Infant epidermal skin-physiology:
Adaptation after birth

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Running Title: Skin physiology adaptation in infants

Key Words: epidermal barrier, hydration, transepidermal water loss, stratum corneum acidification, pH, Raman, natural moisturizing factor, ceramide, lactic acid, cohesion, newborn, infant
• Differences in a variety of skin functions are established between infants and adults
• Even in term neonates, the functional skin adaptation to the extrauterine environment is ongoing after birth
• Our results reveal the dynamic changes in the amounts of the natural moisturizing factor constituents and water profile in the period of infancy
• The age group segmentation, especially for the neonates and young children aged less than 3 months, offers direct comparison of skin physiology parameters and microcomposition of skin components

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Abstract
Functional and structural skin adaptation is a dynamic process which starts immediately after birth in humans and mammalian skin in general. This adjustment to the extrauterine dry environment is accomplished in the first year of the postnatal life of humans. The aim of this study was to assess the dynamic changes in vivo after birth in the molecular composition and skin physiology parameters compared to older children and adults. The molecular composition of the stratum corneum (SC) and the water profile were investigated non-invasively by in vivo Raman confocal microscopy as a function of depth. Functional parameters including transepidermal water loss (characterizing epidermal permeability barrier), capacitance (as an indirect parameter for SC hydration) and skin surface pH were assessed non-invasively. The measurements were performed in 108 subjects divided into six age groups: full-term newborns (1 to 15 days), five-week-old babies (+ 1 week), six-month-old (± 1 month), 1 to 2 years old children, 4 to 5 years old children and adults aged 20 to 35 years.
We could show that skin acidification is still under development during the first weeks of life. While the basal epidermal barrier is competent immediately after birth, stratum corneum is less hydrated in the first two weeks of the postnatal life. Similar continuous decreasing water content towards the surface for all age groups was observed, whereas this gradient was lower for the newborns. Dynamic changes in the amounts of the natural moisturizing factor constituents were revealed in the period of infancy.

We could demonstrate the relation of formation of an acidic pH as well as underlying mechanisms in the induction of a fully hydrated SC over the first weeks of human life as a dynamic functional adaptation.

**Abbreviations:** arbitrary units (a.u.); area under the curve (AUC); stratum corneum (SC); transepidermal water loss (TEWL); β-glucocerebrosidase (β-GlucCer’ase), acid sphingomyelinase (aSM’ase); in vivo Raman confocal microscopy (RCM)

**INTRODUCTION**

The skin of term neonates provides a competent inside-out and outside-in barrier already after birth. However, the adaptation post partum to the dry extra uterine environment is still ongoing. A difference in the structure of infant stratum corneum (SC) in relation to the dermal papillae density and distribution compared to those of adults has been revealed. A functional consequence of these discrepancies is the not fully competent water-handling properties despite the intact SC of newborns.

In vivo Raman confocal microscopy (RCM), enables a detailed analysis of the molecular composition of the skin. The concentration profiles of epidermal ceramides, sweat constituents (lactate and urea), natural moisturizing factor and its specific components, water profile as well as exogenous substance influence on the skin can be assessed non-invasively. Infants aged 3 to 33 months showed a steeper water gradient in the SC and a higher water content within the first 20 μm compared to adults. Lower amounts of Natural Moisturizing Factor (NMF) were evidenced in infants in comparison to adults. In addition, water absorption and desorption properties were disturbed in infants. These discrepancies could partly explain the vulnerability of infant skin to the development of desiccation and dermatitis. On the other hand, it has been
demonstrated that newborn skin is dryer than the one of adults \(^1,12-15\). Later in life (2 weeks to 3 months) the hydration increases and reaches the levels or even exceeds that in adults \(^16,17\). Therefore, the critical interval for skin hydration adjustment comprises the first weeks of postnatal life.

