9 intensity range (Figure 6-5), hence to derive the difference in echogenicity profile we agreed to analyse the proportion of pixels in the intensity range 10-127. This is because the dermal density reflects the amount of collagen in the dermis which would determine the structural integrity of the dermis (Mercado et al., 2014)., as described in Section 2.4.1.

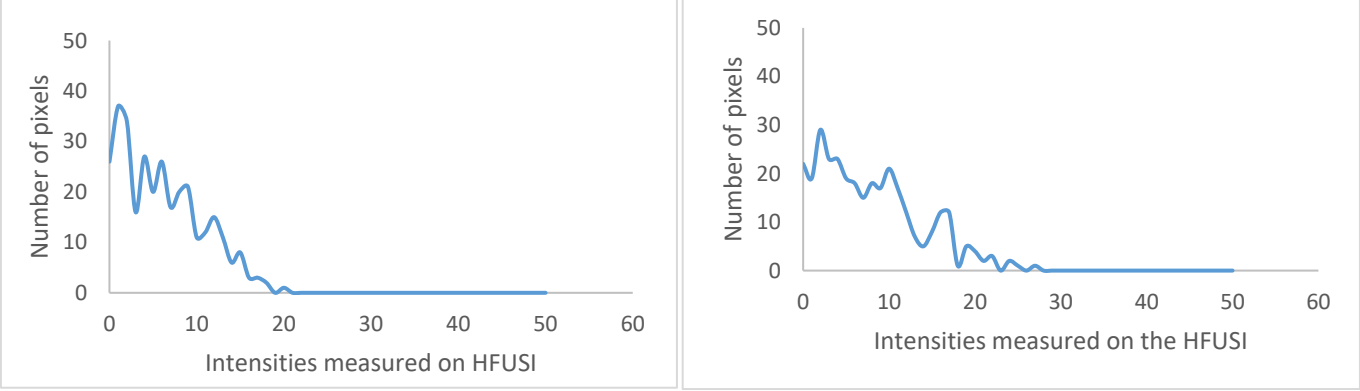


Figure 6-5 Demonstration of distribution of pixels (y-axis) across intensities (x-axis) in two HFUS scans of skin in term infants

The figure demonstrates that the difference in number of pixels at intensities >10 is a major differentiating feature of these profile graphs.

6.4.1 Analysis of US images in Term infants

10 term infants had QC images at <48 h and 13 had them at 4-7 days. There were 5 female infants in <48h and 6 in 4-7 days groups. The chart in Figure 6-6 shows that the there was little variation in dermis thickness between term infants. There was greater variation of TST both between infants and when measured at <48h and 4-7 (average 6 days) days.

The mean dermal thickness and TST increased over the first week significantly (Table 6-3) as shown by the Wilcoxon signed rank test (Dermal thickness: Z=-2.297, p=0.013, TST: Z=-

2.599, p=0.009). The median dermal thickness was 0.66 mm (0.5-0.8 mm) at <48h and increased to 0.79 mm (0.6-0.9 mm) while the TST increased from median 2.38 mm (1.8-3.3 mm) when measured before 48h of age to 3.08 mm (2.2-4.8 mm) when measured after an average duration of 6 days.

The increase in dermis thickness and TST in term babies is small but is only over 6 days. The large rate of growth in babies in the first few months would lead to increase in dermis and TST further reflecting development of its underlying structures.

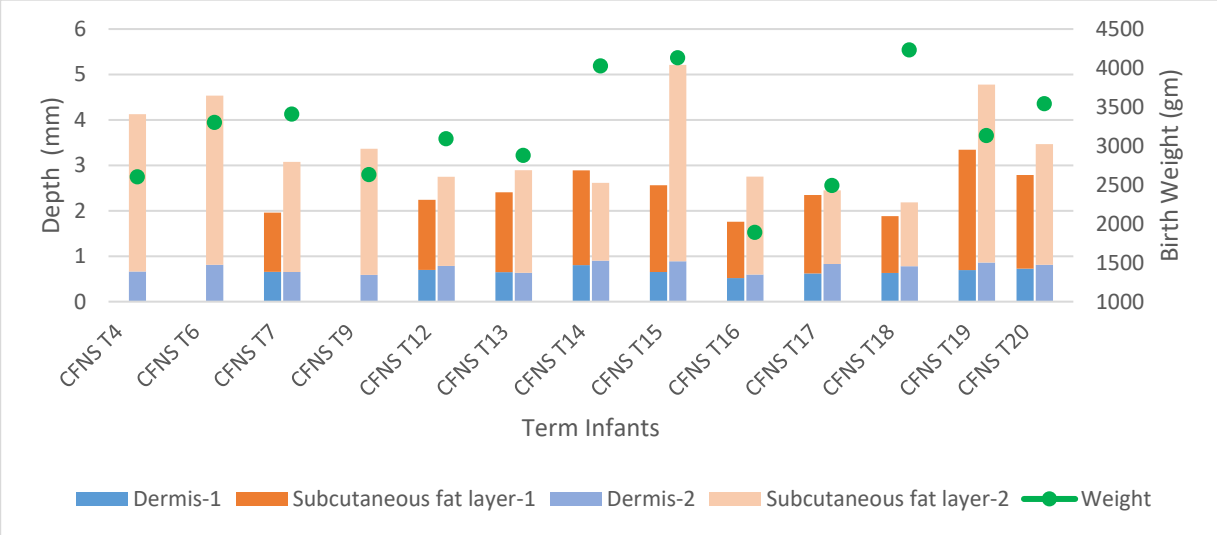


Figure 6-6 Thickness of dermis and subcutaneous fat layer in term infants measured at two-time points

Dermis-1 and Subcutaneous fat layer-1 were measured at <48h of age and Dermis-2 and Subcutaneous fat layer-2 were measured 4-7 days of age. The respective birth weights are plotted as green dots.

The Wilcoxon signed rank test for the echogenicity profile measured as the proportion of pixels with intensity ≥10 showed that the decrease in proportion of pixels with time (30.38% to 22.16%) was statistically significant (Z=-2.395, p=0.017). The median intensity decreased with time from 7.6 to 6.2 suggesting more pixels in the image were at lower intensity by 4-7 days of age compared with the image at <48 hours of age. The echogenicity profile was also more homogenous with time as the relative dispersion was noted to have decreased from 39 to 36 as shown in Table 6-3. It is important to bear in mind that the two time points compared are quite close together- <48h and 4-7 days.

The GA of term infants ranged over 5 weeks from 37-42+1 weeks at birth (Table 4-1). Spearman's correlation test however did not show any statistically significant correlation of dermal thickness or TST measured at <48h with GA at birth (Dermal thickness: r_s = *0.472,* p=0.169, TST*:* r_s =0.176, p= 0.627*).* There was no difference in the dermal thickness and TST between the male and female infants *(p>0.05).*

Table 6-3 Comparison of US images of term infants taken at <48 hours and 4-7 days from birth.

	<48 from birth	4-7 days from birth	
37-42+1 weeks	**Median (range)**	**Median (range)**	**Trend**
Dermal Thickness (mm)	0.66 (0.5-0.8)	0.79 (0.59-0.90)	↑
Total Skin Thickness (mm)	2.38 (1.7-3.4)	3.08 (2.16-4.78)	↑
Pixels with intensity ≥10 (%)	30.38 (14.6-52.2)	22.16 (12.89-55.37)	↓
Median intensity	7.6 (5.2-11.8)	6.2 (4.6-12.6)	↓
Relative dispersion	39.76 (33.8-51.7)	36.33 (31.99-54.03)	↓

There was also no correlation of the proportion of pixels at intensity ≥10 with GA at birth (r_s = *-0.333, p=0.347)* or birth weight (r_s =-0.21, p=value 0.953). Like dermal thickness and TST, pixels with intensity ≥10 at <48h did not show any statistically significant difference when compared between male and female infants or between weight and GA groups.

The term infants appeared to be a homogenous group in terms of dermal analysis despite the variation in their demographic characteristics. The dermis thickness and TST increased with time while the proportion of pixels with intensities ≥10 and median intensities decreased. In general, the images were more homogenous with advancing age. It would be useful to correlate these findings with similar measurements in older infants and children, as well as adults.

6.4.2 Analysis of US images in <28week Preterm infants

Ultrasound images of 20 preterm infants born before 28 weeks gestation were analysed out of total 39 preterm infants recruited. The chosen infants were the most preterm babies in the study ranging from 24+1-27+5 weeks GA at birth. These images from the most premature babies were hypothesized to reveal the maximum differences between the skin characteristics of preterm and term infants. Being preterm, these babies were cared for in the NICU for several days (maximum 141 days) as shown in Table 4-1. Hence there was an opportunity to obtain the US images over several weeks to capture the skin changes with time as detailed in Table 6-2.

Table 6-4 Division of ultrasound images of <28 weeks preterm infants into groups

Time point (TP) for analysis	Time of measurement	Number of patients with QC confirmed scans (maximum 20)	Number of patients where dermis was clearly visualised	Number of patients where total skin thickness was measured	Number of patients where dermal echogenicity was evaluated.
TP 1	<48h	18	0	18	18
TP2	20-28 days	16	11	16	16
TP3	>8 weeks	8	8	8	8

120 images passed QC among the 20 preterm infants born <28 weeks (Table 6.2C). Each of these infants had 4-7 samples taken over an average 50.75 days (range 15-129 days) with a median of 39.5 days. For the analysis the scans were organised into three groups (known as time points) as shown in the Table 6-4. The measurements at three time points (TP1, TP2, TP3) for Dermal thickness (Dermis1, Dermis2, Dermis3), Total skin thickness (TST1, TST2, TST3) and Pixels with intensity≥10 (Pixel1, Pixel2, Pixel3) were recorded from the images included after QC. Median intensity (Median1, Median2 and Median3) and Relative dispersion of intensities (Relative dispersion1, Relative dispersion2 and Relative dispersion3 were also calculated from these US images.

These time points were chosen because it was considered that it would take time for dermal development to occur. Table 6-2 A. shows that the dermis was not well delineated for several weeks in preterm babies.

The scans at <48h in CFNS PT 15 (24+5 weeks) and CFNS PT 29 (26+4) did not pass the QC hence they were not included in analysis leaving 18 scans to analyse in this group. In 12/18 patients in this group dermis could not be differentiated from the rest of the skin layers.

In theses 6 patients dermis was visible but could not be confidently delineated [CFNSPT37 (24+1 weeks), CFNSPT14(24+5 weeks), CFNSPT24 (26+2 weeks), CFNSPT35 (26+5 weeks), CFNSPT36 (26+5 weeks) and CFNSPT25 (27+2 weeks)] as shown in the example in Figure 6-4A. Hence no dermal thickness measurements could be done in the scans taken at <48h of age in <28 weeks GA preterm infants.

In the second group (20-28days) 16/20 samples were available because 3 babies were transferred to another hospital and 1 died. Dermis could be recognised but was poorly defined in 4 infants [CFNSPT37 (24+1 weeks), CFNSPT39 (24+4 weeks), CFNSPT18 (27+0 weeks) and CFNSPT17 (27+4 weeks)] hence they were excluded from analysis. Examples of such scans are depicted in Figure 6-4 C, D.

In the third group (>8weeks) dermis and total skin thickness measurements were available for all 8 infants who were present on the unit at this age. As shown in Figure 6-4B, the layers of skin, especially dermis was more clearly delineated with increasing chronological age.

Echogenicity of the dermis was also measured as per the protocol explained in section 3.3.3 and depicted in Figure 3-4 and Figure 6-4. Proportion of pixels in intensity ≥10 /128, median intensities and relative dispersion of the pixels across the 128 intensities were recorded for each scan and were subjected to statistical tests (Table 6-5).

6.5 Factors associated with skin structure and morphology

The factors considered for this analysis were gestational age, weight, gender, and time from birth. Nutrition was also considered for correlation of skin characteristics with intake of energy, fat, carbohydrates, and proteins.

6.5.1 Association with demographic characteristics

The groups in Table 6-4 were created to systematically compare the skin characteristics in babies whose US images were opportunistically recorded at variable times to accommodate the clinical needs of each infant as mentioned in section 3.3.3. The grouping allowed conclusions to be drawn at significant time points for the <28week preterm infants. The demographic data for each group is present in Table 6-5. Almost similar number of male and female infants were present in each group. The median for gestational age for each Time Point (TP) is 26+4, 29+5 and 35+5 which match with the TP 48h, 20-28days, and >8 weeks.

Table 6-5 Demographic characteristics and aggregate group-wise skin characteristics at three chosen time points in preterm infants <28 weeks

TP1 (<48h of age)				
	N	**Min-Max**	**25-75 centile**	**Median**
Gestational age (weeks + days)	18	24+1-27+5	25+4-27+1	26+4
Age (days)	18	1-2	2-2	2
Weight (grams)	18	540-1090	760-1030	860
Dermis1 (mm)	0			
TST1 (mm)	18	0.8-2.1	1.09-1.5	1.2
Pixel1 (%)	18	1.6-34.5	4.6-15.3	7.21
Median1	18	3.2-7.8	3.8-4.8	4.0
Relative dispersion1	18	26-24	24-33	29.8
Gender M:F	10:8			
TP 2 (20-28 days of age)				
	N	**Min-Max**	**25-75 centile**	**Median**
Gestational age (weeks+days)	16	27+4-31+5	28+6-30+0	29+4
Age (days)	16	20-28	22-28	26
Weight (grams)	16	750-1660	1095-1270	1130
Dermis thickness (mm) (Dermis2)	11	0.2-0.4	0.3-0.4	0.3

TST2 (mm)	16	1.1-2.4	1.4-1.7	1.5
Pixel2 (%)	16	1.7-40.9	4.3-19.3	9.2
Median2	16	3-9.2	3.5-5.3	4.3
Relative Dispersion2	16	26-44	28-35	30.7
Gender M:F	10:6			
TP 3 (>8weeks of age)				
Gestational age (weeks+days)	8	33+5-43+4	34-40	35+5
Age (days)	8	56-129	63-93	67.5
Weight (grams)	8	1220-3460	1877-2925	2190
Dermis3 (mm)	8	0.3-0.7	0.5-0.6	0.5
TST3 (mm)	8	1.8-5.6	1.14-2.3	2.9
Pixel3 (%)	8	6.2-40.6	11.3-25.4	19.4
Median3	8	4-9	4.8-6.5	5.7
Relative dispersion3	8	29-45	32-38	35
Gender M:F	5:3			
Demographics at birth				
Birth weight	20	500-1090	775-1023	870
Gestation at birth	20	24+1-27+4	25+2-26+6	26+3
Gender M:F	11:9			

The table summarises the measurements at three time point (TP1, TP2, TP3) for Dermal thickness (Dermis1, Dermis2, Dermis3), Total skin thickness (TST1, TST2, TST3), proportion of Pixels with intensity≥10 (Pixel1, Pixel2, Pixel3), Median intensity (Median1, Median2, Median3) and Relative dispersion (Relative dispersion1, Relative dispersion2, Relative dispersion3)

The Table 6-5 shows that gestational age and weight increase as the time points progress. Also, the dermis thickness, total skin thickness and pixel intensity ≥10 increase. The total number of infants in each group are small but for comparison statistical tests were applied and showed significant difference in groups as shown in Table 6-6 and Figure 6-7 for difference in gender and weight categories. The difference seen for weight centiles is possibly due to proportional increase in body surface area with increased weight and length as the infant grows. The male infants have smaller measurements of dermis thickness and total skin thickness. The difference in total skin thickness between male and female infants is less than that in dermis thickness in preterm infants born at <28 weeks (Figure 6-7).

