The neonate epidermal barrier: development of superficial chymotrypsin-like protease activity and natural moisturising factors

John Chittock[1], Alison Cooke[3], Tina Lavender[3], Kirsty Brown[1], Andrew Wigley[1], Suresh Victor[4,5], Michael J. Cork[1,2] and Simon G. Danby[1]

[1]The Academic Unit of Dermatology Research, Department of Infection and Immunity, The University of Sheffield Medical School, Sheffield, UK; [2]The Paediatric Dermatology Clinic, Sheffield Children's Hospital, UK; [3]School of Nursing, Midwifery and Social work, The University of Manchester, UK; [4]Institute of Human Development, The University of Manchester, UK; [5]Sidra Neonatology Center of Excellence, Sidra Medical and Research Center, Doha, Qatar *Corresponding Author: j.chittock@sheffield.ac.uk

INTRODUCTION

- The infant stratum corneum (SC) is thinner, and immature in both structure and function compared to adults throughout the first year of life.[1]
- From birth, the SC enters a maturation phase of rapid hydration and acid mantle formation[2] throughout the neonatal period, suggesting underdeveloped or transitional mechanisms underlying neonate epidermal barrier differentiation and desquamation.
- This potentially vulnerable period of maturation as the SC adapts to terrestrial life, coincides with the onset (<1 year of age) of skin manifestations such as atopic dermatitis.[3]

AIMS

1. To assess the biophysical and biological properties of the epidermal barrier from birth to 4 weeks of age
2. Monitor the development of chymotrypsin-like protease activity and the level of natural moisturising factors and investigate early signals of barrier breakdown
3. To place our neonate findings into context by comparing the results to a healthy adult cohort

METHODS

Subjects

- **Neonate cohort:** A total of 115 healthy, full-term neonates (≥37^{+0} weeks gestation) were recruited for the oil in baby skincare (OBSeRvE) randomised clinical trial.[4] Assessments on the forearm and thigh were performed at birth (<72 hours old) and repeated at 4 weeks of age (*n* = 39, no oil untreated group only)
- **Healthy adult cohort:** For comparison an unrelated cohort of adults with no history of skin disease or atopy was recruited and underwent identical assessments during a single visit

Biophysical measurements

- Epidermal barrier function was determined by measuring transepidermal water loss (TEWL) using an AquaFlux evaporimeter (Biox, UK) in climate controlled conditions
- Skin surface pH and SC hydration were determined using a Skin-pH-meter and Corneometer (C&K, Germany)
- Tape stripping (3 D-squame discs in total) was combined with IR densitometry to measure the mass of SC removed

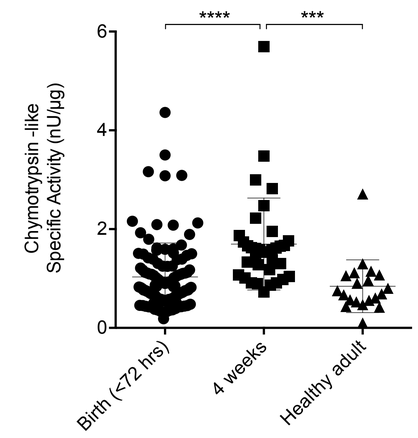
Biological assessments

- Specific chymotrypsin-like protease activity using peptide substrate MeOSuc-Arg-Pro-Tyr-AMC was assayed on D-Squame discs collected from the forearm using a previously published method adapted for *in situ* analysis[5]
- The level of filaggrin-derived natural moisturising factors (NMF) was quantified *in situ* from D-Squame discs collected from the thigh by HPLC[6] (2-pyrrolidone-5-carboxylic acid, urocanic acid) and o-Phthaldialdehyde derivatisation[7] (free amino acids)

RESULTS

Biophysical and biological properties of the neonate epidermal barrier are transitional from birth to 4 weeks of age

	Birth	Mean difference (95% CI)	Infant (4 wks)	Mean difference (95% CI)	Healthy adult
Subjects (*n*)	115	-	35∞	-	20
Age	28.11 (±11.32) Hours	-	30.7 (±2.35) Days	-	24.65 (±6.67) Years
Sex (% male)	57	-	64	-	25
Family history of AD	37/115	-	13/39	-	0/20
TEWL (g/m²/h)	12.14 (±2.31)	1.23 * (0.05, 2.42)	13.38 (±3.02)	0.73 ns (-0.97, 2.44)	12.64 (±3.09)
SC Hydration (RCU)	17.66 (±4.55)§	24.13 **** (21.30, 26.97)	41.79 (±9.65)	10.32 **** (6.21, 14.43)	31.47 (±6.90)
Skin-surface pH	5.93 (±0.51)§	0.94 **** (0.73, 1.16)	4.98 (±0.34)	0.20 ns (-0.11, 0.52)	4.78 (±0.42)
SC cohesion (µg / 3 discs)	292.43 (±77.17)	12.22 ns (-23.47, 47.90)	304.65 (±88.65)	29.23 ns (-22.58, 81.04)	275.42 (±62.45)