Another crucial element in the skin adaptation is the maturation of the acid mantle of the epidermis of newborns. It is widely recognized that the surface pH of human adult skin is acidic with a pH between 5 and 5.5 \(^18,19\). Yet, the skin surface of term newborns is not fully acidified and shows elevated pH values compared to adults \(^16,19\), which can predispose to the development of inflammatory dermatoses in infancy such as the diaper dermatitis \(^18,19\). Different mechanisms are responsible for the formation of an acidic skin surface pH. These include endogenous pathways - free fatty acid generation from phospholipid hydrolysis \(^20-22\), the sodium/proton pump antiporter, sodium/hydrogen antiporter-1 \(^12,20,23\) and the generation of acidic metabolites of filaggrin metabolism, such as cis-urocanic acid from histidine \(^24,25\). However, the last pathway is currently under discussion \(^26\). In addition exogenous mechanisms, such as the eccrine gland-derived lactic acid \(^27,28\) and the free fatty acids of pilosebaceous origin \(^29,30\), may also contribute to the skin surface acidification. None of these potential mechanisms has been previously linked to the pH formation in human newborns \(^31,32\).

The aim of this study was to compare the skin functions and biochemical pathways between term neonates, young children of different age groups and adults. The molecular composition of the skin was assessed by RCM providing a detailed analysis of the water profile, NMF and its constituents and sweat components concentration profiles. The postnatal functional adaptation of the newborn skin, in terms of epidermal barrier maturation, skin hydration and pH formation was investigated.

**MATERIAL AND METHODS**

**Study protocol**

A single-center, non-drug study was performed in 108 male or female volunteers (in total) with healthy skin in six age groups with 18 subjects each: 1.) full-term newborns (1 to 15 days), 2.) five-week-old babies (+ 1 week), 3.) six-month-old babies (± 1 month),
4.) 1 to 2 years old children, 5.) 4 to 5 years old children and adults aged 20 to 35 years. Whenever possible, adults were recruited from the parents of group 1 to 5. Healthy infants and adults with no present and/or past history of skin disease were included. Subject with symptoms of a clinically significant illness that might have influenced the outcome of the study in the four weeks before and during the study were excluded. Treatment with cosmetics and topical medications of any kind in the test sites (volar forearm) was not allowed within the last three days prior to the start and during the study. Taking a short shower was allowed, if the test fields were not washed with soap/detergent or with any other skin care product.

The study was performed in Berlin, Germany from March 2009 to September 2009 with a recruitment-pause during the summer months. The selection of subjects was in accordance with the requirements of §§ 40 and 41 of the German Drug Law as well as the recommendations of the Somerset West revision of the Helsinki Declaration (1996) and the ICH-GCP Guideline as applicable for this study. Subjects and/or parents or legal guardians signed a written informed consent before the start of each study. The study was approved by the local authorities.

**Measurements and instruments**

The subjects were randomized and acclimatized both volar forearms for at least 30 minutes in an air-conditioned room at 20 ± 2°C and 50 ± 10% humidity with the test area uncovered by clothing. There were two test fields, one on each volar forearm, in this study. Test field 1 was used for the measurement TEWL, corneometric measurements, Raman spectroscopy and finally skin pH measurements. Test field 2 was used for the sampling of two sequential D-Squames. Test field 1 and 2 were randomly assigned to the right and left volar forearm.

TEWL was measured using an open chamber device, Tewameter (TM 300, Courage & Khazaka, Cologne, Germany). Measurements were performed at each site until a stable value for TEWL was reached. Two measuring values were taken of each
test field. The hydration of the stratum corneum (SC) was assessed by measuring the electrical capacitance of the skin using a Corneometer (CM 825, Courage & Khazaka, Cologne, Germany). The measurement was initiated by contact pressure to the skin; three measuring values were taken of each test field. The pH of the skin surface was measured using a Skin-pH-Meter (pH 905, Courage & Khazaka, Cologne, Germany). Two measuring values were taken of each site. Measurements with the Skin-pH-Meter pH 905 were made using a probe consisting of a flat glass electrode containing all the sensor elements connected to a probe handle containing measurement electronics. Tewameter TM 300, Corneometer CM 825 and Skin-pH-Meter pH 905 were attached to a Multiprobe Adaptor (MPA9 or MPA5, Courage & Khazaka, Cologne, Germany) central unit and a PC.