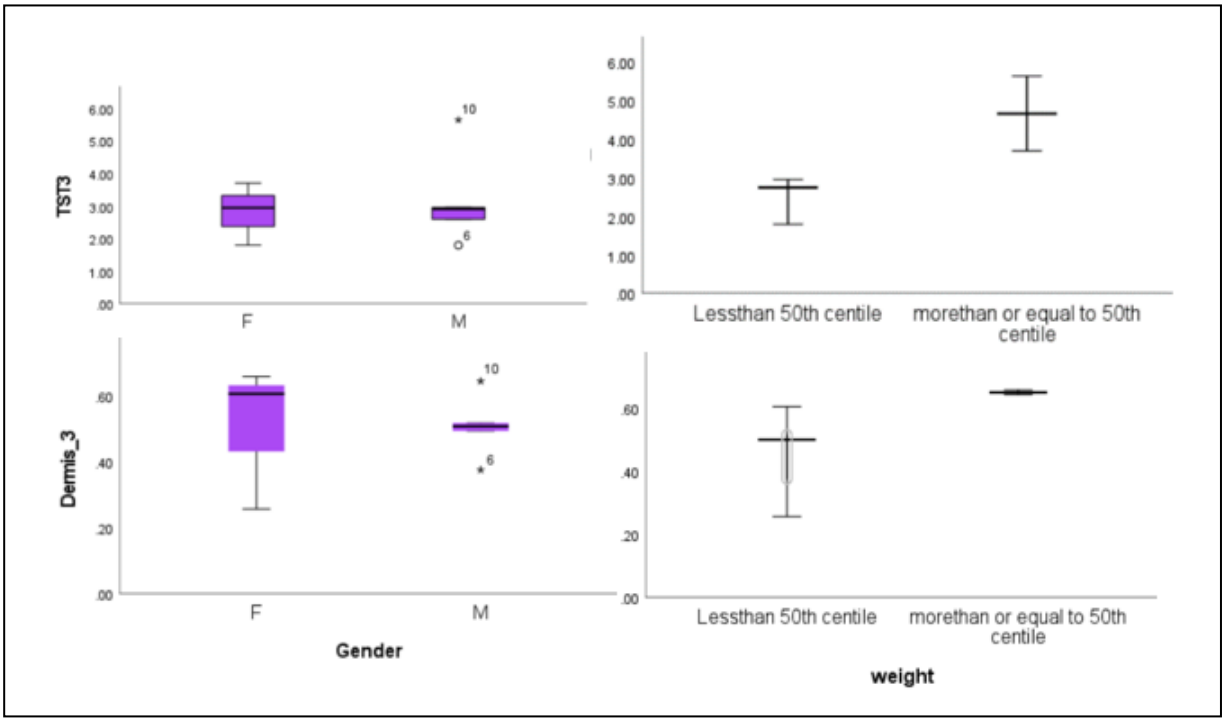


Figure 6-7 Box plots comparing the difference in dermis thickness (mm) and total skin thickness (mm) for weight centiles (<50th centile vs ≥50th centile) and gender.

Independent Mann Whitney U test was conducted on the gender and birth weight categories (<50th centile and ≥ 50thcentile) to explore the significance of the differences seen in Figure 6-7. There was no statistically significant difference between male and female infants for TST and dermis at any of the three time points as shown in Table 6-6. The TST and dermis measured at >8 weeks (TST3 and Dermis3) were significantly different between birth weight groups (p value TST3= 0.046 and p value Dermis3 = 0.044).

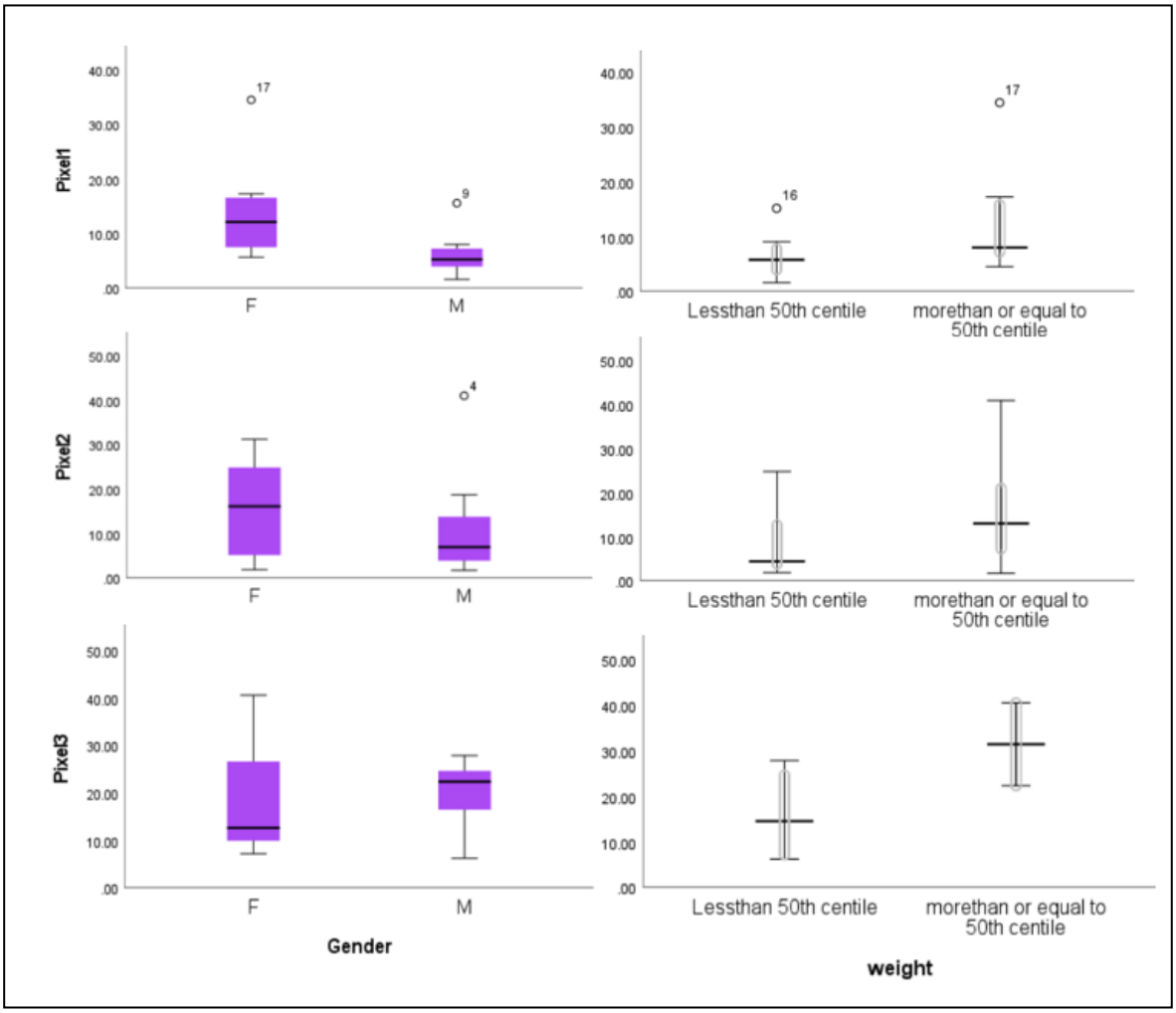


Figure 6-8 Box plots comparing the difference in proportion of pixels with intensity≥10 for weight centiles and gender at three-time points

The pixel intensity in male infants appears to increase with time (Pixel3 is for TP3 at >8 weeks) and is more than for the female infants at >8 weeks' time point (TP3) (Figure 6-8). The statistical test in Table 6-6 shows that the pixel intensity is statistically different only at the first time point (<48h) for the two genders (U=12, p=0.013) but not at TP2 (20-28 days) or TP3 (>8 weeks). Box plot in Figure 6-8 shows there is difference seen for weight centiles categories with increasing gestational age as expected due to increased body weight and length with general growth. There was no statistically significant difference in percent pixels with intensity ≥10 in the two birth weight categories at any of the three time points (Table 6-6).

Table 6-6 Mann Whitney U test to compare difference in skin characteristics between genders and birth weight categories of Preterm infants born at <28 weeks.

Skin characteristics by time points	Category 1 (n) and Category 2 (n)	U	p-value
TST1	Birth weight <50th centile (9) and ≥ 50thcentile (9)	23	0.122
TST2	Birth weight <50th centile (7) and ≥ 50thcentile (9)	30	0.874
TST3	Birth weight <50th centile (6) and ≥ 50thcentile (2)	0	**0.044**
Dermis2	Birth weight <50th centile (3) and ≥ 50thcentile (8)	7.5	0.357
Dermis3	Birth weight <50th centile (6) and ≥ 50thcentile (2)	0	**0.046**
Pixel1	Birth weight <50th centile (9) and ≥ 50thcentile (9)	20	0.07
Pixel2	Birth weight <50th centile (7) and ≥ 50thcentile (9)	19	0.186
Pixel3	Birth weight <50th centile (6) and ≥ 50thcentile (2)	2	0.182
TST1	Gender M (10) and F (8)	27	0.248
TST2	Gender M (10) and F (6)	26	0.664
TST3	Gender M (5) and F (3)	7.5	1
Dermis2	Gender M (8) and F (3)	9.5	0.61
Dermis3	Gender M (5) and F (3)	6	0.66
Pixel1	Gender M (10) and F (8)	12	**0.013**
Pixel2	Gender M (10) and F (6)	22	0.386
Pixel3	Gender M (5) and F (3)	7	0.981

When comparing TST<48h in term infants with TST in <28 weeks infants at TP1 and 2, the difference is statistically significant (Table 6-7). The median intensity measurements on term infants are universally greater than preterm infants with the difference of median intensities significantly reduced in the measurements at the third time point. These observations are supported by the Mann U Whitney test as shown in Table 6-7.

The earliest dermis thickness measured in preterm infants <28 weeks was at 20-28 days of age, and it was a median of 0.37mm (Table 6-5). Wilcoxon signed rank test showed that this was not significantly different from the dermis thickness of 0.51mm measured when these infants were >8 weeks old (Z=-1.83, p=0.068)

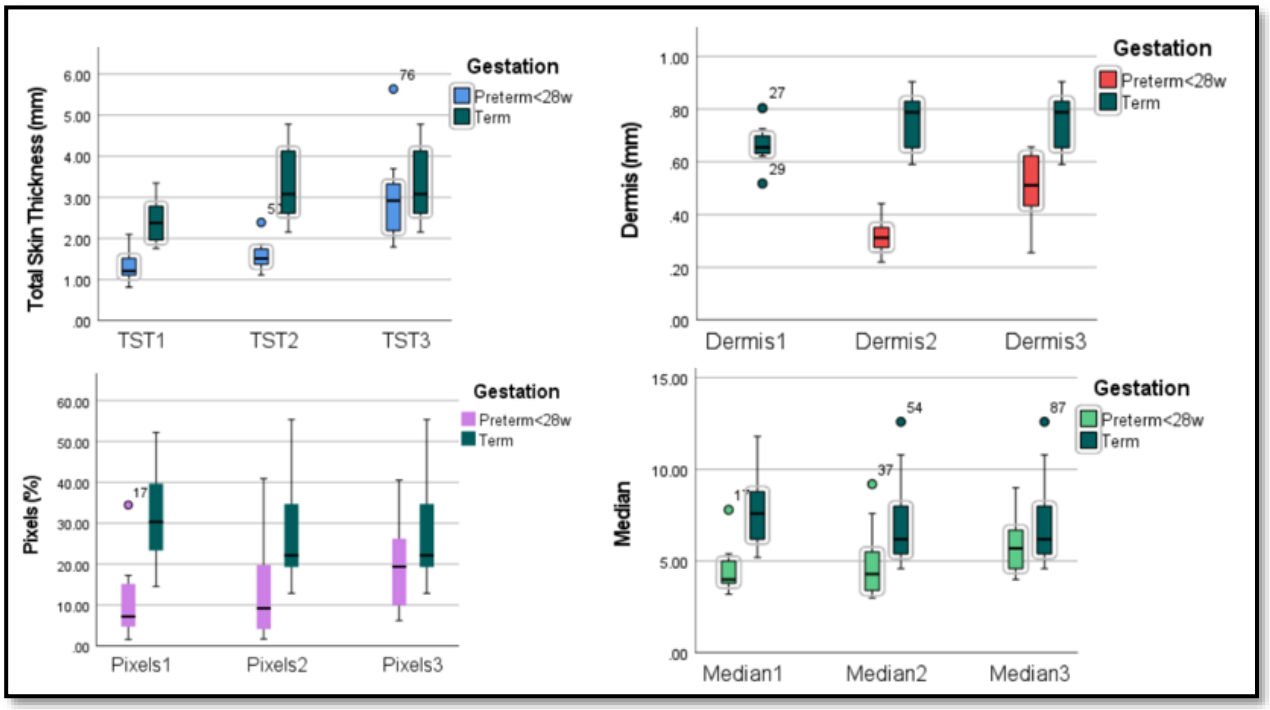


Figure 6-9 Cluster box plots showing comparison of total skin thickness (TST), dermal thickness, proportion of pixels at intensity ≥10 and median pixels as measured on HFUS images in term and preterm <28-week infants.

While the dermis thickness at >8 weeks (Dermis3) correlated well with the GA (rs=0.832, p=0.01) there was no correlation of GA with dermal thickness at 20-28 days (Dermis2) as shown in Figure 6-10. The dermal thickness at >8 weeks also correlated well with weight (r_s=0.897, p=0.003) (Figure 6-10). The dermal thickness in preterm infants <28 weeks at >8 weeks of age (0.51mm) was still significantly smaller than the dermal thickness in term infants at >48h of age 0.79mm (U=9, p=0.002) as show in Table 6-7 and Cluster box plot Figure 6-9. So, the dermal thickness correlates with GA at birth and birth weight from >8 weeks of age and it continues to be significantly smaller than term infants.