Results panel 1: The biophysical and biological properties of the developing neonate epidermal barrier. Neonates were assessed at birth (<72 hours old), repeated at 4 weeks of age and the results compared to an unrelated healthy adult cohort. From birth TEWL (forearm), chymotrypsin-like protease activity (forearm) and the level of NMF (thigh) significantly elevated to beyond adult levels. Significance was determined using a 1-way analysis of variance with Bonferroni's post-hoc analysis. Mean ± standard deviation presented.

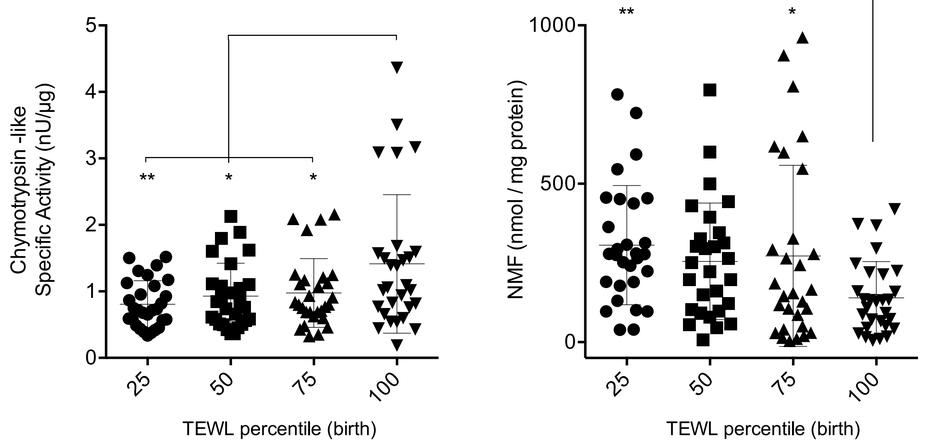
At birth protease activity and NMF levels significantly correlate with TEWL, SC hydration and skin surface pH

		TEWL (g/m²/h)	SC Hydration (RCU)	Skin-surface pH
Chymotrypsin-like protease activity	**Birth**	0.26 ** (0.08, 0.43)	-0.30 ** (-0.46, -0.12)	0.25 ** (0.07, 0.42)
	Infant 4wks	0.12 ns (-0.22, 0.44)	-0.39 * (-0.64, -0.06)	0.10 ns (-0.26, 0.40)
Natural moisturising factor (NMF)	**Birth**	-0.38 **** (-0.53, -0.21)	0.50 **** (0.35, 0.63)	-0.54 **** (-0.66, -0.40)
	Infant 4wks	-0.23 ns (-0.53, 0.11)	-0.10 ns (-0.42, 0.24)	-0.13 ns (-0.44, 0.21)

Results panel 2: The relationship in neonates between protease and NMF at birth (< 72 hours old) and at 4 weeks of age, with TEWL, SC hydration and skin-surface pH. Pearson correlation coefficient calculated (r)

Significantly elevated protease activity and low NMF co-exists in neonates predisposed to atopic dermatitis

Results panel 3: Neonates with the highest TEWL at birth (>75th percentile), also on average displayed significantly elevated chymotrypsin-like protease activity and low NMF compared to the remaining cohort. TEWL was utilised as risk factor for the development of AD by 1 year of age.[8] Significance was determined using a 1-way analysis of variance with Bonferroni's post-hoc analysis. Mean ± standard deviation presented.



CONCLUSIONS

- The biophysical, biological and functional properties of the developing neonate SC are transitional from birth to 4 weeks of age and differ to adults
- The observed elevation in chymotrypsin-like proteases (such as kallikrein-7) from birth to 4 weeks of age occurs seemingly independently from the concomitant change in skin surface pH as part of the acid mantle development
- Weakened epidermal barrier function occurring at some point after leaving the maternity ward, may reflect negative environment stressors culminating in elevated protease activity as a consequence of the neonate barrier adapting to the 'home' environment
- Heightened protease activity and low NMF at birth provides novel mechanistic insight into why certain individuals are prone to barrier breakdown and the development of AD
- Our findings highlight the need for standardised, infant skin care regimens from birth that maintain normal barrier development, and support the strategy of emollient barrier enhancement from birth in high risk individuals[9]

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