Raman spectrum measurements were performed by using a Raman Spectrometer (3510 Skin Composition Analyzer, River Diagnostics BV, Rotterdam, The Netherlands). The skin region of interest was placed above an upright placed probe. Visible (671 nm) and near infrared laser light (785 nm) of low intensity was focused on the skin using a microscope objective. The Raman spectrum of the scattered light was analyzed, revealing the local molecular composition. Molecular concentration profiles were measured by varying the position of the laser focus through the depth of the SC. The maximum amount of light energy emitting from the device was max. 30 mW, both at 671 nm and 785 nm. The measurement depth was set to 0 - 30 μm using 5 μm measurement steps. Water content, concentration profiles of NMF and its constituents, and sweat components (urea and lactic acid) were determined by software and in accordance with previously described protocols.

Statistical analysis

The analysis was performed using the Statistics Analysis System (SAS Inc., Cary, NC) Version 9.1.3.

Demographic and background data were summarized by age group using descriptive statistical methods. For each method the average of the repeated measurements was used.
in the following analyses, i.e. average of the two TEWL, three corneometry, two skin surface pH and two sequential D-Squame measurements.

The TEWL, corneometry, skin surface pH and skin thickness (RCM) outcomes were presented by age groups providing descriptive statistics including number of subjects, mean, standard deviation, median, interquartile range and minimum and maximum. For each RCM molecular concentration profile the area under the curve (AUC) of the intensity by depth curve was determined for the depth ranges: 0-5 µm, 5-15 µm, 15-25 µm, 25-35 µm and 0-25 µm. The AUCs were descriptively presented by age groups.

Water content [mass-%] was summarized descriptively by age groups for each depth.

RESULTS
One hundred-eight subjects (18 subjects per age group) were enrolled for this study (full-term newborns (1 to 15 days), five-week-old babies (+ 1 week), six-month-old babies (± 1 month), 1 to 2 years old children, 4 to 5 years old children and adults aged 20 to 35 years). The demographic characteristics of the subjects such as mean age, weight and height are described in the table 1. All subjects were included in the statistical analyses. All subjects completed the study as planned. There were no dropouts. In one subject of the newborns the first visit took place at the hospital (no Raman device available at the hospital), so the measurements were done when the subject was 9 days old. One subject in the newborn group showed abnormally high corneometric values (mean = 86.5 a.u.) since the subject was agitated during this measurement. Therefore, this subject was not included into the statistical analysis of the corneometric data. In one subject of the 1 to 2 years age group the Raman data were not analyzable because the subject moved during the measurement and the skin had no close contact to the measurement window.

Transepidermal water loss (TEWL) as a parameter for the epidermal permeability barrier
The mean TEWL values, attributed to the epidermal permeability barrier (inside-out), in all groups were below 10 g/m²h that is within the normal ranges for healthy skin under basal conditions (figure 1a, table 2a). Only slight differences were observed in mean
TEWL values between the six age groups. The lowest mean TEWL value of 6.4 g/m²h was noted for the 5 weeks + 1 week age group while the highest mean TEWL value of 9.7 g/m²h was registered in the 1 to 2 years age group. The pairwise comparisons between the different age groups (table 2a) showed statistically significant greater mean TEWL values for the 1 to 2 years group when compared to the 5 weeks +1 week, the 6 months ± 1 month, and the 4 to 5 years groups (p = 0.0011, 0.0468 and 0.0105, respectively). But there are no significant differences between these infants’ groups and adults.