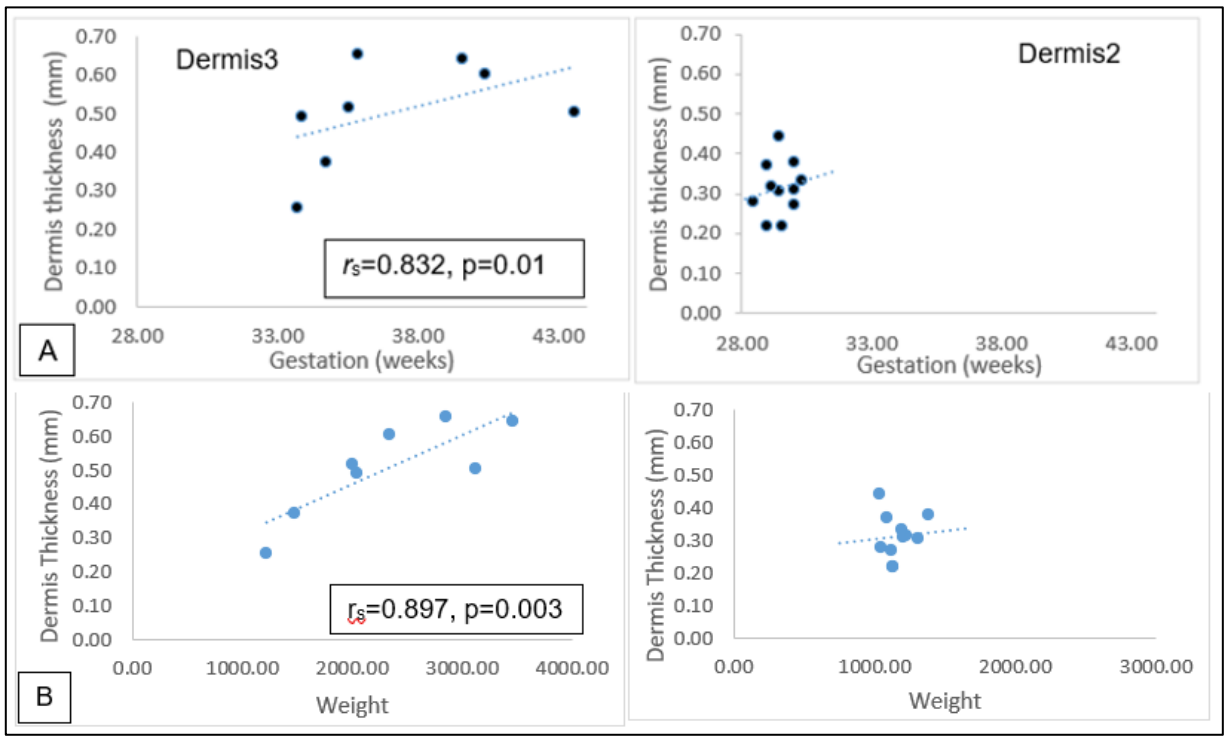


Figure 6-10 Correlation of dermal thickness with gestational age and weight at TP3 (>8 weeks) and TP2 (20-28days) in preterm <28 weeks at birth

The total skin thickness was 1.2mm, 1.5mm and 2.9 mm when measured at <48h, 20-28 days and >8 weeks of age in preterm infants born at <28 weeks GA (Table 6-5). While the increase in TST at <48h (1.2mm) to 20-28 days (1.5mm) was not statistically significant (Wilcoxon signed rank test Z=-1.727, p=0.084), it was noted to be significantly increased from 20-28 days (1.5mm) to >8 weeks(2.9mm) in these infants (Z=-2.524, p=0.012) (Figure 6-9). This was correlated to increase in body weight from a mean weight 1186 ±197 g to 2316±734 g from 20-28 days to >8 weeks. Correlation of body weight with TST at >8 weeks: r_s=0.807, p=0.016 was shown (not depicted in figures), as expected due to general growth.

The Mann Whitney U test showed that the TST in term infants at >48h was not statistically different from the TST3 at >8weeks in preterm infants born at <28 weeks. The TST in term infants at <48h was significantly different from the TST1 measured at TP1 <48h (U=3, p-<0.001) This is shown graphically in Figure 6-9 (Table 6-7).

Table 6-7 Mann Whitney U test for comparison of skin characteristics of Preterm babies <28 weeks at birth with term infants

Preterm<28weeks	Term	U	p-value
TST1 (n=18)	TST Before 48h (n=10)	3.0	**<0.001**
TST2 (n=16)	TST After 48h (n=13)	2	**<0.001**
TST3 (n=8)	TST After 48h (n=13)	43	0.514
Dermis2 (n=11)	Dermis After 48h (n=13)	0	**<0.001**
Dermis3 (n=8)	Dermis After 48h (n=13)	9	**0.002**
Pixel1 (n=18)	Pixel Before 48h (n=10)	10	**<0.001**
Pixel2 (n=16)	Pixel After 48h (n=10)	35	**0.002**
Pixel3 (n=8)	Pixel After 48h (n=13)	33	0.169
Median intensity1 (n=18)	Median intensity Before 48h (n=10)	6.5	**<0.001**
Median intensity2 (n=16)	Median intensity After 48h (n=0)	33.5	**0.002**
Median intensity3 (n=8)	Median intensity After 48h (n=13)	35.5	0.231

The echogenicity was measured as percent pixels at intensity ≥10 in the dermis. In the preterm babies <28 weeks there were 9.7% and 13.1% pixels at intensity ≥10 when measured at TP1 (<48h of age) and TP2 (20-28days of age) respectively as compared to 31.9% in term infants measured at <48h of age (Table 6-5). This difference was statistically significant on Mann Whitney U test (<48h of age U=10, p=<0.001; 20-28 days of age U=18, p=0.001) (Table 6-7). This is more evident graphically in Figure 6-9. The same was not true when the measurements were repeated for preterm babies at >8weeks (19.7%) and compared with term infants' proportion of pixels at both time points (Term <48h= 31.9%, 2-7days=28.5%). This is also shown as clustered box plot in the Figure 6-9.

Hence the echogenicity profile of extreme preterm babies born <28 weeks is statistically like term infants by 8 weeks but not at 20-28 days. Wilcoxon signed rank test explored the significance of difference in proportion of pixels with intensity≥10 at various time points and it shows significant difference between the measurements at <48h and >8 weeks (Z=-2.52, p=0.012) (not depicted as a figure).

Table 6-8 Table of comparison of skin measurements at TP3 with Term >48h (Mann Whitney U Test)

Preterm <28 weeks, >8 weeks old (n=8)	**Term >48h old, (n=13)**	**U**	**p-value**
Dermis3 (0.5mm)	Dermis After 48h (0.79mm)	9	**0.002**

TST3 (2.9mm)	TST (3.08mm)	43	0.514
Pixel3 (19.4)	Pixel (22.16)	33	0.169
Median intensity3 (5.7)	Median intensity (6.2)	35.5	0.231

The Table 6-8 shows that the babies born <28 weeks gestation show significant difference in dermal thickness but no significant difference in their other measurements (echogenicity profile as well as total skin thickness) after 8 weeks of age, when compared with term measurements. At TP1 and TP2 all these the measurements are significantly different (Table 6.7).

In summary, the skin echogenicity profile after 8 weeks of age in preterm infants <28 weeks appears to be like the echogenicity profile in term infants, but dermal thickness continues to be significantly less in preterm infants.

6.5.2 Association with nutrition

Nutrition data was organised as cumulative daily nutrient intake (energy, carbohydrate, protein, and fat) since birth. The nutrient data was available for the period when the infant received parenteral nutrition or measured milk feeds. By the very nature of their physiology, the preterm babies established breast-feeds towards the end of their stay in NICU, hence cumulative nutrient data was available for all the preterm infants <28 weeks as shown in Figure 4-5 in Section 4.4. This data was correlated with the characteristics of the skin.

Body weight and head circumference along with intake of carbohydrate, fat, protein, and energy were used as surrogates for nutritional intake. Body weight has its own fallacies not only in terms of measuring, due to difficulties in maintaining accuracy of weighing a sick fragile newborn baby but also because body weight could fluctuate due to multiple reasons other than nutrition due to excess fluid retention or dehydration. However, it is still the most frequently used method of recording growth in newborn babies and children. Head circumference similarly is difficult to measure in sick newborn babies and ubiquitously known for inter-person variability. However, regular accurate body weight and head circumference measurements to monitor their trends are considered the best growth monitoring methods by prestigious intellectual bodies like Royal College of Paediatrics and Child Health. Research is ongoing to understand how to assess the body composition in preterm babies as it is considered a fundamental part in improving nutrition (Andrews et al., 2019, Johnson et al., 2022).

Table 6-9 Name labels used for nutritional analysis.

Cumulative energy intake since birth at time point 2 (KCal/Kg)	CEnergy2
Cumulative carbohydrate intake since birth to time point 2 (g/Kg)	CCArbohydrate2
Cumulative fat intake since birth at time point 2 (g/Kg)	CFat2
Cumulative protein intake since birth to time point 2 (g/Kg)	CProtein2
Cumulative energy intake since birth at time point 3 (KCal/Kg)	CEnergy3
Cumulative carbohydrate intake since birth to time point 3 (g/Kg)	CCarbohydrate3
Cumulative fat intake since birth at time point 3 (g/Kg)	CFat3
Cumulative protein intake since birth to time point 3 (g/Kg)	CProtein3

For comparison and keeping in line with the analysis of skin characteristics, the nutritional analysis was conducted at similar time points 48h, 21 days and 8 weeks. The nutrition accumulated since birth till the time point were aggregated and used for analysis. It was accepted that not all the nutrient intake goes into tissue building in a metabolically unstable premature newborn baby. A substantial proportion of nutrient intake is used up for metabolic bodily functions especially in the stressful intensive care environment and a lot of it is excreted as well. However accurate daily charting is currently considered the best way to understand nutritional intake in newborn babies. The cumulative nutrient intake was labelled for each time point as below for ease of recording and analysis as shown in Table 6-8.

Table 6-10 Spearman rank Correlations of body weight with skin characteristics in preterm infants

Correlation of	With	P value
Weight at birth	Pixel1	**0.047**
Weight at birth	Dermis3	**0.003**
Weight at birth	TST3	**0.01**
Weight at 21 days	TST2	**0.024**

Weight at 21 days	Dermis3	**0.004**
Weight at 8 weeks	Dermis3	**0.011**
Weight at 8 weeks	TST3	**0.016**

NB Only significant correlations are added to this table.

The correlation was not conducted for any skin characteristics measured at <48h with nutrient intake as it was considered too short a time for postnatal nutrition to impact the skin characteristics. On the other hand, the birth weight and head circumference were considered as surrogate markers of nutrition at this early stage. Birth weight has been discussed in respective chapters already and is briefly mentioned here for reference. TEWL at <48h was noted to be significantly different on Mann Whitney U test in two weight categories 500-800g vs 1200-1500g (U=3, p=0.049) and 500-800g vs 2000-2500g (U=3, p=0.009) (Table 5-2). In fact, the babies with birth weight <1200g had significantly higher TEWL values than those with birth weight 2000-2500g (Figure 5-7). It was prudent to explore if TEWL correlated with structure and composition of the skin. TEWL>21days correlates with cumulative carbohydrate intake (r_s=0.521 and p value 0.046) and energy intake (r_s= 0.56 and p value= 0.29) as shown in Figure 6-11.

The birth weight had no correlation with dermal thickness or TST in term infants (Figure 6-3). There was strong correlation of body weight with Dermis3 (r_s=0.897, p=0.003) (Figure 6-6 and Table 6-3). There was no correlation of percent pixels at intensity ≥10 with birthweight in term infants however Pixel1 correlated with birth weight in <28week preterm infants (p=0.047), as seen in Table 6-10.

Table 6-11 Spearman Rank Correlations of skin characteristics with nutrient intake

	P value	r_s	n
TST2 with CEnerygy2	0.075	0.457	16
TST2 with CFat2	0.057	0.485	16
Pixel2 with CEnergy2	**<0.001**	**0.77**	**16**
Pixel2 with CProtein2	**0.003**	**0.691**	**16**
Pixel2 with CCarbohydrate2	**<0.001**	**0.751**	**16**
Pixel2 with CFat2	**0.005**	**0.661**	**16**
TEWL>21d with CEnergy2	**0.046**	**0.521**	**16**

TEWL>21d with CCarbohydrate2	**0.029**	**0.563**	**15**
Dermis3 with CEnergy3	**0.044**	**0.721**	**8**
Dermis3 with CFat3	**0.003**	**0.885**	**8**
TST3 with CEnergy3	**0.038**	**0.735**	**8**
TST3 with CFat3	**0.018**	**0.797**	**8**

No correlation of head circumference was noted with TEWL, TST1 or Pixels with intensity ≥10 intensity at <48h. Correlations of cumulative nutrient intake at the three time points were analysed using the Spearman Rank test. Only the positive correlations are shown in the Table 6-10 and 6-11. Scatter plots show these correlations graphically in Figures 6-10, 6-11 and 6-13.

