Aus der Klinik für Dermatologie, Venerologie und Allergologie
der Medizinischen Fakultät Charité-Universitätsmedizin Berlin

DISSERTATION

Investigation of skin physiological parameters in term neonates and evaluation of the influence of bathing on skin barrier function in newborns during the first four weeks of life

Prospective examination of the following skin parameters:
stratum corneum hydration, skin pH, transepidermal water loss and skin surface lipids

Zur Erlangung des akademischen Grades
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My family

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SC	Stratum corneum
VC	Vernix caseosa
EGF	Epidermal growth factor
NMF	Natural moisturizing factor
TEWL	Transepidermal water loss
SCH	Stratum corneum hydration
RWL	Respiratory water loss
IWL	Insensible water loss
SGA	Full-term, small for gestational age infant
AGA	Full-term, appropriate for gestational age infant
LGA	Full-term, large for gestational age infant
“b”	Group bath
“w”	Group wash
2d	2^{nd} day of newborn’s life
7d	7^{th} day of newborn’s life
28d	28^{th} day of newborn’s life
SD	Standard deviation
NSCS	Neonatal skin condition score
TNHL	Transient neonatal hair loss
RH	Room humidity
RT	Room temperature
SEC	Skin surface electrical capacitance
IQR	Interquartile range
n	Number of subjects

1. Introduction

The most important function of the skin is to act as a physical barrier between the body and the environment.

The barrier properties of the skin are located almost entirely in the outermost layer of the epidermis, in the stratum corneum (SC). The SC provides many functions essential to human survival. These include water and electrolyte excretion, regulation of body temperature, maintenance of the proper colonization of the skin, protection against potential pathogens and toxins, physical protection against trauma, fat storage and tactile sensation (Darmstadt 2000, Lund 1999).

Functional maturation of the SC begins in the third trimester, around 24 weeks (Holbrook 2000). Epidermal cell layers and thickness increase from 24 weeks to term, but neither the SC nor the dermo-epidermal undulation are discernible until approximately 34 weeks of gestation (Evans 1986). Formation of functional barrier coincides with regression of periderm[1] and development of the vernix caseosa (VC) (Visscher 1999).

The age of the infant is mirrored by the function and efficacy of skin. Thus, preterm infants have skin whose structure and function directly reflect their degree of prematurity (Rutter 2000). For example, infants born before 30 weeks of gestation have a very thin SC (2-3 layers) in comparison to full-term infants (5-6 layers) or adults (8-12 layers) and they lack a protective mantle of VC (Darmstadt 2000, Pickens 1999). However, regardless of the degree of prematurity, the structure of the epidermis of the preterm infant develops rapidly after birth, so that by 2-3 weeks of age it resembles the epidermis of a term infant in structure and function (Kravchenko 2003). The change from an aqueous (intra-uterine) to a xeric environment (air exposure) might be the stimulus for this accelerated maturation that occurs in premature infants. However, factors which accelerate barrier formation in utero and in vitro, i.e. glucocorticoids, thyroid hormone and estrogen[2], may not further accelerate barrier formation in neonates (Hanley 1997).

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[1] Probably, periderm is the first protective layer which provides protection between embryo and amniotic fluid, and is present after 5 weeks of gestation.

[2] Glucocorticoids, thyroid hormone (T3), and estrogen accelerate, while androgens delay barrier formation both in utero and in the in vitro system, explaining the poorer outcome of premature males versus females. But neither T3 nor glucocorticoids are absolutely necessary for barrier development. PPARalpha (peroxisome proliferator activated receptor) and FXR (farnesoid X-activated receptor) activators, which like T3, heterodimerize with the nuclear receptor, RXR (retinoid X receptor), also accelerate barrier development in vitro. Finally, not only the nuclear receptor family, but also Ca ++ could regulate key events late in barrier development (Williams 1998).

Lack of a SC, as found with extremely low birth weight infants or burn victims, results in significant problem with water loss, thermoregulation, increased drug absorption, infections, and electrolyte imbalance (Visscher 1999). Conflicting data exist about the maturity of term newborn's skin.

Some authors consider the SC of healthy term newborns as structurally and functionally similar to that of adults. Histologically, epidermal thickness and number of cell layers in each epidermal compartment are comparable in adult and newborn skin, as are the cellular structure, number of cell layers, and thickness of the adult and newborn SC (Ertel 2003). However, other authors state that development of the epidermis and dermis in term newborn infants is not complete at birth (Evans 1986). The late gestational human fetus must develop physiological mechanisms for successful transition from an aqueous to a terrestrial environment at birth. These mechanisms include adaptation to air breathing and enteral nutrition, elimination of wastes, and maintenance of body temperature and water balance. The in utero development of a relatively impermeable cutaneous barrier, the SC, is the key to this transition (Harpin 1983). The changes, which SC undergoes in endogenous hydration and surface water binding, as well as the changes in the hair structure and cycle may be an important mechanism of skin adaptation following birth. Hair appears first on the scalp at 20 weeks of gestation and then grows over the rest of the body. These initial hairs are lanugo, unmedullated hairs and initially all of them are in anagen (growing phase). The change of synchronized (in utero) to unsynchronized hair cycle can be responsible for the development a physiological transient hair loss in newborns (Cutrone 2005).

In conclusion, whether a structurally fully developed skin in healthy term newborns has a complete functional capacity similar to adults is still under discussion.

Intra-uterine skin barrier formation results in a surprisingly dry skin surface at birth, as determined by skin surface electrical measurements of capacitance and conductance (Saijo 1991, Okah 1995). The surprising feature of this barrier is that it develops under conditions of total fluid immersion, i.e., under conditions that result in maceration and loss of SC integrity and function in the adult (Willis 1973).

Epidermal changes are similar to those seen during recovery from partial thickness burns or wound healing (Evans 1986). Whether these changes are mediated by local factors or whether they occur in response to a systemic growth factor, such as epidermal growth factor (EGF), is unknown[3]. However, analysis of cutaneous barrier function in hairless mice in two different

[3] Although EGF produces these changes to the skin when injected into neonatal mice, no change in urinary excretion of EGF was seen in preterm infants in the early neonatal period (Evans 1986).

humid environments, showed that exposure to a dry environment enhances epidermal permeability, thickness of epidermis and barrier recovery following either acetone treatment or tape stripping (Denda 1998). The mechanisms responsible for the establishment of epidermal barrier function in the perinatal period, as well as the definition of postnatal developmental process as far as maturation or adaptation are concerned, are still being discussed.

