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## Developmental alterations of physical properties and components of neonatal-infantile stratum corneum of upper thighs and diaper-covered buttocks during the 1st year of life



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#### ABSTRACT

Background: Although physical properties of neonatal-infantile stratum corneum (SC) change drastically after birth, precise developmental alterations of specific sites have not been fully elucidated. Objective: To determine the longitudinal alterations of neonatal-infantile SC functions and components of upper thighs and diaper-covered buttocks during the first year of life. The data were compared with those of adults.

Methods: Nineteen full-term neonates and their mothers were subjected to the measurements. Skin hydration, water sorption/retention capacity, TEWL were measured. Superficial SC analyses for NMF, ester binding sebum, and free fatty acids were performed by ATR-FTIR spectrometer. Total amount of ceramides (CERs) and CER subclasses were analyzed by NPLC-ESI-MS.

Results: SC hydration of neonatal thighs was lower than that of their mothers, which rapidly increased during the 1st month. Skin hydration of neonatal buttocks was similar to that of their mothers. This also rapidly increased during the 1st month. The neonatal TEWL was less than those of their mothers indicating more efficient barrier function at both sites, which significantly increased during the 1st year development. This was mostly correlated decreased in the  $\omega$ -hydroxy fatty acid-esterified CERs. Superficial ester-binding sebum content of neonates was similar to that of their mothers, which significantly decreased during the measurement; the decrease was more marked on buttocks. Neither NMF nor FFA of the superficial SC showed significant alteration during the 1-year development.

Conclusion: Our results indicate that physical functions and components of neonatal-infantile SC show considerable alterations between diaper-covered buttocks and upper thighs during the 1st year development.

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#### 1. Introduction

The physical properties of neonatal-infantile skin drastically change after birth. Previous reports indicate that full-term neonates already show functionally mature SC at birth [1], and

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immature SC of preterm neonates become mature during the 1st month after birth [2,3]. Nevertheless, few reports exist, which show location-specific alterations during development specifically dermatitis-prone diaper-covered areas [4,5] We analyzed physical properties and various components of SC of 19 full-term neonates longitudinally during the 1st year of life and compared with those of their mothers. The analyzed areas were upper thighs and diaper-covered buttocks. Alterations of physical properties and various components including total CERs and CER subtypes were also analyzed. To the best of our knowledge, this is the first report clarifying the physical properties and components of SC longitudinally at these specific locations using the same infants during the 1st year of development. Detailed analyses of infantile SC function of arms [3-12 months] have been described by Nikolovski et al. [6].

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Abbreviations: SC, stratum corneum; TEWL, transepidermal water loss; NMF, natural moisturizing factor; FFA, free fatty acids; ATR-FTIR, attentuated total reflectance Fourier Transform Infrared spectrometer; CERs, ceramides; NPLC-ESI-MS, normal-phase liquid chromatography-electrospray ionization-mass spectrometry; SDT, sorption-desorption test.

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#### 2. Materials and methods

#### 2.1. Subjects and study design

Signed informed consents were obtained from 19 mothers of babies born at Asahikawa Medical University Hospital. The measurements were performed daily between the 3rd and 7th day, and then at 1, 3, 6, 9, 12 month, respectively. All children and their mothers were subjected to the measurements described below consecutively for 6 times.

Biophysical measurements were performed in a climatecontrolled room and the babies were acclimated at temperature of 25 °C and relative air humidity of 40% for 20 min [7].

The measurements of neonates-infants were performed on two locations: diaper-covered buttocks and inner side of upper thighs; the measurements of mothers were performed on the same sites. During the analyses, subjects were avoided to use any skin care products on these locations for at least 24 h before the measurement.

The data of their mothers considerably fluctuated during the first 2 months after delivery. Therefore the data of thighs and buttocks 3 months post-delivery and thereafter were used for comparison. These were essentially similar to those of 5 agematched female control values and no significant difference was observed between thighs and buttocks (data not shown).