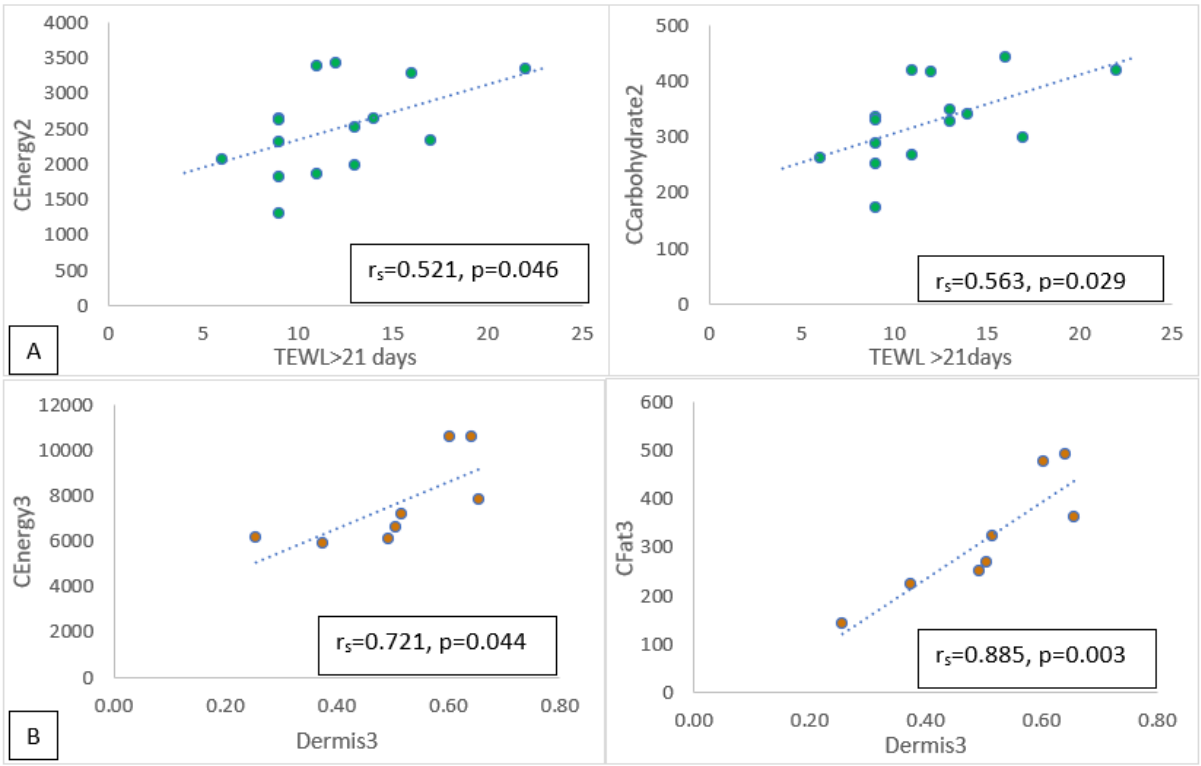


Figure 6-11 Correlation of Dermis 3 (at >8 weeks) and TEWL at >21 days with nutrition

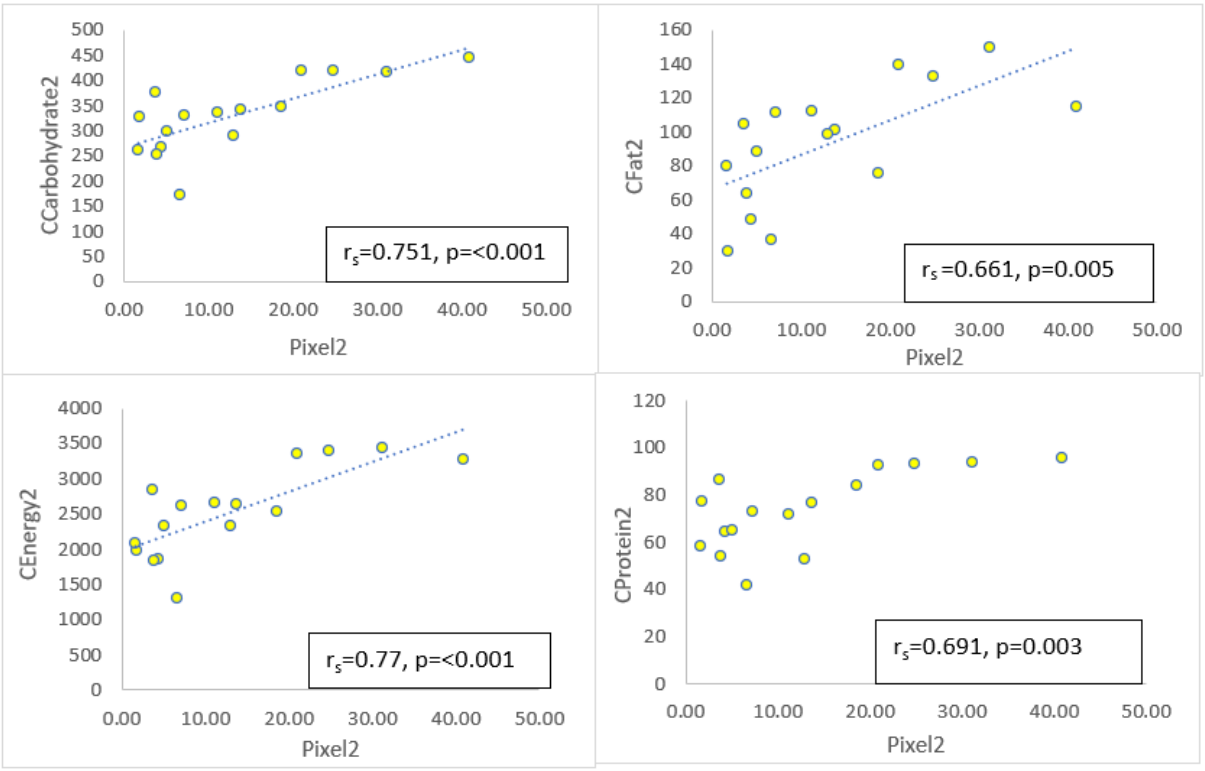


Figure 6-12 Scatter plots showing significant correlation of multiple nutrients with Pixel2

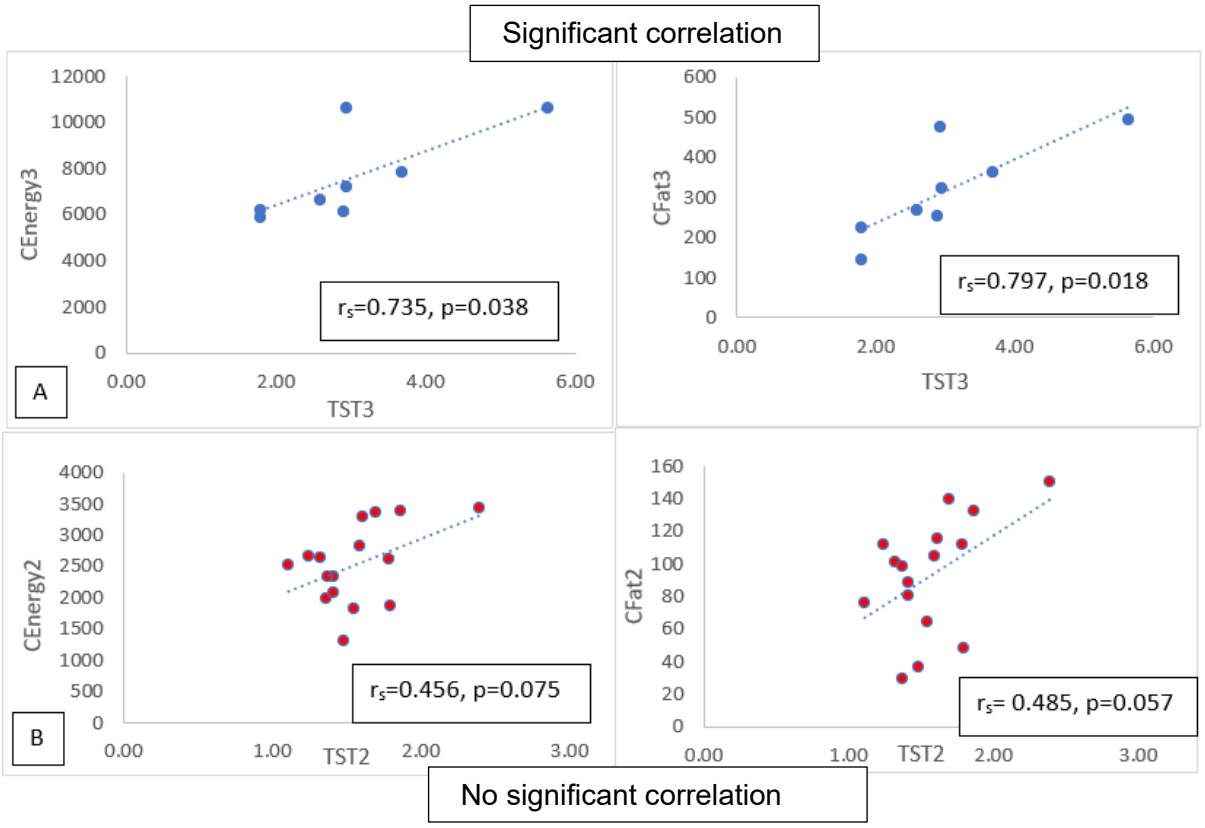


Figure 6-13 Spearman Rank correlation for TST3 and TST2

All correlations were statistically significant with r_s coefficient labelled for each scatter plot. The correlations were obviously greater with advancing age due to increased intake. The correlation of dermal thickness was strongest with fat intake at TP3.

An important observation during the study of graphs in Figure 6-12 was that percent of pixels ≥intensity 10 were correlated to all the nutrients accumulated at TP2 (20-28 days). There was no such correlation noted at TP3 (>8 weeks). This may be because the data for nutrition intake at >8 weeks was not complete as several babies had started to breast feed and many others had been discharged or transferred to another hospitals.

While TST3 demonstrated good correlation with cumulative energy and fat, TST2 showed no correlation with any nutrient. The Pixel 2 similarly showed strong correlation to all the nutrients studied (Figure 6-12), while Pixel3 had no correlation. The correlation of TST3 with cumulative energy intake, weight and cumulative fat intake would be expected as part of normal growth in an infant. Dermis3 also correlated with the same factors as TST3.

In summary the correlation of nutrients with some skin measurements is present but does not demonstrate a pattern. Cautious accurate conclusions should be drawn about this parameter due to small numbers in each group. Also, the nutrient intake is accurately calculated only when on parenteral nutrition or nasogastric feeds, nutrient intake assessment in breastfed babies is limited due to physiological reasons.

The salient points recognised from analysis of skin structure and function considering nutrient intake in this study were:

1. Dermal thickness, total skin thickness and echogenicity increased with increasing age and weight. Head circumference had no such correlation.
2. No difference of dermal thickness and total skin thickness between genders
3. Male infants had less percentage of Pixels with intensity≥10 at <48h than female infants. Percentage of Pixels with intensity≥10 in males increased with time but in female infants remained the same. Male infants had greater proportion of Pixels with intensity≥10 at >8 weeks than female infants (Figure 6-8).
4. Dermal thickness in <28week babies at chronological age >8 weeks continued to be significantly smaller than term infants, while the total skin thickness and echogenicity are similar by this age (Figure 6-9).
5. TEWL and pH were higher in smaller babies (Figure 5-2) and continued similarly for longer
6. Total skin thickness and dermal thickness correlated with all measured nutrients but not with protein intake at any time point.

7. Correlation of dermal thickness was strongest with fat intake (Figure 6-11)
8. Percent Pixel with intensity≥10 at 20-28 days correlated with all nutrients (Figure 6-12).

Chapter 7 Discussion

This study adds to the knowledge about skin characteristics particularly in the extreme preterm babies and strengthens the evidence by documenting the statistically significant chronological changes in preterm infants when compared within the group and with infants born at term gestational age. In the current times when survival of extreme preterm babies is increasingly common, the non-invasive techniques of research are gaining significant importance as alternative methods to expand knowledge without challenging the ethical dilemmas faced by the more traditional invasive techniques to study the physiologic processes in neonates. Non-invasive in-vivo tests available to study the structure and function of skin in preterm babies were successfully implemented in a single centre longitudinal study design, with corresponding clinical and nutritional data. The findings from this study demonstrated the following:

- Transepidermal water loss is significantly higher in extreme preterm babies and takes longer to decrease, indicating that SC is significantly immature in these babies for several weeks. TEWL was also affected by incubator humidity settings, which were highlighted by the corrected TEWL values presented in this thesis.
- All newborn babies (term and preterm) are born without adequate acid mantle, leaving the skin vulnerable to biochemical and microbiological insults. The acid mantle starts to develop over first few weeks, more quickly in moderate preterm babies than in extreme preterm babies.
- The skin structure and morphology of extreme preterm infants is significantly different and takes several weeks to be comparable with term infants. This includes a thinner, less dense dermis. In addition, the extreme preterm infants demonstrate limited differentiation between the skin layers of the epidermis, dermis and hypodermis, which is indicative of structural immaturity and susceptibility to mechanically induced damage (pressure injuries).
- Quick bedside assessment of skin function and structure using non-invasive devices was shown to be feasible, with potential clinical application for supporting practice in both skin care and physiological monitoring.

The aim of this study was to characterise the skin structure and function for different gestational ages and birth weights. The functional characteristics of skin in this population are well described in literature and are known to arise from the stratum corneum composition and maturity. These have been assessed by measuring firstly the TEWL to assess the stratum corneum maturity and secondly pH which assesses the acid mantle and indication of natural microbiome. The structural characteristics of skin are described using the novel method of high frequency ultrasound scan to delineate the dermal and subdermal characteristics including dermal thickness and density which measures the collagen content and organisation in the dermis. Chronological evaluation of the function of stratum corneum and the structure of the dermis presented as a time series data helps to enrich the description of the evolving changes in skin with increasing maturity.

The objective measurement of the aspects of skin structure using high frequency ultrasound scan is shown to be an optimum technique which is reliable, reproducible as well as easy to use as a bedside tool. There are novel correlations which have also been tested with nutrition in Section 6.5.2.This study has demonstrated correlation of dermal density with TEWL as demonstrated in Section 6.5.2, Figure 6-11. These correlations will pave way to help the clinicians in management of pressure injuries mentioned in Section 1.1, Figure 1-1. In this study there was no effect of maternal BMI on development of skin barrier. The correlation with other aspects of maternal nutrition was not within the scope of this study. Antenatal interventions such as antenatal steroids did not demonstrate any effect on development of skin maturity. There was no influence of mode of delivery as well as mentioned in Section 5.1.2. Besides measurement of TEWL the influence of incubator humidity on evolution of skin barrier was demonstrated in detail both using the measured TEWL values and calculated TEWL at 50% humidity (cTEWLH50) Section 5.1.4, Figure 5-4 and 5-5.

7.1 TEWL, pH, HFUS

Our study explores the skin function and maturation, along with the changes in skin structure and morphology in serial measurements over several weeks in preterm babies over a range of gestational ages and compares the measurements with term infants. The previous literature typically involved smaller sample sizes, with a limited range of gestational ages (Table 7-1).

Table 7-1 Table of studies on preterm skin maturation

References	GA (weeks) mean (range)	Weight(g) mean (range or SD)	n	Type of study
TEWL				
(Rutter and Hull, 1979)	<30 (26-29)	890 (660-1340)	4	Cohort study
(Hammarlund et al., 1982)	26 (25-27) 28 (28-30)	860 (±110) 1320 (±240)	9 13	Cohort study
(Kalia et al., 1998)	27(23-32)	900 (615-1650)	10	Case series
(Agren et al., 1998)	24-25	711(481-805)	13	Case Series
(Agren et al., 2006)	25 (23-27)	740 (395-995)	22	RCT
pH				
(Kanti et al., 2014)	30-37	1000 - >2500	48	Observational study
HFUSI				
(Vitral et al., 2018)	36(24-41)	2680 (510-4340)		Observational study of dermal thickness
Current study				
(Data collection in 2018)	26 (24-27) 32 (28-36) 39 (37-42)	885 (500-895) 1680 (690-2440) 3230 (1888-4230)	20 19 20	Observational cohort study with serial measurements

Out of 75 eligible patients we were able to obtain consent from 59 patients. Agren et al enrolled 27 infants but excluded 5 due to death or transfer to another unit (Agren et al., 2006). Similarly, in the current study 3 preterm babies died in the duration of the study and 11 were transferred out but data was retained for analysis for all of them. There were 6 sets of twins and one set of triplets in our study like another study which also included 1 set of twins and a set of triplets amongst the 10 recruited (Kalia et al., 1998), as multiple pregnancies often result in preterm labour. Due to purposeful recruitment, we were able to recruit 20 babies in 24-27 weeks GA group, as another study which recruited 22 in the same GA range (Ågren et al., 2006). In addition, we also recruited to 19 in 28-37 weeks and 20 term babies, as shown in Table 7-2. Besides Agren et al, there are no other studies who recruited as many extreme preterm babies as the current study. Unlike other studies we are unique in being able to provide comparisons with late preterm and term babies, as there are similar number of babies in each group as depicted in Table 7-1.