The relationship between the water desorption rate and skin hydration observed during the period of adaptation following birth is particularly interesting. Rapid desorption of water is associated with a dry surface (Vissher 1999). Because the skin surface of newborn infants is drier than in adults, it can indicate an impaired SC water holding capacity in newborns (Saijo 1991). Application of topical isopropyl alcohol can decrease the water desorption rate in newborn infants, supporting a potential role for a lipid film in repelling exogenously applied water (Okah 1994, Visscher 1999). The higher level of SC lipids found in infant skin may be responsible for the greater penetration of lipophilic materials. In addition, infant and adult skin exhibits differential permeability to some compounds. Hydrophobic skin surface layer confers potential survival advantages in the newborn in terms of decreasing evaporative heat loss (Visscher 1999). Therefore, two mechanisms may be operating simultaneously in the newborn infant during the period of early adaptation following birth. Firstly, a drying of the SC is the response to a low environmental water activity. This is associated with a presumptive generation of natural moisturizing factor (NMF) due to proteolysis of endogenous filaggrin (Scott 1986). The controlled drying of the SC, therefore, would result in a flexible, conformal surface “membrane” without abnormal patterns of desquamation or surface flaking. Secondly, exogenous surface lipids may confer a hydrophobic property to the SC, which could result in the ability to repel surface water (Visscher 1999).

One obvious candidate for the latter role is the VC. During the last trimester, the human fetus produces increasing amounts of lipids. These lipids include those found in the space between the corneocytes of the SC and in the VC. The composition of these two lipid materials is different with respect to the nature of the fatty acid chains. VC contains a significant fraction of branched chain fatty acids and lipids with longer chains than those typically found in the SC barrier. The functions of these two lipid-rich materials may be different as the infant prepares for a transition to a dry environment. At birth, the infant is dried rapidly to reduce heat loss due to the evaporation of water. Much of the vernix is removed from the skin surface during this process, due to the use of a rubbing motion with absorbent towels. Whether vernix is beneficial to the newborn infant is unclear. It seems unlikely however, that nature would have invested such energy in producing a complex superficial biofilm without multiple functions (Visscher 1999).

Generally, the skin of infants is more delicate, sensitive and susceptible to irritant penetration, such as allergic contact dermatitis, itching, redness and dryness, percutaneous infection, damage secondary to trauma or toxicity from topically applied active agents (Hoeger 2004, Holbrook 2000). The higher cutaneous permeability can be the result of a four times larger surface area-to-body weight ratio of the neonate than of an adult and the limited ability of the immature organs to detoxify chemicals (Visscher 2000). The skin of a preterm infant comprises approx. 13% of the body weight, compared to only 3% that of an adult. In term and in preterm infants, the skin is characterized by less stronger connection between the dermis and the epidermis, low melanin production, neutral or alkaline skin surface pH and absence of normal flora during the first few days after birth (Kravchenko 2003). Functionally, both thermal and emotional sweating are reduced and thermoregulation by vasoconstriction of dermal capillaries is limited (Pöschl 1991, Green 1973). Morphologically, during the adaptation process, the epidermis increases in thickness and dermo-epidermal connections are enhanced (Evans 1986).

The deficient SC barrier function is a transient phenomenon in newborns (Gfatter 1997). The water-handling properties of term infant skin are in a state of flux during the neonatal period, in contrast to adults and can be objectively indicated by measuring, non-invasively, skin parameters, such as: transepidermal water loss (TEWL), stratum corneum hydration (SCH), skin surface pH and sebum levels (Chiou 2004). As it is so far unknown, which parameter the best reflects the functional adaptation of neonatal skin, for the first time, all four parameters were used simultaneously in the present study to evaluate SC barrier.

1.1. Transepidermal water loss

In a state of rest, healthy humans lose about 25% of their produced heat through evaporation of water from the skin (TEWL $g/m^2/h$) and respiratory passages (Respiratory Water Loss, RWL mg/kg/min). The sum of insensible loss of water from the skin and from the respiratory passages defined as perspiration insensibilis (Insensible Water Loss, IWL) is an important factor in the thermoregulation and water balance of newborn infants. TEWL comprises about 75% of IWL, whilst RWL about 25%.

Compared to adults, infants lose slightly more of their produced heat by evaporation under basal conditions (Sedin 2003). It seems probable that their skin is thin in comparison to adults, as they contain much more water. A full-term newborn infant has a water content of 77% of the body weight in comparison to 86% in a preterm infant at 24 weeks. After birth, total body water decreases with extra-cellular water and body weight. These events are considered to be a "physiological weight reduction". In the case of preterm infants with gestational ages of less than a 32-week, disturbance of water balance was often observed in the 1970s. The previously applied methods did not allow a separate determination of water loss through the skin from the respiratory tract. The methods used were developed and applied for direct determination of TEWL and for determination of RWL in the 1970s within the framework of a number of projects at the University of Linköping and Uppsala University, Sweden (Sedin 2003, Hammarlund 1977, Riesenfeld 1987). The technology of measuring TEWL has changed from whole-body enclosing metabolic chambers to ventilated chambers with closed skin capsules to measurements of water vapor pressure gradients with open chambers. In 1977, Hammarlund and Nilsson and in the 1980's Hammarlund and Sedin published a series of articles introducing the gradient method for measuring infant TEWL (Hammarlund and Nilsson 1979, Sedin 1983, Sedin 1985). Today, the gradient method is used as the standard for determination of skin barrier function in vivo. The small space required, expenditure of time and handling, which correspond to specifications for bedside-use in neonatology, were applied in the present study.

Compared to older infants and adults, the term newborn achieves, during the first hours after birth, equal or a lower TEWL over most of the body (10 $g/m^2/h$), except for the forehead, palms and soles of the feet. It could be concluded that term newborns are born with a functionally mature SC, and reduction of TEWL that takes place the first four hours after birth, may reflect an adaptation to extrauterine life. Baseline TEWL values of adults range over most of the body between 2.8±1.0 and 8.13±2.89 $g/m^2/h$. In adults, on the palms, soles and forehead, TEWL can reach the following values, 48.0, 27.0 and 16.5 $g/m^2/h$, respectively. The regional variation in TEWL is related to the varying skin structure, particularly the epidermis and its horny layer and the regional distribution of the eccrine sweat glands, which are concentrated on the palms and soles, face and upper trunk. Different anatomical regions are variably exposed to the environment, including the sun, which contribute to regional variation as time passes. Thus, the anatomic site is an important variable with respect to baseline TEWL (Pinnagoda 1990).