The “acid mantle”

The greatest mean skin surface pH-value of 6.0 was noted in the newborns (figure 1b). It was significantly higher compared to all other age groups (p <0.0001, each with the exception of the comparison to adults p = 0.0022). The mean skin surface pH-value in the 5 weeks + 1 week age group was 5.1, which corresponds to a decrease of 0.9 units when compared to the newborns. In the older age groups the mean skin surface pH-value remained on this level. The lowest mean skin surface pH-value (4.9) was noted in the 1 to 2 years age group. Greater mean pH-values were also found for the adults when compared to the 5 weeks + 1 week age group (p = 0.0026), the 6 months +/- 1 month age group (p = 0.0057), the 1 to 2 years age group (p = 0.0003) and to the 4 to 5 years age group (p = 0.0185) (table 2b).

Stratum corneum hydration

*Capacitance as indirect parameter for skin hydration state*

The mean corneometric value for the newborns was significantly lower compared to other age groups (figure 1c, table 2c). Higher mean corneometric values were found for the 5 weeks +1 week age group as well as the 6 months ± 1 month age group when compared to the 1 to 2 years, 4 to 5 years and 20 to 35 years age groups (p <0.0001, each).
**Water profile assessed by RCM**

A similar continuous increase in percentage of water content was observed in correlation with increasing depth for all age groups, whereas the increase in percentage of water content was lower for the newborns (figure 2). With the exception of the newborns the percentage of water content revealed a saturation of approximately 60% in a depth between 15 - 20 µm. The newborns showed 60% saturation at depth between 25 - 30 µm.

**Natural moisturizing factor (NMF) profile**

Overall greater mean AUCs of Raman depth profiles for NMF were noted in the skin surface (0 – 15 µm) as witnessed in figure 3a. The mean AUC was greater for the newborns in a depth of 5 – 15 µm and 15 - 25 µm as well as for the complex depth 0 – 25 µm compared to all other age groups (figures 3a, b). Clearly lower mean AUCs were noted for the 6 months ± 1 month age group for each depth as well as for the complex depth 0 – 25 µm when compared to the other age groups. The analysis of the separate constituents of the NMF showed in general similar profiles compared to the bulk NMF for pyrrolidone carboxylic acid, serine, glycine, histidine, urea, and trans-uranic acid (data not shown). In contrast, the mean AUCs for lactic acid were not greater for the newborns compared to the other age groups. Greater mean AUCs were noted for the 6 months ± 1 month age group especially in the skin surface as well as for the complex depth 0 – 25 µm followed by the adults (figure 4). Only slight differences between the different age groups regarding the mean AUCs of Raman depth profiles for ornithin, proline and alanin were observed with generally lower mean AUCs in newborns.

**DISCUSSION**

Measurement of TEWL is generally accepted as a reliable tool to assess objectively the epidermal barrier function especially for the inside-out-barrier. Full term neonates have completely developed permeability barrier function at birth. High TEWL was shown in the first 4 hours after birth, and afterwards returned to values of 6 g/m²h, suggesting ongoing desiccation of the skin immediately after birth. Despite the different methodologies used, there is a wide agreement that basal TEWL values in non-stressed skin in term newborns is below 10 g/m²h which is similar to that in healthy adults. Our findings are consistent with previous reports. In infants the fully
developed, albeit thinner SC assures competent skin barrier function. A trend towards an increase in TEWL after the age of 5 weeks + 1 week was noted. The highest value of TEWL was observed in the 1 to 2 years group (9.7 g/m²h), although not significantly higher than the TEWL in adults (8.9 g/m²h). This could be attributed to the ongoing skin functional adaptation to the gaseous environment (measurements performed on the volar forearm that has direct exposure to the surrounding environment). The mean values of TEWL in children were comparable to those in adults (figure 1a) which is in accordance with previously published data.