Average gestation amongst extreme preterm babies recruited in the current study is 26 (24-27) weeks while Agren et al recruited at average GA 25 (23-27) weeks and Kalia et al recruited 10 patients from 23-32 weeks. While one study recorded measurements till 28 days of age (Agren et al., 2006), 4 babies were measured till after 8 weeks of age in another study (Kalia et al., 1998). In the current study 18 babies had measurements after 8 weeks of age, hence providing chronological changes for preterm babies for longer. Also, in the current study we can compare the measurements for preterm and term babies which has not been presented in the other studies.

The birth weight for extreme preterm babies in the current study was 885g which is like 900g in the study by Kalia et al but more than 740g in study by Agren et al. In the current study the gender distribution was 10:8 M:F for GA <28 weeks, while female babies were in majority(6:4) in another study (Kalia et al., 1998). On the other hand, boys are reported to be in majority (M:F 14:8) in the other study (Ågren et al., 2006). While 12/20 babies in the current study were delivered by caesarean section, only 8/22 did so in another study (Agren et al., 2006). Antenatal steroids were administered for all extreme preterm babies in our study, like two other studies by Kalia and Argen. All the extreme premature babies in the current study were in 80-90% incubator humidity to start with and it decreased gradually in week 2. Similarly, Agren et al started all the extreme preterm babies in 85% humidity till day 7 and them randomised them to 75% or 50% for further measurements. On the other hand, babies in another study were in variable ambient humidity including humidified tent in one baby (Kalia et al., 1998).

In our choice of site for measurements thigh was chosen because it was easily accessible regardless of the babies' position in the incubator and of the multiple therapeutic interventions occurring elsewhere on the body surface, like another study including extreme preterm babies (smallest baby, 23 weeks, 615g) (Kalia et al., 1998). Another previous study measuring TEWL in extreme preterm babies at 18 different sites, included thigh as one of the sites (Rutter and Hull, 1979).

7.1.1 TEWL

Our choice of equipment was an open chamber device which was like the devices used in several previous studies of newborn and preterm infants in incubator humidity. Like other researchers we did not face any difficulties like condensation of ambient water vapours. We paid close attention to avoid any drafts near the device during measurements and obtained reliable and repeatable TEWL measurements which were also comparable to other studies.

Table 7-2 Comparison of measurements in each study

Gestational age at birth (weeks)						
(Agren et al., 2006)		Current study				
Measurement protocol	**23-27 weeks**	**Measurement protocol**	**<28 weeks**	**28-33 weeks**	**34-37 weeks**	**>37 Weeks**
Days of life	n=22	**Days of life**	n = 20	n = 11	n = 8	n = 20
0	14	**<2**	20	11	7	19
3	3	**3-4**	10	10	4	18
7	22	**6-7**	17	11	3	
14	10	**9-11**	16	13		
-	-	**13-15**	19	12		
28	22	**28-34**	15	10		
-	-	**>50**	11	7		
Total	**71**		**108**	**74**	**14**	**37**

Comparing the frequency of data collection, the current study collected 108 samples from 20 extreme preterm babies which is not very different from 71 by (Agren et al., 2006) (Table 7-2). On the contrary, Kalia et al collected 211 measurements from 7 babies <28 weeks at birth, 4 babies having measurements till >8 weeks of age (Kalia et al., 1998). TEWL on day 1 in extreme preterm babies (<27 weeks) has been reported to range between 32 g/m^2/h (Rutter and Hull, 1979) and 62 g/m^2/h (Agren et al., 2006). However, the corresponding measurements of TEWL in the present study was 19.4 ± 4.6 g/m^2/h. Rutter and Hull et al have reported measurements of TEWL within 1 hour after birth in RH 27-63%, in babies born at 26-29 weeks. Agren et al have reported TEWL measurement in babies born at 23-27 weeks, on the day of birth, with RH in incubator at 85% which was decreased to 65% to avoid condensation of vapours on sensors of open chamber device and ambient incubator temp increased by 1° C to avoid drop in axillary temperature. In contrast the first measurement in the current study was between 24-48hrs after birth, in average incubator humidity of 83%± 5.3 giving time for skin adaptation to occur to some extent. Also, the incubator humidity and temperature were kept unchanged before and during the duration of the measurement, so the measurement in the current study is reflection of the concurrent TEWL (artificially reduced TEWL in presence of ambient humidity) rather than skin immaturity, resulting in lower TEWL in lower gestational ages compared to that measured by Agren et al and Rutter and Hull et al. This method of TEWL measurement is considered advantageous, as it gives an idea of true insensible loss to the clinician, though it doesn't give the true assessment of degree of SC immaturity. A correction factor was used as mentioned in section 3.3.4 to calculate cTEWLH50 to obtain an estimate of TEWL in ambient humidity of 50% which is equivalent to room air.

In a previous study Agren et al reported mean TEWL at 34h of age was 39.9±9 g/m^2/h and cTEWLH50 was 58.4±14.8 g/m^2/h in thirteen 24-25 weeks babies (Agren et al., 1998). Of note the ambient humidity in the incubator is reported to be 65±7%. The babies of similar gestation (24-25 weeks) in the current study (n=6) were measured at <48h in incubator humidity 83%± 5.3 and had measured TEWL 22.3±4.5 g/m^2/h and cTEWLH50 74±29.3 g/m^2/h. The babies in the current study were similar by gestational age (mean 24+6, range 24-25+2 weeks) but slightly smaller by weight (689 ± 110 g), as compared to the babies in Argen's study (mean 711g) (Table 7-1). On day 28 TEWL was 20g/m^2/h (n=3) in the current study which is like that reported by Agren et al [TEWL= 26 g/m^2/h (n=8)].

Stamatas et al have reported changes in skin structure and function with age in a population ranging from newborn to adults in a review of *in vivo* studies (Stamatas et al., 2011) and in a recent cohort study (Stamatas et al., 2023). Other authors have presented data to this effect for childhood years (Walters et al., 2016). The comparison of newborn infants born at preterm and full-term GA has not been presented for a few decades (Hammarlund and Sedin, 1979). Our analysis showed that TEWL significantly decreases with increasing gestational maturity as shown in Figure 5-2 in the clustered box and whisker plot and higher the gestational age at birth, lower is the TEWL. TEWL was measured at 7g/m^2/h at 34-37 weeks and 19 g/m^2/h at 24-26 weeks when measured at <48 hours indicating that the SC barrier is much more mature in late preterm babies than extreme preterm babies at birth, which goes hand in hand with the clinical experience in NICU. This is similar to the findings in the series of studies by Hammarlund et al, where TEWL measured within first 24 hours was 20 g/m^2/h in 29 week babies and 5g/m^2/h in babies born at 37 weeks (Hammarlund and Sedin, 1979).

Premature infants less than 28 weeks lack the coverage of vernix and the TEWL in these babies has been noted to be consistent with wounded skin (Hammarlund and Sedin, 1980) (Section 2.3.3). cTEWLH50 was 120g/m^2/h in 24–25-week infant in the current study (Figure 5-5) which is like that reported by Hammarlund in 25–27-week babies when nursed in 50% humidity. This strengthens the argument for nursing these babies in incubator humidity to maintain haemodynamic stability. Another study has shown that moisture accumulation rate is higher in vernix retained skin (Visscher et al., 2005), though vernix retention did not affect thermoregulation, demonstrating the advantages of vernix retention in postnatal SC adaptation.

Also, the current study has demonstrated that TEWL decreases with increasing chronological age in all gestational age groups (Figure 5-2), with statistical significance. The TEWL values reported by Rutter et al of 32g/m^2/h at 4 hours in <30week babies and 11g/m^2/h at 2 weeks of age in the same group (Rutter and Hull, 1979) are similar to the current study. While a correlation between TEWL and postconceptional age in <30 weeks has been reported by Rutter et al, this was not confirmed by statistical analysis. Despite lower first TEWL noted in the babies in our study (19g/m2/h in the smallest babies) we found statistically significant comparisons of TEWL measured at <48 hours of age between all GA groups, and some birth weight groups (500-800g vs 1200-1500g, 2000-2500g and 2500-4500g) (Table 5-1 and 5-2). This provides the basis to inform clinicians with critical data about the barrier function of the skin and therefore provides important evidence for clinical management in NICU. Similar to the present study showing statistically significant drop in TEWL with increasing age (<48h

and >21 days), Agren et al showed that the decrease in TEWL between day 7 and day 28 in 23-27week babies was statistically significant (Agren et al., 1998).

In a randomised controlled trial of using 75% or 50% relative humidity in nursing extreme preterm babies, Agren et al concluded that decrease in TEWL was more pronounced in babies nursed in lesser relative humidity (Agren et al., 2006). Similar findings were reported by another group (Fluhr et al., 2010). The current study did not compare TEWL measurement at different RH for the same GA however, it was noted that TEWL decreased to <10 g/m2/h within the first week in most of the babies born at >27 weeks (exposed to lesser humidity), while it took 3-4 weeks in those born at 24-26 weeks (nursed in incubator humidity for 2 weeks). The more preterm babies (24-25 weeks) had higher TEWL at birth, hence they were further away from the mature SC barrier (TEWL <10g/m²/h), see Section 5.1.4, Figure 5-4.

It was also interesting to note that in the current study TEWL<10g/m²/h was achieved while nursed in humidity, and it increased when relative humidity was lowered or removed (Figure 5-3, 5-4). In Figure 5-4 TEWL increased back to 20-30 g/m²/h in two 24 weeks infants at 2 weeks of age with cessation of humidity. This shows that ambient humidity can affect TEWL artificially and favourably, especially in supporting fluid and electrolyte management, while the stratum corneum barrier function is still immature. Besides the RCT (Agren et al., 2006), numerous other studies have reported time to reach TEWL ≤10 g/m²/h (enough to suggest SC barrier development). While in one study the average TEWL was plotted to ≤10 g/m²/h at 40 days of age in 25-27week babies (Hammarlund et al., 1977), another study reported TEWL decreasing to approximately 10 g/m²/h by 2-4 weeks in <30week babies (Rutter and Hull, 1979). The case series by Kalia et al meticulously reported TEWL <10 g/m²/h at 8-9 weeks in a 23week baby and 3-5 weeks in 26 week babies (Kalia et al., 1998). As mentioned previously, the current study shows that while time to reach TEWL <10 g/m²/h is 1-2 weeks in >27-week infants it is at least 3-4 weeks in 25–26-week infants, and even longer in more premature babies, 40-60 days in 24–25-week babies. Though the primary outcome of the RCT (Agren et al., 2006) mentioned above was not when the TEWL decreases to less than 10 g/m²/h the group has reported that at 28 days of age TEWL is 22±2 g/m2/h and 13±1 g/m²/h in 75% and 50% relative humidity respectively. In the RCT, TEWL dropped from 62 g/m²/h to 17 g/m²/h in the second week of life in the 23–27-week babies which is similar to the present study (Figure 5-4).

A recent cohort study reports mean TEWL in 20 babies (mean age 1.1 years) to be 14.5 g/m²/h with range 13-18 g/m²/h (Stamatas et al., 2023). The same study also reported TEWL 11.5-13.5 g/m²/h in 3–6-year-olds. TEWL was measured using a closed chamber device and

was measured on cheek and dorsal forearm. TEWL in the current study for Term infants was generally <10 g/m²/h in most cases (Mean 7.4±1.9 g/m2/h) (Figure 5-2). A reference dataset of TEWL values of newborn babies reports TEWL 7±3.4 g/m²/h (Kelleher et al., 2013) in term babies using open chamber device, like the current study. Another study reports that though stratum corneum looks intact shortly after birth the way it transports water becomes adult like only after 1 year of age. Several studies have highlighted the importance of not removing vernix after birth to allow SC cornification and slow reduction in surface hydration as also noted in section 2.3.3. Visscher et al have shown that the water binding moiety, free fatty acid levels were extremely low at birth in vernix removed skin and remained markedly lower than typical adult levels, indicating the importance of retaining vernix after birth to allow adaptation of SC after birth (Visscher et al., 2011).

TEWL remains higher for longer at earlier gestation which matches with the clinical experience where metabolic - hemodynamic instability and need for careful management of fluid balance for longer in extremely premature babies. This study demonstrates that the difference is TEWL is statistically significant and cements the need for incubator humidity for extremely premature babies. The functional immaturity of the SC requires further investigation, matching the optimisation of environmental conditions, skin interventions (barrier products) and care regimen to promote barrier maturation.

7.1.2 pH

Our choice of equipment for skin pH was glass pH meter which was calibrated daily in a reference solution. All comparative studies used similar equipment (Yosipovitch et al., 2000, Hoeger and Enzmann, 2002, Visscher et al., 2013, Kanti et al., 2014). There were no studies using calorimetric method (Section 2.5.2).

The present study identified that skin pH reduced in the weeks post birth (developing the acid mantle). This involved a decrease from pH 6.5 to pH 6 over 3 weeks in 24–26-week group (Figure 5-6). Also, when comparing different gestational age groups pH decreased from 6.5 to 6 in 24-26 weeks babies and 5.9 to 5.8 in 34-37 weeks babies over first 3 weeks of life. These values of pH in the current study are comparable to the pH 5.4 measured at 3 weeks of age in <38 week babies (mean GA 31+6 weeks) (Visscher et al., 2013). Kanti et al measured skin pH in 48 infants ranging from 30-36 weeks (mean 34 weeks) and noted decrease in skin pH from 5.7 to 5.5 from day 2-14 (Kanti et al., 2014), which is again comparable to the current study. The values for term infants in the current study (mean pH 6.7 at <48h) also matches with pH 6.3 in term infants soon after birth reported in a recent

review (Oranges et al., 2015) and pH 6.6 in term newborn babies when measured <10 hours after birth (Yosipovitch et al., 2000).

The decrease in pH has been reported to be significant between the first and second day of life in term infants (Yosipovitch et al., 2000) and preterm infants (Kanti et al., 2014), the current study could not compare with these findings as comparative measurements were not made within the first 2 days in the current study. The gradual decrease during the first month of life suggested by several studies for term babies (Yosipovitch et al., 2000, Oranges et al., 2015, Hoeger and Enzmann, 2002) and two studies for preterm babies (Kanti et al., 2014, Visscher et al., 2013) has been demonstrated in the current study, suggesting that the acidification pattern of preterm infants follows that of term infants and is not influenced by GA at birth.