Compared to preterm neonates, TEWL is up to 15 times higher in day-old infants born at 25 weeks' gestation (~90 $g/m^2/h$). Also TEWL values of full-term infants small for gestational age (SGA) differ from those of full-term infants appropriate for gestational age (AGA). Lower TEWL in full-term SGA is still not fully understood, but might be related to the water content of the skin. After total removal of SC by tape stripping, TEWL values can achieve extremely high levels, even up to 140 $g/m^2/h$ (Öhman 1994).

Values of TEWL vary according to the equipment, environmental conditions, such as ambient temperature and humidity, body temperature and infant activity during the measurement.

Between ambient humidity and evaporation rate, an inversely linear relationship exists, and ambient temperature is positively correlated with TEWL (Cartlidge, 2000).

Based on available literature, it is known that during infant activity TEWL can increase by about 37% (Sedin 1985, Hammarlund and Nilsson 1979). Increase of body temperature above 37.1°C can cause a significant rise in TEWL, i.e., an increase from 37.1°C to 37.2°C already causes an increase in TEWL by about 80%. If a body temperature exceeds 37.2°C, visible sweating occurs and the effects of body temperature and activity on TEWL cannot be kept separate (Sedin 2003).

1.2. Stratum corneum hydration

The skin barrier is the dual result of the terminal differentiation of keratinocytes and the biosynthesis of specific lipids that together form the traditional "brick and mortar"[4] model of the mature SC (Elias 1996). Lipids are an extremely important part of the barrier function and represent approx. 20% of the total volume of SC. About 50% of the lipids are ceramides, 25% cholesterol and 10-20% fatty acids. Lipids in adult human SC prevent desiccation and serve as a barrier to diffusion of substances across the skin (Kravchenko 2003). Under normal conditions 100-150 mg of lipids must be generated by the skin every day to replace those which are lost in desquamation (Tharp 2004). Another important factor for skin barrier is NMF, derived in corneocytes by hydrolysis of filaggrin. It is a complex mixture of low molecular weight, water soluble and hygroscopic compounds, which allow taking up and releasing water. The appropriate water binding capacity is essential for the development of NMF, for hydration of the outer layer of the SC, maintenance of flexibility and for its maturation process.

With age, the level of NMF declines and indirectly there is a decline in the ability to restore the barrier. Race, disease and diet can also significantly affect this process. Evidence is available that stress may elevate glucocorticoid levels and this can play an important role in delaying the barrier recovery. The winter months and the desiccating environment certainly affect barrier function, which includes decreased intra-cellular lipids. In the case of patients with X-linked

[4] The useful model of SC structure was proposed by Michaels *et al* in 1983/1975. The "bricks" represent flattened corneocytes with a 15 nm thick cornified envelope and outer ceramide capsule or corneocyte lipid envelope (Nemes 1999), while the "mortar" consists of a heterogeneous mixture of predominantly non-polar lipid, arranged to form a complex lamellar bilayer structure in intercellular space (Wertz 2000).

recessive ichthyosis, a dysfunction of sterol metabolism and in patients with erythrodermic form of ichthyosis, a reduced fraction of sphingosine, was detected (Shapiro 1978, Paige 1993). Cleansers and retinoids also have the ability to alter the skin barrier (Tharp 2004). Each change in NMF, in lipid barrier or in terminal differentiation of SC can lead to ablation of corneocytes and the disturbance of the protective function of SC causing scaly skin (Madison 2003). The skin moisture is routinely determined by measuring the capacitance of the skin surface, which is based on the different dielectric constants of water and other substances. This technique was first used as an index of barrier maturation in 1995 (Okah).

Hydration of the SC is reduced in term neonates, but increases during the neonatal period, achieving adult levels (60-80 units) according to Hoeger's and Enzmann's (2002) data at about the 3rd month. Decreased SCH can be responsible for the environmental changes following birth. However, for normal ranges of SCH in neonates no exact data exists.

1.3. Skin pH

Interest in skin pH is of long standing. In 1892, Heuss claimed that the entire surface of the body is acidic. This early finding based on the use of hardly adequate technology, was corroborated by the investigations of Schade and Marchionini (1928). They had already addressed the differences according to the region of the body area in question, in particular they found that occluded skin was less acidic than skin exposed only to the atmosphere. Additionally, they were the first to propose the term "acid mantle" for skin surface pH (Korting 1996). However, there are some anatomical regions, such as: intertriginous, axilla, genital, anal and plantar inter-digital areas, where the so-called "acid mantle" is not present (Marchionini and Hausknecht 1938, Marchionini and Schmidt 1938).

Determination of skin pH is important for the evaluation of SC integrity, as the acidity of the SC plays an extensive role in homeostasis. Normal formation of SC lipids in a lamellar structure, regulation of desquamation and control of bacterial skin flora are all dependent on an acidic environment. There is also evidence that changes in pH are involved in the pathogenesis, prevention and treatment of skin disease such as irritant contact dermatitis, atopic dermatitis, ichthyosis, as well as wound healing. The significance of the "acid mantle" has been under scientific discussion ever since 1928.

The "acid mantle" of the SC is thought to arise from a combination of factors: secretion of sebum (fatty acids), sweat (lactic acids), amino acids, urocanic acid and pyrrolidine carboxylic acid from keratinization and hydrogen pumps from lamellar body exocytosis.

The usual pH range at the surface of non-occluded human skin is between 4.0 and 6.0 (Parra 2003). According to the Braun-Falco data (1986), pH in adults ranges from 5.4 to 5.9 and according to the product information (Courage and Khazaka 2002) pH range is between 4.5-5.5 for female and 4.3-5.5 for male. In contrast, the body's internal environment maintains near neutral pH, ranging between 7.35 and 7.46. From a biological point of view, a change in pH of about 2 units over so short a distance as 10-20 µm is an important event. The physiological pH cannot be attained if all corneocytes are fully removed, making the stratum Malpighi cell layers accessible (Parra 2003). This situation can be observed in adults after removal of SC using tape stripping (pH has increased) as well as at birth, when SC has an insufficient amount of layers and the intensified process of maceration and desquamation takes place. A subsequent increase in the pH occurs when deeper layers of SC are removed (Parra 2003).