#### 2.2. Instrumentation

Skin hydration (capacitance) and water sorption/desorption analyses were measured by by Corneometer<sup>®</sup> (CM825/Courage + Khazaka electronic GmbH, Germany). TEWL was measured, Tewameter<sup>®</sup> (TM300/Courage + Khazaka electronic GmbH, Germany). Analyses of NMF, ester binding sebum, and FFAs were performed by Attentuated total reflectance Fourier Transform Infrared (ATR-FTIR) spectrometer (Nicolet380, Thermo Scientific, K.K, Japan) equipped with a fiber-optic probe coupler (FiberMate II, Systems Engineering Inc., Japan) and AgCl-AgBr polycrystalline infrared fiber-optic probe (PIR fiber probe, Systems Engineering Inc., Japan). These non-invasive measurements by the ATR-FTIR device, albeit superficial, showed more constant values than the previous measurements especially of NMF [8]. Normal-phase liquid chromatography-electrospray ionization-mass spectrometry (NPLC-ESI-MS) was used for the quantification of CER species [9], which can analyze as small as 2.5 cm  $\times$  6.0 cm tape-stripped SC. Tape-stripping was performed 3 times on the same sites and were analyzed for CERs, which was performed by Agilent 1100 Series LC/MSD single-quadrupole system equipped with an electrospray ionization source, ChemStation software, a 1100well-plate autosampler (Agilent Technologies, Palo Alto, CA), and an Inertsil SIL 100A-3, 1.5 mm i.d. × 150 mm column (GL Science, Tokyo, Japan) as was described previously [9,10].

#### 2.3. Water sorption and retention capacity

A distilled water-containing 16 mm-sized round sheet was applied to the upper thighs and buttocks for 1 min. Following the removal of the sheet, water content was measured every 10 s until 3 min [11]. The water sorption value indicates the means of the first 6 measurements (up to 1 min following the removal). Water retention capacity indicates the means of the residual water content of the last 6 measurements (from 2 to 3 min following the removal of the sheet).

#### 2.4. Characterization of overall CER species in SC

Human SC CERs can be divided into 12 groups according to their fatty acid and sphingoid structures. The following CERs were

analyzed by NPLC-ESI-MS. CER[NDS] stands for non-OH fatty acids [N] and dihydrosphingosines [DS]. CER[NS] stands for [N] and sphingosines [S]; CER[NH] for [N] and 6-hydroxy sphingosines [H]; CER[NP] for [N] and phytosphingosines [P], CER[ADS] stands for  $\alpha$ -OH fatty acids [A] and [DS]; CER[AS] for [A] and [S]; CER [AH] for [A] and [H]; CER [AP] for [A] and [P]; CER [EOS] for ester-linked  $\omega$ -OH fatty acids [EO] and [S]. CER [EOH] stands for [EO] and [H]; CER[EOP] for [EO] and [P]. CER[EODS] stands for [EO] and [DS], which was recently detected in human [12]. CER[EODS] was not measured in this study.

#### 3. Results

#### 3.1. Skin hydration and water holding capacity of upper inner thighs

The water content of upper inner thighs of full-term neonates was lower than that of their mothers, which rapidly increased during the 1st month after birth, and remained relatively constant thereafter (Fig. 1A). Water holding capacity of neonatal thighs determined by Sorption-Desorption Test (SDT) was higher than that of their mothers, which rapidly decreased during the 1st month and remained relatively constant thereafter (Fig. 1B). The residual water content following the SDT also decreased during the 1st month and remained relatively constant thereafter. The water content and the SDT values of 5 age-matched adult female inner thighs were similar to the values of those of the mothers (data not shown).

## 3.2. Skin hydration and water holding capacity of diaper-covered buttocks

The water content of neonatal diaper-covered buttocks (Fig. 1C) was slightly higher than that of upper inner thighs (Fig. 1D), which also rapidly increased during the 1st month after birth. The skin hydration of neonatal thighs was lower than that of their mothers (Fig. 1A), while that of neonatal buttocks was similar to that of their mothers (Fig. 1C). Skin hydration of both sites increased and remained higher than that of their mothers. The water content of 5 age-matched adult female buttocks was similar to the values of those of the mothers (data not shown). No significant difference in the water content was detected between the adult thighs and buttocks (Fig. 1A and C). The water holding capacity of neonatal buttocks slightly decreased during the development and was significantly lower than that of their mothers at 1 year (Fig. 1D). There was no significant difference in the water holding capacity between infantile thighs and buttocks at 1 year. While the water holding capacity of infantile thighs was close to that of their mothers after a month and thereafter (Fig. 1B), that of infantile buttocks was slightly but significantly lower than that of their mothers after 3 months and thereafter (Fig. 1D). The residual water content following the SDT slightly decreased during the 1st month and remained constant thereafter, which was also significantly lower than that of their mothers (Fig. 1D).

#### 3.3. Transepidermal water loss (TEWL)

Neonatal TEWL of both upper thighs and buttocks was significantly lower than that of their mothers (Fig. 2A and B), suggesting more efficient barrier function of full-term neonates than that of adults. The infantile TEWL of both upper thighs and buttocks rapidly increased during the 1st month, and were close to those of their mothers at 1 year. The TEWL of 5 age-matched adult female buttocks was similar to that of the mothers (data not shown).

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