Our study shows that pH in term infants decreases significantly from <48h to >21 days (Table 5-4) however there was no statistically significant difference between the two measurements taken in week 1 (Table 5-8) which indicates that pH continues to decrease over first few weeks rather than a rapid decline within week 1. This is same as shown in another study where pH was measured in term infants over first 3 months and showed decrease in pH from day 3 to day 30 (Hoeger and Enzmann, 2002). On the other hand, rapid decrease in pH was demonstrated from day 1 to day 2 by another group of researchers, however no further measurements were recorded in this study (Yosipovitch et al., 2000). This group showed that pH was significantly different between day 1 and 2 in term infants ($p<0.05$) at all anatomic sites measured and between neonates and adults ($p<0.01$).

Vernix caseosa has been shown to enhance skin surface acidification, as decrease in pH occurred earlier in group with vernix retention (Visscher et al., 2005). While the demographics and pH values for term infants in the current study (pH 6.7) are comparable with pH 7.0 in another study of term infants (Yosipovitch et al., 2000), it should be noted that the skin was washed with wet towel and tap water in study by Yosipovitch et al but only dried with warm towels in the current study. We speculate that gentle washing with water did not remove the hydrophobic vernix, leading to process of acidification not being affected and skin pH measured is same as our study.

Stratum corneum function is a combination of variables of which TEWL and pH have been measured in this study. Both the current study and several previous reports on term infants mentioned in discussion above in Section 7.1.1 show that TEWL is relatively low soon after birth. Skin pH continues to be raised for longer as shown in the current study and argued above. Hence though TEWL is low, the raised pH in term infants enhances the irritant

potential of compounds on newborn skin. The acid mantle is important to measure as it plays an important barrier function in protecting against microbial invasion, regulating permeability and the rate of barrier repair after injury.

7.1.3 HFUS

To the authors' knowledge, we have demonstrated for the first time using HFUS that dermis is not clearly delineated in extreme preterm infants for several weeks. In these infants once the dermal thickness could be measured at 3 weeks of age it continued to be significantly smaller than the dermal thickness in term infants. The echogenicity of dermis using HFUS has also not been described in literature for extreme preterm infants. The dermis is poorly echogenic soon after birth. The echogenicity increases with increasing gestational age and chronological age but is significantly different from term infants for at least 8 weeks after birth (Section 6.4.2).

For HFUS imaging we used a 35Hz probe with a resolution in vertical dimension of 40 microns which produced good image of epidermal echo and delineated the dermis and hypodermis well. This was like the 48 MHz probe used by Paulina et al. B-mode images were used for analysis like the other 2 studies showing neonatal skin measurements (Paulina Przybysz, 2020, Vitral et al., 2018). Multiple images were produced, and mean was calculated for dermal thickness from the best image chosen, both in the current study and another one which recruited neonatal patients (Paulina Przybysz, 2020). Similar method of dermal thickness assessment was used by other studies for paediatric and adult skin using HFUS. Echogenicity was measured by counting pixels of varying echogenicity in a pre-decided ROI in our study. While similar protocol was described for echogenicity measurement by Paulina et al, no echogenicity data has been published by this group. Other studies used 20 MHz probe for neonatal and paediatric or adult skin assessment and used ROI to count echogenic pixels, like the current study (Seidenari et al., 1994, Crisan et al., 2012).

Strict QC of HFUS images was conducted to ensure that only good quality images are included. Table 6-1 shows the QC process. No such quality control processes were mentioned in published articles. In literature, Quality assurance of ultrasound images for maintaining the image quality are being considered however there is no standardization yet (Sassaroli et al., 2019). There are a number of audit reports that demonstrate the earnest effort to systematically improve ultrasound image quality via the implementation of a quality control measures (Caserta et al., 2020, Freeman et al., 2022).

The current study noted that that traditionally defined layers of skin are not visible in extreme preterm babies till 20-28 days of age. Figure 6-4 shows examples of images that only showed clearly delineated epidermis and subcutaneous layer and no dermis. This is similar to observations by (Holbrook, 1982, Paulina Przybysz, 2020) in the newborn period of absence of clearly defined dermo-epidermal junction. These findings in the current study correlate with Section 2.3.2.4. Fibronectin structural protein was noted in all the layers of dermis in fetal skin but not as prevalent in adult skin(Coolen et al., 2010). Other reports read that definitive elastin fibres first become detectable from 22-24 weeks (Akiyama et al., 1999, Holbrook, 1982). Several animal and human foetal skin studies also report higher levels or glycosaminoglycans and water in foetal dermis which is thicker by end of gestation but still much thinner than adult dermis (Coolen et al., 2010). Expansion of dermis has also been attributed to increase in fibrous collagen in extracellular matrix (Mast et al., 1991, Whitby and Ferguson, 1991, Coolen et al., 2010, Smith et al., 1986, Singh et al., 2023, Revell et al., 2021). We recognise that there are no ethical methodologies that can directly link the immunohistochemical findings with imaging. HFUSI appears to be a safe, non-invasive easily replicable method that can be used as a bedside tool.

In the current study the dermal thickness on HFUS imaging was noted to be 0.3mm in <28 weeks babies when first delineated at 20-28days of age and it continued to be statistically significantly different from term dermal thickness of 0.79 mm when compared at >8 weeks of age despite having increased to 0.5mm (Figure 6-9). No studies comparing dermal thickness at different gestational ages were found in literature, either on pathological samples or on imaging. Direct contact with perinatal histopathologists also did not indicate any concurrent ongoing studies to show skin thickness parameters. There is no database of dermal thickness on histopathological examination of newborn babies. Direct communication at SOFFOET 2022, Paris (Collardeau, 2022) with the perinatal pathologists uncovered that regular skin biopsies are not taken during perinatal autopsy, but the group found the prospect very interesting. A recent systematic review also revealed the lack of evidence on skin thickness dimensions obtained by histology (de-Souza et al., 2019).

The dermal thickness is reported in literature but not for the similar gestational age as in this study. Dermis thickness on thigh for term babies was noted to be 0.66mm at <48h after birth in the current study which is less than 0.97mm in the forearm as reported by Vitral et al who studied 222 babies soon after birth with median GA 36weeks (range 24-41) within 24 hours after birth (Vitral et al., 2018). Another study reports dermal thickness as 0.7mm on the forearm, abdomen and thigh measured in 72 newborn babies ranging from 37-41 weeks within 24 hours of age which is similar to the current study though the measurements in the current study were on the thigh (Paulina Przybysz, 2020). These are the only two studies we

found that report dermal thickness in newborn babies on HFUS imaging. Median dermal thickness on microphotographs at autopsy of premature babies at 37-43 weeks corrected GA was 0.7mm and in term babies <7days old was 1.2mm (Reed et al., 2021). Both these measurements were greater than the measurements taken on HFUS imaging in the current study as well as in the two studies found in literature (Vitral et al., 2018, Paulina Przybysz, 2020). Though they have the potential to be extremely useful, autopsy samples also run the risk of changes due to tissue processing.

As noted in Section 2.3.1.4 preterm infants do not get the opportunity to enter third trimester when the adipocytes and fat globules are aggregated in the hypodermis. Majority of the differentiation of adipocytes occurs after birth (Burdi et al., 1985). The total skin thickness is an estimation of subcutaneous fat and has been measured for all the scan images included in the analysis in the current study (Table 6-5). Figure 6-9 shows increase in total skin thickness from 1.2mm at <48h to 2.9mm at >8 weeks and is comparable with 3.08mm in term infants. No comparative measurements are available in literature. While skin thickness measurement has been found to be reliable and repeatable method of assessment of subcutaneous fat and could be used as a bedside tool, a recent systematic review suggests air displacement plethysmography, dual-energy x-ray absorptiometry, magnetic resonance imaging, or isotope dilution as trustworthy and validated methods (Yumani et al., 2023). However, these methods may not be available universally.

The current study has shown that echogenicity of the dermis increases as gestational age and chronological age increase. While echogenicity of the dermis has been reported by a Przybysz et al in newborn babies, there are no measurements or comparison of the amount of echogenicity (Paulina Przybysz, 2020). Echogenicity in children at various body sites has been reported in other studies Seidenari et al reported lower echogenicity in adults than in children on face, but increasing echogenicity with age in limbs (Seidenari et al., 2000). Another study has reported decreasing echogenicity with increasing age in 1-12 year old children (Sili Ni, 2016). Further studies on neonates will be helpful to obtain comparative data. Echogenicity was not compared in upper and lower dermis in the current study. This may be considered in the images with clearly delineated dermis. No such comparison is found in literature on HFUS imaging though Confocal laser scanning microscopy has demonstrated that the border between papillary and reticular dermis observed in adult skin, is absent in 3-24 month old infants (Stamatas et al., 2011). It is thought that the ultrasound imaging could provide the non-invasive method of investigating skin structure and morphology.

To the best of our knowledge, this is a first study to report the chronological changes in structure of the dermis. As a point of care assessment tool, already in daily use in neonatal intensive care units, ultrasonic assessment of skin thickness and density has potential for

being available to clinicians as a surrogate for epidermal barrier maturation, an essential information about neonatal physiology which can help guide intensive care decision making.

7.1.4 Correlation of demographic data/TEWL/skin pH and HFUS with nutritional data

The dermal thickness increases with gestational maturity and chronological age (Figure 6-5) This is comparable to the TEWL measurements as shown in Figure 5-2. Comparisons of dermal thickness with TEWL have been attempted in this study (Figure 6-11) to demonstrate relationship between dermal thickness and functional maturity despite the knowledge that dermal thickness is the result of collagen accumulation in dermis and TEWL is a marker of stratum corneum maturity. The dermal thickness correlates with GA and birth weight from >8 weeks of age in babies born at <28 weeks GA (Figure 6-10) and the echogenicity profile at this age (indicating collagen content in the dermis) is similar to that in term infants. This pattern is similar to the pattern observed with the TEWL measurements in term and 24–25-week preterm infants at 6-8 weeks of chronological age (section 7.1.1).

The correlation of dermal thickness was strongest with fat intake at TP3 in extreme preterm babies after 8 weeks of age. Dermal thickness correlates well with fat and energy intake at >8 weeks in <28-week preterm babies. Total skin thickness correlates well with energy and fat intake at >8 weeks not at 20-28 days.

The nutritional and anthropometric data collected in the SENNAT database has been analysed to establish relation of infant nutrition and growth to the functional (TEWL and skin pH) and structural (Dermal thickness and echogenicity as measured on HFUS) characteristics of skin in preterm infants. Though specific nutrient levels, for example essential fatty acids, zinc, selenium, and manganese have not been analysed in these babies, positive correlation of rate of weight gain with changes in skin parameters were predicted and demonstrated. The salient points recognised from analysis of skin structure and function in relation to nutrient intake in this study are as follows:

1. All measured structural skin characteristics (dermal thickness, total skin thickness and echogenicity) increase with increasing age and weight. Head circumference has no such correlation. There is no gender correlation.
2. Premature boys accumulate pixels faster than premature girls (Figure 6-8)
3. Thickness of dermis continues to be significantly smaller at term corrected age while other skin parameters (Total skin thickness, Pixels with intensity≥10 and median intensity of pixels) start to match term measurements (Figure 6-9).

4. TEWL is higher in smaller babies (Figure 5-2) and decreases chronologically. Time to reach functional maturity is longer, the more preterm they are.
5. pH decreases with increasing chronological age in term and preterm infants. Acid mantle might not be achieved before or simultaneously with decrease in TEWL.
6. Echogenicity of skin appears to correlate with all nutrients and dermal thickness correlates with fat intake (Figure 6-11, 6-12).

The comparative studies for nutrition in newborn babies in relation to skin characteristics are only available for term infants. Hughes-Formella et al measured infants and toddlers to assess difference in skin characteristics in relation to nutrition and showed that dermal thickness decreased from week 1 till 6 months of age in well-nourished infants (Section 2.6.3). This contrasts with the current study where all skin parameters are shown to increase with chronological age. In fact, the dermal thickness in undernourished infants was noted to be more than well-nourished infants (Hughes-Formella et al., 2019), which is also different in the current study where preterm babies at term corrected age have significantly smaller dermal thickness than babies born at term. The increased dermal thickness in undernourished infants is thought to be due to water retention because of delayed transition from intrauterine environment (Hughes-Formella et al., 2019), which on one hand is same as the low echogenicity in preterm infants in the current study. However, the low echogenic dermis of preterm infants in our study is much smaller than term infants (Figure 6-9).
Like the current study Hughes-Formella et al noted that Total skin thickness (proxy for subcutaneous fat thickness) increased chronologically from week 1 till week 4. They also noted that total skin thickness measurements were smaller for moderately nourished infants, like the current study where the smaller preterm babies have smaller total skin thickness.

Essential fatty acids contribute to the structure of the dermal and epidermal layers of the skin (Hughes-Formella et al., 2019) and also are central in maintaining skin barrier integrity (McCusker and Grant-Kels, 2010). These findings are like the current study showing increase in dermal thickness and decrease in TEWL with increasing maturity and dermal thickness measurements correlating well with fat intake (Figure 6-11)
Hew et al have shown that high protein intake increases dermis and epidermis thickness in male mice, and high carbohydrate intake in females resulted in thinner dermis and increased subcutaneous fat (Hew et al., 2016). Other studies showed that thinner dermis when subcutaneous tissue was increased was due to decreased fibroblast proliferation, decreased collagen precursors and suppression of genes for collagen synthesis (Yamane et al., 2010, Ezure and Amano, 2010).(Yamane et al., 2010, Ezure and Amano, 2010). This contrasts with

the current study where dermal thickness as well as total skin thickness increased chronologically in all infants without any gender differences. The nutrient intake was not controlled in the current study so male and female infants received similar proportion of carbohydrates and proteins and increase in total skin thickness did not result in dermal thinning in the current study.

While TEWL was high in premature infants and decreased chronologically in all patients in the current study, though at different rates, another study of term infants reported little difference in TEWL between well-nourished and moderately undernourished infants (Hughes-Formella et al., 2019). The most likely explanation for this is that both the groups of term infants studied by Hughes-Formella et al had attained SC maturation before start of the study, and the moderately malnourished still had an intact SC.
The poor delineation of dermis and decreased echogenicity noted in preterm infants in the current study are due to decreased collagen in the dermis as detailed in Section 2.3.2.2 and reported by (Coolen et al., 2010, Deutsch and Esterly, 1975, Smith et al., 1986). This is similar to the histopathological studies in skin of children with clinical protein energy malnutrition which have shown dermal oedema and decreased collagen content (Thavaraj and Sesikeran, 1989). Vasantha et al demonstrated decreased soluble collagen and delayed collagen maturation in dermis of rats exposed to protein energy malnutrition (Vasantha, 1970).