The progressive postnatal adaptation of SC pH to ex-utero conditions is independent of fetal age at birth (Behne 2003, Fox 1998). Therefore, the validity of using the postnatal change in skin pH as a maturational marker in preterm infants is unknown. Skin pH differs between males and females; however, it is unknown when this differentiation process between females and males actually begins. Exogenous, cosmetics and soaps as well as pathological factors, such as atopic dermatitis, ichthyosis vulgaris can influence the skin pH (Chiou 2004).

The measuring principle is based on a potentiometric method, which was invented in 1935 by Arnold Beckman.

1.4. Sebum level

Sebum is the major source of skin surface lipids and the first demonstrable glandular product of the human body. The development and function before birth and in the neonatal period appear to be regulated by maternal androgens and by endogenous steroid synthesis by the fetus. Sebaceous glands produce most of the lipids comprising VC. It is interesting that only the skin of full-term neonates is covered by vernix, although this naturally occurring biofilm already starts to form between the 17th and the 20th weeks of gestational age (Chiou 2004). Sebum composition is age-related, but the differences between infants and adults can only be observed until the age of 7-10 years. Also the postnatal glandular activity is changed in the course of life. Accepting an analysis from Courage and Khazaka (2002) 100-200 μg/cm^2 lipids can be recovered on the forehead of adults, whilst at birth, in term neonates the level of sebaceum is significantly reduced.

Sebum serves as a waterproof barrier and an immunological modulator, which plays a major role in skin homeostasis (Downing 1987, Metze 1988, Thody 1989). Antimicrobial properties of sebaceous free fatty acids (e.g., palmitoleic acid) are also known. Each cleansing agent, even normal tap water, shifts sebum level to lower values, this effect is maintained over several hours. The dissolution of fat from the skin surface may influence the hydration status, leading to a dry and squamous skin.

The measurement principle of sebumeter is based on grease-spot photometry. The sebumeter computes the change in sheet transparency, which is proportional to the quantity of lipids absorbed.

Owing to anatomical and physiological differences of the skin between neonates and adults in general, newborns are at risk of suffering from skin damage, percutaneous infection, or toxicity from topically applied active agents.

The sensitive skin of newborns responds very quickly to external stimuli, such as heat, friction, sunlight, humidity, water, occlusion and chemicals. Better understanding of perinatal skin adaptation or maturation and water binding can provide a scientific basis for infant skin care practices.

Appropriate skin care is very important for successful transition from the uterine aquatic to the aerobic environment, as well as for adaptation or maturation processes following birth.

A variety of non-invasive physical methods for the assessment of skin physiology have been described. However, they have been evaluated in the adult population and in school children, and there is only a limited amount of data available for neonates and infants.

1.5. Aim of investigation

1. To prospectively monitor the changes of different skin parameters:
   - Transepidermal water loss
   - Stratum corneum hydration
   - Skin surface pH
   - Sebum level

in full-term newborn infants, from 37 completed weeks of gestation, in different body sites (forehead, abdomen, upper leg, buttock) during the first week of life for both groups together (from the 2^{nd} to 7^{th} day) and for the subsequent three weeks for each group separately (from the 7^{th} to 28^{th} day).

2. To check the effect of bathing with clear water in comparison to washing with clear water by the parameters:
   - Transepidermal water loss
   - Stratum corneum hydration
   - Skin surface pH
   - Sebum level

   from the 7^{th} to 28^{th} day with standardized skincare regimen.

3. To investigate the influence of the following factors on the skin parameters:
   - Anatomic sites
   - Gender
   - Ethnicity
   - Mode of birth
   - Gestational age
   - Ambient conditions

Because of regional differences in skin physiological parameters the measurements in the present study were performed on four specially selected anatomical sites: abdomen, upper leg, and buttock as occluded area, as well as on the forehead as non-occluded area. The choice of these anatomical sites was guided by two considerations: firstly, forehead, abdomen and upper leg appeared to be the most neutral and non-sensitive to external influences and secondly, diaper rash has been previously associated with increased skin wetness and higher skin pH.

2. Materials and methods

2.1. Study design

This monocenter, prospective, randomized study was performed in close cooperation between the Departments of Neonatology, Gynaecology and Dermatology, Campus Charité Mitte, Charité-Universitätsmedizin Berlin from October 2005 to April 2006.

2.2. Ethic

The project was developed and conducted in compliance with the ethical principles adopted by the 18th World Congress in Helsinki in 1964 and amendments adopted in 1975, 1983, 1989, 1996, and 2000. It was also performed in accordance with guidelines issued of GCPs (Good Clinical Practices) published by ICH Topic E6, and in compliance with local regulatory requirements.

This protocol and all appropriate amendments were reviewed and approved by the local Ethics Committee of the Campus Charité Mitte. Written, informed parental consent was obtained for all infants participating in the study. The informed consent form, approved by the Ethics Committee, was fully explained to the parents.

The study was covered by the insurance of the Charité-Universitätsmedizin Berlin.

2.3. Demographic characteristics of subjects

Population

A total of 57 healthy term neonates (32 boys and 25 girls), 50 of Caucasian descent and 7 of non-Caucasian[5] descent, were recruited from the neonatal wards of the Charité Campus Mitte.

The infants were randomly assigned to one of two groups: bath (“b”) and wash (“w”). In the first group the newborns were bathed by submerging in the bathtub twice a week with clear water (n=29) and in the second control group the newborns received dry care by wiping with a washcloth moistened with clear water (n=28). The randomization of newborns and treatment’s schema was performed before starting the study together with the statistician.