Skin hydration depends on two major mechanisms namely the formation of a highly hygroscopic molecular complex (NMF) mainly by degradation of filaggrin and the hydrophobic extracellular lipid matrix of SC which provides a barrier to an uncontrolled loss of water and electrolyte from the viable epidermis. Newborns had significantly lower mean capacitance values compared to other age groups (figure 1c, table 2c) and a lower increase in the percentage of water content than the older subjects (Figure 2). So it seems that there is a correlation between hydration at the skin surface and water content into the SC. On the other hand, the newborns displayed the highest mean AUCs of Raman profiles for NMF (figure 3b). Therefore the lower hydration immediately after birth can not be attributed to a low content of NMF constituents. In newborns, other studies have already shown similar results on hydration (lower than older infants and adults). But this is the first time we analyze NMF contents in newborns (aged from 1 to 15 days) and we observe such high level. Birth induces ‘environmental changes’ such as the transition between aqueous intra-uterine ‘milieu’ to gaseous and dry extra-uterine environment. Our hypothesis is that the incomplete acidification of SC and the lower SC hydration in newborns (figure 1b, table 2b) induces adaptation processes and increased compensatory mechanisms. Thus, NMF production in newborns could be necessary to rebalance pH and hydration at the skin surface during the post-natal period. In accordance, animal studies have shown that activation of filaggrin proteolysis was dependent on the drop in external humidity during the transition from intra- to extrauterine environment. The lower SC hydration in infants requires adequate skin moisturization even in the first days after birth.
On the other hand, the two key enzymes of the lipid processing (β-GlucCer’ase and aSM’ase) which do not have the requested acidic pH for their optimal activity are not fully activated. Hence, the synthesis of the constituents of the hydrophobic extracellular lipid matrix of SC is hindered and this results in decreased skin hydration in newborns. Furthermore the protein degradation by proteases is also pH-dependent and not fully developed at birth. The clinical consequence of the uncompleted skin surface acidification could be the propensity towards development of certain inflammatory dermatoses such as seborrheic dermatitis or diaper dermatitis. Neutral-to-alkaline pH amplifies the activity of serine proteases (kallikrein 5 and 7) resulting in a blockade of lamellar body secretion (SC lipids) and induction of T-cell driven inflammation. High pH values favor the colonization with skin surface pathogens such as *Staphylococcus aureus* and *Candida albicans*. Additionally, of practical importance is the avoidance of alkaline soaps and detergents in the postnatal period.

Previous studies revealed that the approximate SC thickness at the volar forearm is 10 -15 μm. NMF is produced in the lower parts of the SC just above a zone of stable filaggrin in the lowermost parts of the SC. This is in accordance to our findings demonstrating the greatest mean AUCs of Raman depth profiles for NMF in the depth of 5-15 μm from the skin surface (figure 3a). On the other hand, exogenous factors such as washing and seasonal variations result in continuous depletion of NMF constituents from the skin surface. Newborns show the highest mean AUCs of Raman profiles for NMF which reaches the lowest levels at the age of 6 ± 1 months and then again raising in the older age groups but never reaching the values observed immediately after birth (figure 3b). A possible explanation for this dynamics is that in newborns the effect of the exogenous factors is not yet observed (high AUCs of Raman profiles for NMF). Later on, it takes about a year for the complete adaptation to the dry gaseous environment, witnessed by the lowest NMF profiles in 6 ± 1 months old and then the relatively similar levels in the older age groups. This could likely to influence the water-handling properties of the skin and to impact its water barrier function quality and competence. Such ongoing adaptation in the first year of life has been suggested by others showing lower amounts of NMF in the outermost skin layers of infants (aged 3–12 months) in...
comparison to adults 3. In addition, faster water absorption and desorption were measured in infants group.

Previous reports demonstrated a decrease in the amounts of sweat constituents (urea and lactate) from the outer to inner layers of the SC suggesting an origin from the eccrine sweat glands 8,52. Our results showed that mean AUCs for lactic acid were not greater for the newborns compared to the other age groups. This can be explained by the lower duct length and coil volume of the sweat glands 32 in newborns as well as by the imperfect sweating response to external stimuli 31. On the other hand, we could not demonstrate the previously observed depth gradient 8,52 neither for urea nor for lactic acid. This discrepancy implies the alternative origin of these substances, possibly sebaceous glands at least for the latter as previously suggested 51.