7.2 Clinical implications of the study

In a systematic review Glass et al highlighted the lack of information about level and duration of incubator humidity in NICU. They have shown that practices are variable not only amongst the units in UK but also in Europe and USA as there is no evidence for the current practices (Glass and Valdez, 2020). They suggest large, randomised control trials in this field, which will be ethically difficult, in the knowledge that humidity is definitely beneficial to extreme preterm babies, especially in their early days. The current observational longitudinal study fulfilled all the ethical requirements whilst providing benchmarking data on skin structure, function, and changes over time. Another RCT in this field clearly demonstrated that babies nursed in higher humidity, take longer for stratum corneum maturation (Agren et al., 2006).
The chronological evaluation of neonatal skin by simultaneously measuring transepidermal water loss, pH and dermal thickness as well as density, as done in this current study will provide comparable dataset for neonatal skin function and structure, which can be used as bedside point of care method of skin assessment and provide individualised guide for the

incubator humidity requirements of preterm babies. This study is the only one we know of which gives an opportunity to compare skin structure and function using simultaneous measurements and how they change with increasing chronological age. Collagen has already been shown to provide the echogenicity in the dermis (Plessis et al., 2013). It is challenging to assess the benefits of nutrition provided in neonatal units. The corelation of dermal thickness and echogenicity with nutritional intake has been demonstrated in this study. This has potential for use in clinical area to monitor growth benefits of adequate nutritional input.

A recent study has explored the difficulties in staging pressure injuries in preterm infants and suggested a revised staging system for neonatal pressure injuries as preterm skin is structurally different from term infants and older children (Nie et al., 2022). The knowledge gained in the present study about the structure of dermis in healthy preterm skin and its correlation with nutritional intake has potential to be used to define the structural changes in a pressure injury site in preterm infants. Figure 6.5 and Table 6.9 show significantly decreased dermis and total skin thickness as well as less collagen (implied by decreased pixel intensity) (Section 2.3.2.4) at <28 weeks GA compared to infants born at term. This correlates well with the findings of nCPAP related pressure injuries being more likely in <30 week gestation (83%) and/or very low birth weight infants (90.6%) and lesion developing at an average 1 week postnatal age (Fassino et al., 2023). Anecdotally, all newborn babies are fragile and skin injury occurs easily at this age, device related skin injury is significantly more prevalent in preterm infants (Imbulana et al., 2018, Visscher and Taylor, 2014, García-Molina et al., 2018) and is well recognised amongst health carers (Imbulana et al., 2018, Visscher and Taylor, 2014, García-Molina et al., 2018, Liversedge et al., 2018). Preventative strategies are being demonstrated to be effective in systematic reviews in adults (Lin et al., 2020) and children (Setchell et al., 2023). Studies show that the tolerance of device loads depend on structure and function of skin (Gefen et al., 2020, Bader and Worsley, 2018).(Gefen et al., 2020, Bader and Worsley, 2018). Hence the findings of the current study can guide the preventative strategies being sought for prevention of pressure injuries in NICUs.

It is important to consider the impact of kangaroo care on TEWL (Sivanandan and Sankar, 2023) . Kangaroo care is evidence based (Linnér et al., 2022) and is a routine practice in neonatal units, however more recently it is being advocated for extremely premature babies because of widespread benefits not only to the babies but also to their parents. In the current study some measurements were made while babies were having skin to skin with their parents Section 3.3.1.1. All such measurements were taken after the babies had completed the period of enhanced incubator humidity. When extra humidity is not added, the incubator humidity is assumed to be approximately 40-50%, like the room air, and in principle should not impact TEWL. However extreme premature babies do get cold when not in the incubator

unless they are in direct contact with skin and have adequate covers. In our study axillary temperature was measured before, mid-way and after the measurements of TEWL, pH and HFUS scan so the body temperature should not have affected TEWL, however there isn't enough data available to opine about this. The drop in axillary temperature during Kangaroo care is likely due to immature SC and inadequate subcutaneous fat besides probably some contribution from immature central thermoregulatory control. This is contrary to the findings of Parsa et al who reported no significant difference in all physiological parameters including temperature, however they included babies between 34-36 weeks of gestation who were stable to leave the incubator (Parsa et al., 2018). Similar findings were reported in the systematic review by Zengin et al which included similar babies (32-37 weeks and 1400-2100g) (Zengin et al., 2023).

A recent meta-analysis included babies from 23 weeks gestation though mean gestational age of included studies was reported as 31 weeks with mean birth weight as 1400g. This is not different from the current study with mean gestational age 33+5 (24-42+1 weeks) and mean birth weight 2253g (500-4230g) (Sivanandan and Sankar, 2023). Though the conclusion in this meta-analysis is that there is very little evidence of negative effect of kangaroo care on neonatal stress, the Forrest plot in the meta-analysis showed that conventional care would be preferred to Kangaroo care for parameter of temperature. The Sivanandan et all report that Kangaroo care has overall benefits. In the current study, in the few cases where measurements were taken during Kangaroo care, no increase in TEWL was noted. Further studies with larger samples, careful consideration to the study design and confounding factors like the current study will help clarify the advantages of Kangaroo care in relation to TEWL and temperature control during Kangaroo care in extreme premature babies.

7.3 Limitations of the study

The study was limited to a single centre cohort, which reduces the generalisability of the findings. Indeed, as described in the demographics section of the thesis (Table 4.1), most of the babies were from a Caucasian ethnicity. Further research is needed on more diverse cohorts to establish whether other factors such as melanin content of the skin may influence maturation of skin characteristics in neonates. As this was a single centre study it was also limited in terms of the clinical management of skin health. There is evidence to demonstrate a high heterogeneity of practice for skin care in NICU settings (Liversedge et al., 2018), with different cleaning and topical product application depending on the setting. More research is

needed to assess whether these represent confounding factors in the maturation of skin development.

Due to the longitudinal cohort design, it is hard to attribute causality between skin properties and the demographic, environmental and clinical care (nutrition) factors. There were some clear correlations observed, particularly with GA and skin properties. However, further studies, which include both intervention and control arms are required to fully establish how incubator settings, nutrition and skin care regimes impact on skin structure and function.

Familiarity with the equipment clearly demonstrated the advantages of a learnt effect from use of equipment in the current study. Indeed, the initial TEWL and HFUS images often did not meet quality control processes during the analysis. More could have been done in the piloting phase to ensure accurate and reliable data acquisition from the start of the clinical trial. This also plays into the challenge with using these tools in practice settings where a degree of technical mastery is needed to obtain meaningful data. This has been shown before for both TEWL (Alexander et al., 2018) and HFUS (Griffith et al., 2014)studies .

Limitations for this study are that TEWL was not measured at the same post conceptional age in all the patients to allow better comparison of data. This was a purposeful decision in the current study, to accommodate the ethical and clinical demands in a NICU. However, taking more frequent measurements will negate the need for averaging the TEWL measured at different time points, decreasing the distortion of results due to inevitable individual variations in skin structure and function. Also, we believe that more frequent data points, especially when the SC maturation appeared to be taking longer than usual, would have informed the chronology of SC maturation in greater detail. If investigated in this manner, we believe that this could help explore further the reasons for such delay which has not been within the scope of this study.

The HFUS imaging obtained in this study were also at different time points of post conceptional age for all patients. This has led the analysis to be done by collating data. These groups have needed to be over weeks for example 20-28 days and >8 weeks. Accuracy of assessment of time when changes in skin structure occur will increase if scans are conducted frequently. We have demonstrated in this study that HFUS can be conducted serially in very premature and sick newborn babies without any adverse effects. We have also demonstrated that this mode of investigation was both acceptable and welcomed by parents as well as staff.

Further, this study was originally designed to characterise inflammatory biomarkers released from the skin that were exposed to potentially harmful loads e.g., medical devices such as CPAP. Preliminary data was derived, however the methodology for sampling the biomarkers (swabbing the device surface) was inadequate to retain proteins above the limit of detection

for commercial ELISA assay analysis. Therefore, this protocol was removed from the study design, with future research needed to establish a more robust methodology for biomarker collection.

7.4 Summary of results

To our knowledge, this is the largest evaluation of preterm neonatal skin, incorporating a longitudinal approach to measurement of structure and function of skin. The study also provides a comparative term cohort from which to differentiate the functional maturity of skin in the preterm infants. This study is novel in terms of presenting the chronological analysis of functional changes in skin and comparing it with the non-invasive evaluation of structure of the skin using a point of care device. This study adds targeted knowledge to fill the gaps identified in knowledge of skin characteristics in preterm infants.

The current study shows that preterm neonates have immature structure and function of skin, and it improves chronologically but all parameters fail to achieve the complete maturation simultaneously. While TEWL has achieved functional maturity in the most extremely premature babies (24-25 weeks) by 40-60 days (section 7.1.1), pH decreases over 3-4 weeks to develop the acid mantle, dermis is not delineated till 3 weeks of age in <28-week babies (Table 6-2 and Table 6-5) and dermal thickness is significantly smaller than a term infant at >8 week of age (Figure 6-9). This is the explanation for increased the risk of pressure injury by 15 times on admission to NICU as compared to another intermediate care unit as reported recently (García-Molina et al., 2018, Visscher and Taylor, 2014). These studies also reported that birth weight and gestational age are inversely related to pressure injury in these babies. The current study helps to improve the understanding of chronological evolution of structure and function of skin in preterm infants and will help guide the implementation of healthcare interventions needed to prevent and decrease pressure injuries in these babies (Setchell et al., 2023, Fassino et al., 2023).(Setchell et al., 2023, Fassino et al., 2023).

Fragile preterm infants are born without the opportunity to complete the in utero structural and functional maturation of various organs. They miss out on the cumulative third trimester activities when nutrients and minerals are stored in preparation for the perinatal period. The defence mechanisms available to a term infant are also not available. The harsh stressful cold environment must be endured without the help of pulmonary surfactants, subcutaneous fat, poor muscle mass, inability to feed and many more. They do not have the benefit of vernix caseosa and must live in an alien environment inside the incubator where they are not able to hear, smell or feel their mothers. Over the decades we have accumulated a great deal of

knowledge that helps us to look after these babies, however further innovation and creation of knowledge is needed. The present study is an attempt towards this endeavour.

Appendices

7.5 Appendix 1: Consent form

CONSENT FORM (*Version 1*)

Study title: Chronological Evaluation of Functional changes in Neonatal Skin (CFNS)
Researchers: Professor Dan Bader, Professor Howard Clark, Dr Peter Worsley, Dr. Anushma Sharma
Ethics number: IRAS 207028

Please initial the box (es) if you agree with the statement(s):

1 **.** I have read and understood the information sheet (18/02/2017, Version 2) and have had the opportunity to ask questions about the study. ☐

2 **.** I agree that my child may take part in this project and agree for his or her data to be used for the purpose of this study ☐

3 **.** I understand that my child's participation is voluntary, and I may withdraw at any time without my legal rights or the care of my child being affected ☐

4 **.*** I understand that data from this study may be used in: academic papers, academic presentations, staff education and assessment tools ☐

5 **.** I have had the opportunity to ask questions and have received satisfactory answers. ☐

Data Protection

I understand that information collected about my child during his or her participation in this study will be stored on a password protected computer and that this information will only be used for the purpose of this study. All files will be made anonymous before analysis.

Name of parent(s) (print name)……

Name of child (print name)……

Signature of parent(s)……

Name of researcher……

Signature of researcher……

PTO

Postal address of parents (to receive a summary of the results):

……
…

……
…

Date……

7.6 Appendix 2: Patient information leaflets

7.6.1 Participant Information sheet – Term Infants

Participant information sheet – Term infants

Study Title: Chronological evaluation of functional changes in neonatal skin (CFNS)

Researchers: Professor Dan Bader, Professor Howard Clark, Dr Peter Worsley, Dr Anushma Sharma.

Ethics number: IRAS 207028

Ethics Committee: South Central – Oxford A

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

What is the research about?

When infants are born their skin undergoes changes to meet the new challenges of the new environment. Infants born prematurely have a number of vital organs that are underdeveloped. For example, skin, in preterm infants is very vulnerable. This is due to the immaturity of the skin layers and the sudden changes in environment it is exposed to during birth.

Researchers at the University of Southampton and Princess Anne Hospital are working together to assess how the skin matures in preterm infants and how it differs to changes in term infants. The aim of this study is to collect information regarding the development of your infant's skin while he/she is in the hospital. This information will provide vital details regarding the safe practice of skin assessment and care.

Why has my infant been chosen?

Most infants who are admitted to this neonatal unit during the study period are eligible for participation. In total, we are hoping to include approximately 20 infants born at term.

What will happen to my infant if he or she takes part?

There will be no changes to your infant's care if he or she takes part. This study will only take measurements at a convenient time without disturbing routine clinical practice of the nurses and medical team. We wish to take three different measurements of skin function at the right thigh (left thigh if right is not suitable). These measurements will include:

1. the pH (acidity) at the skin surface
2. the amount of water which is lost through the skin
3. the thickness of the skin

Measurement Tools

The pH and water loss measurements will be taken using two wireless probes which will be placed gently in contact with your infant's skin for 30 second duration (Figure 1). Each probe will be cleaned in-between assessments in accordance with the strict infection control policies on the neonatal unit. The thickness of your infant's skin will be measured using an ultrasound machine. This is the similar machine that is used for cranial ultrasounds during routine assessments by the medical team. We will also collect standard information regarding your infant's gestational age, weight at birth, sex and ethnicity. We will also document the incubator settings, nutritional and growth parameters

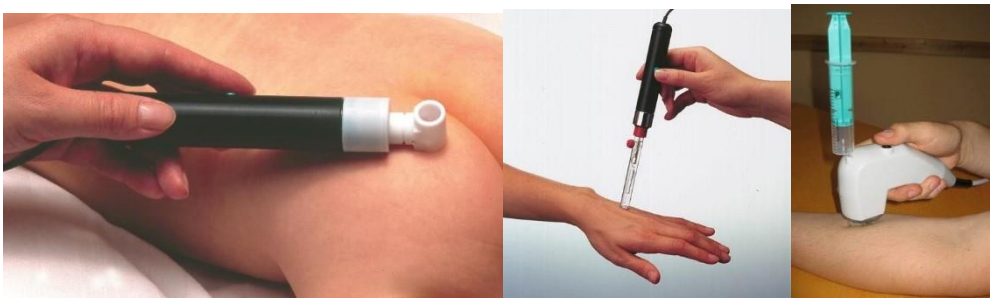


Figure 1. Water loss probe (right), pH probe (middle) and an ultrasound scanner (left).

When will my infant be measured?