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[5]In the group of non-Caucasian were included newborns from Latin America, Asia and North Africa

Inclusion criteria

All healthy (i.e. meeting none of the non-inclusion criteria) term newborns were candidates for the study. The following criteria had to be met prior to enrolment in the study:

1. Male and female term newborns from 37 completed weeks of gestation, age <48 hours
2. Written informed parental consent

Non-inclusion criteria

Newborns meeting the following criteria were not included:

1. Any critically ill term newborn, i.e. septic infants, infants born with serious congenital malformations/defects, asphyxia, hydronephrosis, severe intracranial hemorrhage
2. Newborns with known immunodeficiency
3. Newborns with preexisting skin disease with eruptions covering more than 50% of body surface i.e. congenital ichthyosis, congenital candidiasis
4. Newborns with relevant skin maceration and/or inflammation, irritation and urticaria
5. Any acutely or chronically ill newborn with temperatures below 35^0C or above 40^0C
6. Newborns with infections skin diseases, e.g. herpes

The planned number of male and female newborns, available at least completely for the first four weeks, to be included in this study, was 40. Because of discontinuation of observation of 13 infants (9 parents cancelled participation after the first measurement and 4 parents after the second measurement) 57 newborns were recruited.

Following tables give a review of data available per measured parameter and point in time (Tab.2.1-2.4).

Table 2.1: Tewameter

	2d	7d	28d
forehead	57	48	44
abdomen	57	47	44
upper leg	56	48	44
buttock	57	48	44

Table 2.2: Corneometer

	2d	7d	28d
forehead	57	48	44
abdomen	57	48	44
upper leg	56	48	44
buttock	57	48	44

Table 2.3: pH meter

	2d	7d	28d
forehead	56	47	44
abdomen	56	47	44
upper leg	56	47	44
buttock	56	47	44

Table 2.4: Sebumeter

	2d	7d	28d
forehead	57	48	44
upper leg	57	48	44

Demographic characteristic of participants:

The infants were born after a mean of 39 weeks and 6 days of gestation (±8.5 days SD) (range 37-42 weeks) with a mean birth weight of 3506 g (±469.5 SD) (range 2450-4680 g) and a mean body length of 51.5 cm (±2.2 SD) (range 46-56 cm). 55 of them were AGA and 2 were large for gestational age (LGA)[6]. Their median APGAR scores were 9, 10 and 10 at 1, 5, and 10 minutes, respectively. Delivery was vaginal in 41 children (72%), by caesarean section in 12 (21%) and by forceps or vacuum extraction in 4 (7%). The mean age of mothers was 31 years (±5.7 SD) (range 23-47). 24 of them were under 30 years old (42%). 31 (54%) were primiparous.

The exactly characteristics of the population is shown in Table 2.5.

[6] Division of newborns into AGA and LGA was made on the basis of week of gestation and birth weigh using a model presented by Bauer K, Groneck P, Speer PC (2001).

Table 2.5: Demographic characteristics of the infants and their mothers grouped in the study populations.

Characteristic	Group "b" (n=29)	Group "w" (n=28)
Female, No. (%)	12 (41)	13 (46)
Male, No. (%)	17 (59)	15 (54)
Caucasian, No. (%)	27 (93)	23 (82)
Non-Caucasian, No. (%)	2 (7)	5 (18)
Age of gestation, mean (SD), days	278 (8)	276 (9)
Birth weight, mean (SD), g	3543 (459)	3466 (485)
Birth length, mean (SD), cm	51 (2)	51 (2)
Eutroph, No. (%)	27 (93)	28 (100)
Hypertroph, No. (%)	2 (7)	0 (0)
Head circumference, mean (SD), cm	35 (1.2)	35 (1.4)
Vaginal delivery, No. (%)	20 (69)	21 (75)
Caesarean section, No. (%)	7 (24)	5 (18)
Forceps or vacuum, No. (%)	2 (7)	2 (7)
Apgar score at 1st minute, median (25%; 75%)	9 (9;9)	9 (8;9)
Apgar score at 5th minute, median (25%; 75%)	10 (9;10)	10 (9;10)
Apgar score at 10th minute, median (25%; 75%)	10 (9;10)	10 (9;10)
Age of mother, mean (SD), y	30 (6.5)	31 (4.8)
Primiparous, No. (%)	18 (62)	13 (46)
Newborn with negative history of skin disease in the family, No. (%)	12 (41)	13 (46)
Newborn with positive history of skin disease in the family, No. (%)	17 (59)	15 (54)
Discontinuation, No. (%)	7 (24)	6 (21)

2.4. Care regime

All infants in both groups were washed the first time from head to toe with clear water about 2.5 hours after delivery in the neonatal ward in order to remove the rest of blood, birthing fluid, meconium and excess of VC. Until the 7th day of life there were no differences in the daily skin care between both groups. On the 7th postnatal day, the parents whose infants were allocated to the first group (n=29) started to bath their babies by submerging them in bathtub half filled with water, up to the shoulder (immersion bath[7]). At the same time, the parents whose infants were allocated to the second group (n=28) started to wash their babies from head to toe by wiping them gently with a soft washcloth moistened with clear water.

The cleansing procedure in both groups was performed with a soft cloth without using skin care products and took place regularly twice a week during the 28 days. Every bath and wash lasted about 5 minutes. Water temperature and pH were between 37-38^{0}C and 7.9-8.2, respectively, in both groups. The water hardness at home was controlled by the parents using the easy stripes (Sofchek, Water Hardness Test Strip) with mean water hardness 13.4 ^{o}dH (±4.2 SD) (range 7-25^{o}dH). If necessary, independent of routine wash or bath, the parents could wash individual parts of infant's body (especially the head or buttock, usually i.e. during the changing the diapers) with a soft washcloth, moistened with clear water.

During the study period parents were allowed to apply one or several of the following products:

1. to areas of skin trauma or open wounds: zinc paste (i.e. Pasta zinci mollis)**,** antiseptic ointments (i.e. Triclosan 1%-Cream, Octenisept®-Solution)
2. to mycosis i.e. Candida-intertrigo: antimycotic preparations (i.e. Candio-Hermal®-Softpaste or systemic Nystatin®-Suspension)
3. to parts with meconium: skin cleansing with oil (only after delivery) and prophylactic skin care with vaseline in the buttock area

The parents got exact instructions about how to perform the skin care of their infant (daily notes in the infant's diary) and were asked to avoid using skin care products at least 12 hours before the measurements.

[7] Immersion bath is placing the infant's entire body, except the head and neck, into a tub of water.