In summary, the functional skin adaptation is a continuous process that takes place in the first year of postnatal life even in term newborns. The mechanisms of the skin acidification, water handling properties and permeability barrier are intimately related. Stratum corneum is the active field where the postnatal adjustment to the gaseous environment is accomplished. Our results revealed for the first time the molecular composition of the skin in relation to the dynamic functional adaptation during the neonatal period and the early childhood.

ACKNOWLEDGMENTS

The present study was sponsored by Expanscience and performed by bioskin. JWF was an employee of bioskin at the time when the study was performed. No products have been evaluated and the purpose was clearly to best understand the infant's skin and not of a commercial interest.
Table and figure legends:

**Table 1:** Demographic characteristics of the enrolled subjects.

Abbreviations: SD – standard deviation; kg – kilogram; cm – centimeter

**Table 2a:** Pairwise comparisons of age groups: TEWL measurements [g/m²h]

Abbreviations: TEWL – transepidermal water loss

**Table 2b:** Pairwise comparisons of age groups: skin surface pH

**Table 2c:** Pairwise comparisons of age groups: corneometric measurements [a.u.]

**Figure 1a:** Graphical presentation of the mean transepidermal water loss (TEWL) ± standard deviation for each age group. Values in gram (g) per square meter (m²) per hour (h).

**Figure 1b:** Graphical presentation of the mean pH value ± standard deviation for each age group.

**Figure 1c:** Graphical presentation of the mean corneometric value ± standard deviation for each age group. Values in arbitrary units (a.u.)

**Figure 2:** Summary of the water content in mass percent (%) (mean value ± standard error) as a function of the depth (in micrometers [µm]) from the skin surface. Data obtained by in vivo Raman confocal microspectroscopy. Vertical lines pointing the 60% water saturation as a function of depth for newborns (continuous line) and other age groups (dashed line)

**Figure 3a:** Summary of Raman spectroscopy data for natural moisturizing factor (NMF) bulk profile- area under the curve (AUC) in arbitrary units (a.u.). Data pooled for three depth segments: 0-5µm, 5-15µm and 15-25µm and for each age group

**Figure 3b:** Summary of Raman spectroscopy data for the total natural moisturizing factor (NMF) bulk profile- area under the curve (AUC) in arbitrary units (a.u.) for each age group.

Figure 4: Summary of Raman spectroscopy data for the lactic acid profile- area under the curve (AUC) in arbitrary units (a.u.) for each age group.
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Figure 1a

The diagram illustrates TEWL (g/m²/h) for different study groups. Each bar represents a different time period post-treatment: 1-15 days, 5-6 weeks, 6 ±1 months, 1-2 years, 4-5 years, and 20-35 years. The TEWL values for each group are as follows:

- 1-15 days: 8.2 g/m²/h
- 5-6 weeks: 6.4 g/m²/h
- 6 ±1 months: 7.7 g/m²/h
- 1-2 years: 9.7 g/m²/h
- 4-5 years: 7.2 g/m²/h
- 20-35 years: 8.9 g/m²/h
Figure 1b
Figure 1c

Capacitance values [a.u.]

Study group

1-15 days
5-6 weeks
6 ±1 months
1-2 years
4-5 years
20-35 years
figure 2
Skin depth (from the skin surface)

NMF Profile (Bulk) - AUC [a.u.]

- 0-5 μm
- 5-15 μm
- 15-25 μm

1-15 days
5-6 weeks
6±1 months
1-2 years
4-5 years
20-35 years
Figure 3b

NMF Profile (Bulk) - AUC [a.u.]

Study group

0 10 20 30

1-15 days 5-6 weeks 6 ±1 months 1-2 years 4-5 years 20-35 years
Lactic acid - AUC [a.u.]

Study group

1-15 days 5-6 weeks 6 ±1 months 1-2 years 4-5 years 20-35 years

figure 4