If you have chosen to take part in the study we will agree a convenient time with you, the medical team and the nursing team to begin data collection. We will take up to two measurements on your infant, starting from within 48 hours of birth and finishing at 6 days of age or discharge, whichever comes earlier. By repeating the measures this will give us critical information regarding the development of your infant's skin. **Are there any benefits in taking part?**

There will be no direct benefit to your infant from this study. The aim of this study is helping neonatal nurses and other healthcare staff to make more informed decisions about skin care of newborn infants in the future. The results of the study may be used to develop new ways of protecting skin in this vulnerable neonatal population.

Are there any risks involved?

There will be no change to your infant's care if he or she participates in the study. For infants who are very sick, there is always a risk associated with being handled and assessed. However, if your infant needs to be turned or moved for assessment, this will be done by the nurses looking after the infant. If the nurse or doctor looking after your infant thinks that he or she should not be moved for assessment, we will not collect data from him/her at that time.

All of the measurement devices have been used in similar studies involving newborn infants with no adverse effects. We shall use standard infection prevention measures as per the unit guidelines for personnel and equipment.

Will my participation be confidential?

This research will be conducted in accordance with the Data Protection Act and the data storage policy of the University of Southampton. Data will be coded and kept on a password-protected computer. If any hard copies are made of the data these will be stored in a locked cabinet in a research office, which is staffed during the day and locked out of hours.
If you agree to participate in the study, we will ask for your postal address so that we can send you a summary of the results once data collection has finished. We will store this with your consent form, and it will not be linked with your child's information.
Your infant's data will be anonymised immediately after collection. The data will only be seen by the research team while the study is ongoing and will be kept confidential throughout. When data is presented from the study your infant will not be identifiable. When the study is completed, in accordance with the data protection policy of the University of Southampton and the Data Protection Act 1998, all the data will be kept in locked filing cabinets or password protected computers for a period of 10 years.

What happens if I change my mind?

You can change your mind at any stage throughout the study. This will not affect your legal rights or the care of your infant. If you no longer want your infant to take part, please tell either the nurse looking after your infant or the researcher. You do not need to give a reason if you decide to withdraw your infant from the study.
After all the data have been collected, your infant's data will be anonymised, so we will not be able to remove his or her information from the database after this point.

What happens if something goes wrong?

The study is insured by the University of Southampton and supported by the Research Governance Office. If you have a concern or a complaint about this study you should
contact The Research Governance Office Building 37, Highfield,
Southampton.
University of Southampton, SO17 1BJ
Tel: +44 (0)23 8059 5058; Email: rgoinfo@soton.ac.uk).

If you remain unhappy and wish to complain formally the Research Governance Office can provide you with details of the University of Southampton Complaints Procedure.

You can also contact the Patient Advice and Liaison Service.

PALS Tel: 023 8120 8498

For general information about research, you can contact INVOLVE:

INVOLVE

Wessex House

Upper Market Street

Eastleigh
Hampshire

SO50 9FD

Telephone: 023 8065 1088

Fax: 023 8065 2885

Email: admin@invo.org.uk

Website: http://www.invo.org.uk

Where can I get more information?

If you have more questions after we have discussed this information, or at any point during the study, you can talk to us when we am on the unit, or contact us by email/telephone:

Dr Peter Worsley

p.r.worsley@soton.ac.uk

02381 208287

Dr Anushma Sharma

anushma.sharma@soton.ac.uk

02381 208899

Thank you for taking the time to read this information sheet.

7.6.2 Participant information sheet - Preterm infants

Participant Information Sheet-Preterm Infants

Study Title: Chronological evaluation of functional changes in neonatal skin. (CFNS)

Researchers: Professor Dan Bader, Professor Howard Clark, Dr Peter Worsley, Dr Anushma Sharma.

Ethics number: IRAS 207028

Ethics Committee: South Central – Oxford A

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

What is the research about?

When infants are born their skin undergoes changes to meet the new challenges of the new environment. Infants born prematurely have a number of vital organs that are underdeveloped. For example, skin, in preterm infants is very vulnerable. This is due to the immaturity of the skin layers and the sudden changes in environment it is exposed to during birth.

Researchers at the University of Southampton and Princess Anne Hospital are working together to assess how the skin matures in preterm infants and how it differs to changes in term infants. The aim of this study is to collect information regarding the development of your infant's skin while he/she is in the hospital. This information will provide vital details regarding the safe practice of skin assessment and care.

Why has my infant been chosen?

Most infants who are admitted to this neonatal unit during the study period are eligible for participation. In total, we are hoping to include approximately 20 infants born premature.

What will happen to my infant if he or she takes part?

There will be no changes to your infant's care if he or she takes part. This study will only take measurements at a convenient time without disturbing routine clinical practice of the nurses and medical team. We wish to take three different measurements of skin function at the right thigh (left thigh if right is not suitable). These measurements will include:

1. the pH (acidity) at the skin surface
2. the amount of water which is lost through the skin
3. the thickness of the skin

Mucous samples will be taken with a swab from the endotracheal tubes (the tube placed in the mouth and connected to the ventilator), nasogastric tubes (feeding tube) or CPAP or Hi-Flow devices (breathing support devices) before they are discarded. This sample will be used for measurement of markers which may be present when the skin is inflamed.

Measurement Tools

The pH and water loss measurements will be taken using two wireless probes which will be placed gently in contact with your infant's skin for 30 second duration (Figure 1). Each probe will be cleaned in-between assessments in accordance with the strict infection control policies on the neonatal unit. The thickness of your infant's skin will be measured using an ultrasound machine. This is the similar machine that is used for cranial ultrasounds during routine assessments by the medical team. We will also collect standard information regarding your infant's gestational age, weight at birth, sex and ethnicity. We will document the incubator settings, nutritional and growth parameters.

When will my infant be measured?

If you have chosen to take part in the study we will agree a convenient time with you, the medical team and the nursing team to begin data collection. We will take up to seven measurements on your infant starting from within 48 hours of birth and finishing at 50-56 days of age or discharge, whichever comes earlier. By repeating the measures this will give us critical information regarding the development of your infant's skin.

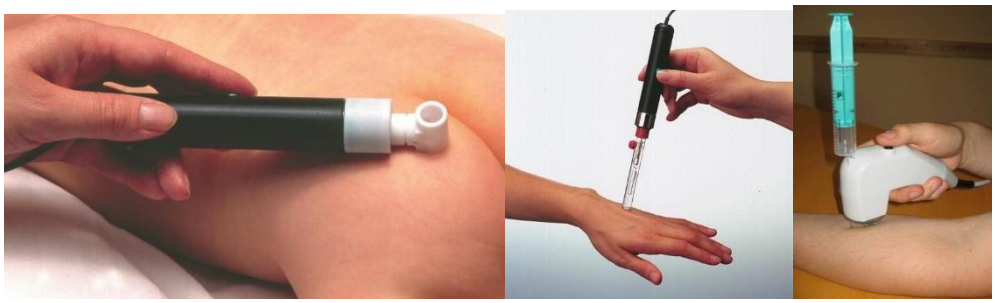


Figure 1. Water loss probe (right), pH probe (middle) and an ultrasound scanner (left).

Are there any benefits in taking part?

There will be no direct benefit to your infant from this study. The aim of this study is helping neonatal nurses and other healthcare staff to make more informed decisions about skin care of newborn infants in the future. The results of the study may be used to develop new ways of protecting skin in this vulnerable population.

Are there any risks involved?

There will be no change to your infant's care if he or she participates in the study. For infants who are very sick, there is always a risk associated with being handled and assessed. However, if your infant

needs to be turned or moved for assessment, this will be done by the nurses looking after the infant. If the nurse or doctor looking after your infant thinks that he or she should not be moved for assessment, we will not collect data from him/her at that time.
All of the measurement devices have been used in similar previous studies involving preterm babies with no adverse effects. We shall use standard infection prevention measures as per the unit guidelines for personnel and equipment.

Will my participation be confidential?

This research will be conducted in accordance with the Data Protection Act and the data storage policy of the University of Southampton. Data will be coded and kept on a password-protected computer. If any hard copies are made of the data these will be stored in a locked cabinet in a research office, which is staffed during the day and locked out of hours.
If you agree to participate in the study, we will ask for your postal address so that we can send you a summary of the results once they are ready. We will store this with your consent form, and it will not be linked with your infant's information.
Your infant's data will be anonymised immediately after collection. The data will only be seen by the research team while the study is ongoing and will be kept confidential throughout. When data is presented from the study your infant will not be identifiable. When the study is completed, in accordance with data protection policy of the University of Southampton and the Data Protection Act 1998, all the data will be kept in locked filing cabinets or password protected computers for a period of 10 years.

What happens if I change my mind?

You can change your mind at any stage throughout the study. This will not affect your legal rights or the care of your infant. If you no longer want your infant to take part, please tell either the nurse looking after your infant or the researcher. You do not need to give a reason if you decide to withdraw your infant from the study. After all the data have been collected, your infant's data will be anonymised, so we will not be able to remove his or her information from the database after this point.

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Southampton, SO17 1BJ.
Tel: +44 (0)23 8059 5058; Email: rgoinfo@soton.ac.uk

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For general information about research, you can contact INVOLVE:

INVOLVE
Wessex House
Upper Market Street
Eastleigh
Hampshire
SO50 9FD
Telephone: 023 8065 1088
Fax: 023 8065 2885
Email: admin@invo.org.uk

Website: http://www.invo.org.uk

Where can I get more information?

If you have more questions after we have discussed this information, or at any point during the study, you can talk to us when we are on the unit, or contact us by email/telephone:

Dr Peter Worsley
p.r.worsley@soton.ac.uk

02381 208287

Dr Anushma Sharma
anushma.sharma@soton.ac.uk
02381 208899

Thank you for taking the time to read this information sheet.

7.7 Appendix 3: Case report form

Case Report Form

Chronological Evaluation of the Functional Changes in Neonatal Skin (CFNS)

Consent: **By:** **Date:** **Time:**

Copy of consent and PIS in notes

Copy of consent and PIS in given to the parents

Original consent in Site file

Exemption forms

Demographic details:

DOB	dd/mm/yy	**Antenatal steroids** (Please circle)	**Yes/No**
TOB	hh/mm	**Maternal chorioamnionitis** (Please circle)	**Fever >38°C** **Foul smelling liquor**
GA	**Weeks days**	**Mode of delivery** (Please circle)	**Vaginal** **Forceps** **Ventouse** **Caesarean**
Weight	**Kg**	**Plastic bag at birth** (Please circle)	**Yes/No**
Vitamin K (Please circle)	**Right Left**	**Admission temp**	**°C**

EGDE , SENNAT

SAMPLE 1 **Date and time** **Age** **Time taken to complete measurements**

Measurement	Incubator temp	Incubator humidity	Baby temp	Room temp
15 min before				
During				
15 minutes after				

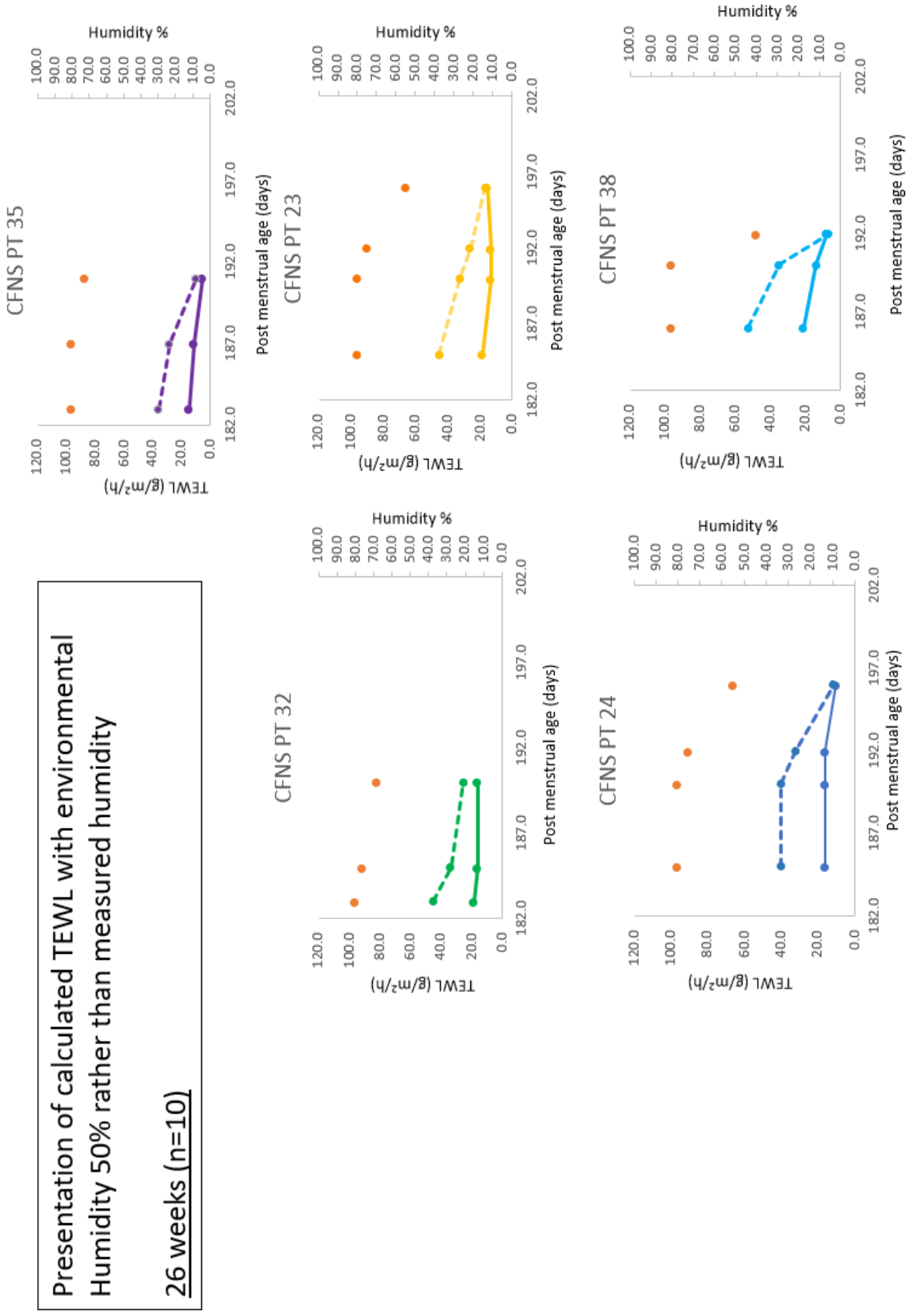
Bloods

DATE			
Na			
K			
Urea			
Creatinine			
CRP			
Zn			
Se			
Mn			
Bo			
C			

SITE	pH	TEWL	Thickness
Fe			
mg			
ANTIBIOTICS			
Steroids			
Vitamin			

Further data was collected on similar continuation sheets.

7.8 Appendix 4: Calculated TEWL with 50% environmental humidity rather than measured environmental humidity



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