2.5. Examination procedure

To avoid selection, every newborn infant matching the inclusion criteria born at the Charité Campus Mitte was proposed the participation at this study protocol. The first discussion took place usually at the first day postpartum. After having explained the purpose and schedule of the study, parents received the parental information and parental consent forms.

If the parents consented, their child was randomly assigned to one of two groups (“b”, “w”). The parents provided their written consent after having fully understood the study information. One signed consent form remained in the Case report form, the other with the parents. After 1st measurement the parents received infant’s diary and stripes for evaluation of water hardness. The exact study schedule is presented below (Tab.2.6).

The response of the parents for the study was usually positive. The reasons for non-participation or discontinuation of the study were mainly due to social situation (difficult public transport connection, long journey to the clinic, organisational problems (other children at home, mother alone at home)), or without the intention to keep the skin care regime after having returned home.

Table 2.6: Study flow chart

	1.month									
Week	1							2	3	4
Day	1	2	3	4	5	6	7			
Written Informed Consent		X								
Inclusion/ Exclusion criteria		X								
Demographic characteristics		X								
Family Medical History		X								
Medical Examination		X								X
Parents self assessment										X
Randomization		X								
TEWL		X					X			X
SCH		X					X			X
pH		X					X			X
Sebum level		X					X			X
Neonatal Skin Condition Score (NSCS)		X					X			X
Physician Assessment on Photographs		X					X			X
Transient Neonatal Hair Loss (TNHL)		X					X			X

Medical evaluation

A complete medical examination was performed on the day of enrolment to assure the infant's eligibility for the study; it was repeated at the end of study for the close out visit. In addition, the newborn's parents were interviewed for any prenatal abnormalities or events and the family's medical history especially about cutaneous, allergic or immunologic diseases.

These examinations were performed by the investigator. History (APGAR score, birth weight, birth length, head circumference, mode of birth, gravida, para, age of mother, pH of umbilical cord, complication during the pregnancy period or during the birth) was obtained from the infant's medical chart.

Investigational sites

Evaluation of the skin condition, measurements and photos was made on intact, undamaged skin from:

1. the forehead, abdomen, upper leg and buttock for biophysical parameters, such as TEWL, skin pH, sebum production and SCH; except for sebum level, which was measured on the forehead and upper leg only
2. the dorsum of the hands, the sole of the feet and abdominal area for skin condition
3. scalp for hair- growth pattern

Study conditions

The measurements at the hospital were performed under standardized condition in baby's room on the neonatal ward. After discharge from neonatal service the parents were asked to arrange follow up visits at the Clinical Research Centre for Hair and Skin Physiology; exceptionally the measurements were performed also at baby's home.

Every measurement was performed on the diaper changing table. On the neonatal ward the first measurements were performed under the thermal lamp.

Room temperature (RT) and room humidity (RH) were recorded with standard devices, and measurements were automatically normalized with these values.

Prior to testing, sites were garment free. Infants were bathed for at least 12 hours prior to testing. If clinical condition allowed, test regions were skin product-free (creams, lotions) for at least 12 hours before data collection. Diaper was taken off 5-10 minutes prior to measurement. During the measurement infant was at rest and its body temperature did not exceed 36.9°C.

For every examination the same procedure was repeated and every visit lasted ca. 35 minutes. The parents were asked about skin changes, skin care regime deviations and general condition of their baby. If another skin product was used, the parents were asked for the reason and for a detailed explanation of the exact product and frequency of usage.

Every exception of the skin care regime was noted by the parents in the infant's diary, which was checked at every study visit and was given back to the investigator at the end of the study.

During the measurement the investigator used sterile gauze in order to avoid direct contact between his hand and examined part of body.

The order for measurements was as follows: TEWL, SCH, pH and sebum, and order for part of body: forehead, abdomen, upper leg and buttock. After measurements, skin physiological parameters, the clinical scoring and hair-growth were visually evaluated and documented with standardized photography.

All information and results of measurements were registered in the Case report form.

2.6. Materials and technical procedures

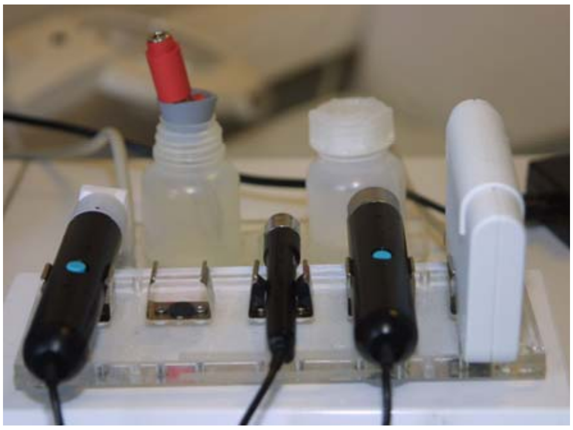
Data on TEWL, SCH, skin pH and sebum production were collected using non-invasive Multi Probe Adapter System MPA® (Courage & Khazaka, Cologne, Germany) connected with the following probes: Tewameter® TM 300, Corneometer® CM 825, Skin-pH-Meter® PH 905 and Sebumeter® SM 815, respectively (Fig.2.1).

As measurements on the living skin depend very much on the environmental conditions, especially temperature and relative humidity were recorded using a special external sensor for ambient condition monitoring (room condition sensor) to control and hold these parameters as constant as possible.

The Multi Probe Adapter System works with a software programme "MPA", version 1.3.2.8, for all probes.

All equipments involved only non-invasive probe contact with infant skin so none of the measurements caused any discomfort to the infants. Probe heads were wiped with disinfectant (CaviCide® for PH 905 and Softasept® for TM 300, CM 825 and SM) between subjects to prevent microbial transfer.

Figure 2.1: Multi Probe Adapter System MPA®



Tewameter

The measurement of TEWL is the most important parameter for evaluating the efficiency of the skin water barrier. This gradient method is generally accepted since 1980/1977, after a series of descriptive articles by Hammarlund and Nilsson (Rogiers 2001).

The measuring principle is based on an open chamber measurement and correlates with the Fick equation of diffusion (1855):

dm/dt = -D A dp/dx

where: A=surface (m^2), m=water transported (g), t=time (h), D=diffusion constant (=0.0877 g/m h mm Hg), p=vapor pressure of the atmosphere (mm Hg), x=distance from skin surface to point of measurement (m).

The temperature and moisture sensors, in addition to the electronic measuring system and calibration data are located inside the probe. The measuring head of the probe is a hollow cylinder (10 mm in diameter and 20 mm in height), which minimizes the influence of air turbulence inside the probe. Also the low weight of the probe (90 g) has no influence on the skin surface structure and allows easy handling. Every measurement lasted about 30 seconds and between each subject and each measurement the ring for the probe head was changed.

The measurement of the "gradient" of water diffusion, the rate of water evaporation from the surface of the skin, is made possible by the two separate sensors in the probe head, which receive information on moisture and temperature values. TEWL values are expressed as $g/m^2/h$ of water loss and can range from 0 to 320 $g/m^2/h$, however the value 30 $g/m^2/h$ is defined as critical skin condition. Service conditions for tewameter is as follows: temperature 10° to 40°C and RH 30% to 90% (Fig.2.2.a).

Corneometer

The Corneometer provides measurement of skin surface hydration by examining electrical capacitance. This measurement is based on the difference in the dielectric constant of water (81) and other substances (most<7). The measuring capacitor shows changes of capacitance according to the moisture content of the tested site.

A glass lamina separates the metallic gold tracks in the probe head from the skin, preventing any current conduction. An electric scatter field penetrates the skin during the measurement and the dielectricity is determined. The penetration of the scatter field is very small so that only the moisture on the skin surface is measured. The measurement depth reaches the top first 60 and 100 μm of the stratum corneum. The low weight of the probe and the small measuring surface (49 mm^2) allow easy handling, measurements on all body sited and simple cleaning after the measurement. The probe was applied for 1-2 seconds onto the infant's skin surface for each site. Values are expressed in random units specific to the device from 0 (maximum dryness) to 130 (maximum humidity). The value 60 units for the head and 50 units for extremity are evaluated as normal (Fig.2.2.b). This measurement procedure was used for the first time in 1995 by Okah et al for evaluation of maturity of the skin barrier in newborns (Berardesca 1997).

pH meter

The pH meter was invented in 1935 by Arnold Beckman. It has been used in studies of skin pH ever since its conception, with the earliest study on infants completed in 1958.

The measurement of the pH level on the skin surface is an important parameter for evaluating the quality of the hydrolipidic film on the skin.

The value of acidity or alkalinity is determined by the number of hydrogen ions (or protons) and hydroxide ions in a solution.

The pH meter consists of a glass electrode filled with an inner buffer (Hg/Hg_2Cl_2 or $Ag/AgCl$). The inner buffer is separated from the measuring solution by a glass membrane and it carries away the potential of the internal side of the glass membrane. A reference electrode carries away the potential of the external side of the glass membrane which is in contact with the measuring solution. The reference electrode is filled with electrolyte and equipped with a diaphragm which allows transportation of the ions between the measuring solution and the inner buffer but prevents the mixing of the two solutions.

The electrode was calibrated using reference buffer solutions with defined pH at least once a week. For each measurement, the probe was applied for 3 seconds.

Values are expressed in random units specific to the device and range from 0 (strongly acidic) to 12 (strongly alkaline) (Mauro 2003, Parra 2003) (Fig.2.2.c).

Sebumeter

Sebumeter technology was first reported by Schaefer in 1973 and has been recognized as a method providing direct measurement of serum secretion on skin, hair and scalp (Pierard 2000). Its measurement principle is the photometric method, a grease spot photometer that is not sensitive to ambient humidity. The sebumeter device contains a photocell which measures the transparency of the measuring tape. The light transmission represents the sebum content on the surface of the measured area. The sebumeter computes the change in sheet transparency (a strip in the probe), which is proportional to the quantity of lipids absorbed. This value, expressed as µg sebum/cm^2, is the native value of the total sebum and corneal lipids on the skin surface and can be reported from 0-350 µg sebum/cm^2, where 350 µg sebum/cm^2 represents 100% saturation tape. Probe contact is maintained for 30 seconds at a constant pressure on the skin test site (Fig. 2.2.d).

The measuring head of the cassette exposes a 64 mm^2 measuring section of the tape.

Figure 2.2: Multi Probe adapter System MPA®

Neonatal skin condition score

Skin conditions were visually evaluated in respect of dryness, erythema and excoriation using a 9-point scale called the NSCS adapted from a visual scoring system used by Lund and Osborne (2001, 2004). In the present study the skin condition evaluations were performed in a very detailed manner at three different body sites: on the abdomen, on the dorsum of the hands and on the soles of the feet. These body sites were documented using standardized photography (Fig.2.3).

Dryness

1=normal, no sign of dry skin

2=dry skin, visible scaling

3=very dry skin, cracking/fissures

Erythema

1=no evidence of erythema

2=visible erythema <50%body surface

3=visible erythema ≥50%body surface

Breakdown/excoriation

1=no evidence

2=small, localized areas

3=extensive areas

Note: perfect score for every body sites is 3, and the worst is 9.

Figure 2.3: Neonatal Skin Condition Score, sites of evaluation

a: dorsum of the hand of a 2-day-old neonate, NSCS is 3; b: sole of the feet of a 2-day-old neonate, NSCS is 3; c: abdomen of a 2-day-old neonate, NSCS is 3; d: abdomen of 7-day-old neonates, NSCS is 4

Hair-growth evaluation

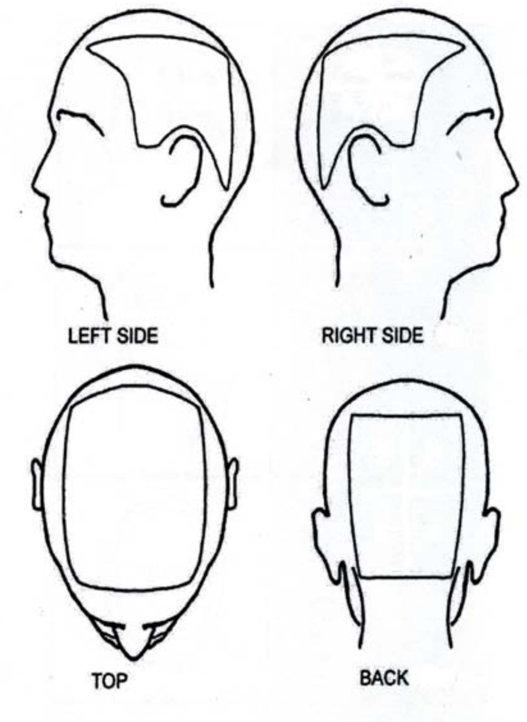
A model of hair-growth was visually evaluated using TNHL proposed by Cutrone and Grimalt (2005), percentage of scalp hair loss, adapted from Olsen and Canfield (2004) and documented with standardized photography on the left and right side, top and back (Fig.2.4).

Cutrone and Grimalt (2005) proposed the following 4 main pattern of TNHL with frequency of occurrence:

- Frontal pattern- hair only in the occipital area (10.89%)
- With „V“ pattern- with the V vertex at the forehead (8.91%)
- Occipital pattern- distribution similar at the so-called "physiologic occipital alopecia" of the 8-12 weeks old children (1.98%) (Fig.2.5.b)
- Parietal pattern- loss in the parietal area, bilateral (0.99%)

Figure 2.4: Visual aid for estimating percentage of scalp hair loss, adapted from Olsen and Canfield (2004) (A) and below clinical correlation with normal hair distribution (B)

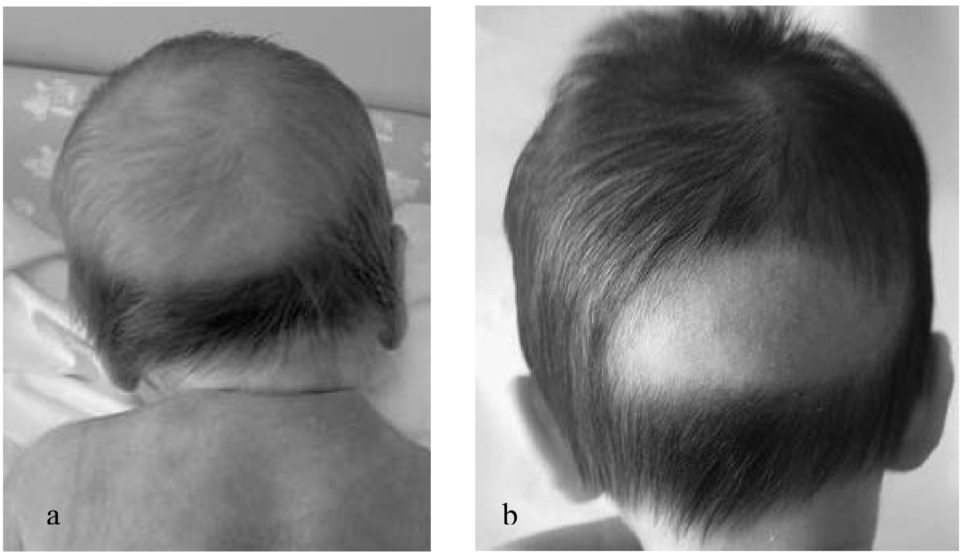
A



B

a-c: scalp of 2-day-old neonates; d: scalp of a 7-day-old neonate

Figure 2.5: Transient neonatal hair loss with a hairless patch with a marked lineal aspect (a), a typical aspect with the oval area of hair loss in a 2-month-old infant (b)



Photos taken from Cutrone and Grimalt. Transient neonatal hair loss: a common transient neonatal dermatosis. Eur J Pediatr 2005; 164, p. 631.

2.7. Biometric- statistical analysis

All data in accordance with scaling and distribution were analysed descriptively and presented graphically with box plots and lineal diagrams.

For comparison of metric, non-normal distributed data between both independent groups ("b" and "w") the Mann-Whitney U-Test was used and between two dependent samples (2d and 7d), the Wilcoxon-Test was applied. For comparison of data between more than two independent groups the Kruskal Wallis-Test was used. For unbalanced distribution or small number of data the accurate methods were applied.

For closer inspection of the temporal courses of both groups ("b" and "w"), analysis of variance with repeated measurements were used, as follows:

		T1	T2		Tp
	proband 1	x111	x112	…	x11p
group 1	…	…	…	…	
…					
	proband n1	x1n11	x1n12	…	x1n1p
	proband 1	x211	x212	…	x21p
group 2	…	…	…	…	
…					
	proband n2	x2n21	x2n22	…	x2n2p

General parallel-design in k groups and p time points T1-Tp.

Because the requirements for the use of analysis of variance for these data are not confirmed, it is a not necessary to use the non-parametric method also.

In non-parametric models, instead of parameter of location (μ_{il}) or differences ($ð_{il}$), the marginal distribution was used to define the effect of bathing.

		marginal distribution			
factor G		T1	T2		Tp
	proband 1	F11	F12	…	F1p
group 1	…	…	…	…	
…					
	proband n1	F11	F12	…	F1p
	proband 1	F21	F22	…	F2p
group 2	…	…	…	…	
…					
	proband n2	F21	F22	…	F2p

Should the aim exist to check the non-parametric analysis for independent factor G (group) and dependent factor F (time), and to observe whether factors G and F have an influence on the changes in features of interest, and whether differences exist in the developing profile between groups, i.e., the effects of group, time and interaction between groups and time points were checked.

For this purpose, three null hypotheses versus corresponding alternative hypotheses were checked, as follows:

H_{0G}: marginal distributions (averaged by time points) do not differ between groups.

H_{0T}: marginal distributions (averaged by groups) do not differ between time points.

H_{0G*T}: there is not interaction between G and T. Differences in marginal distributions from one time point to the next time point do not differ between groups.

The analysis of interaction will be checked, if the changes of observation features between grades of factor G through time response are constant, i.e. when the course of features is parallel between groups.

Because the baseline values could have an influence on the results, they were included in the analysis as covariates.

Relative marginal effects could be used for graphical presentation of the results (lineal diagram).

Relative marginal effects are based on the probability that measured values inside one defined group, at one defined time point, are higher than all other measured values.

It is possible to accept the values between 0 and 1, however, the relative effect of an approximate difference of 0.5 between all measured values is characterised.

A value of <0.5 (>0.5) means that measured values in comparison with all other measured values tend towards smaller (higher) values.

Non-parametric variance analysis was used also for the evaluation of the pH parameter and interactions between this and 2 factors: group and sex.

As a test procedure for non-parametric analysis of variance, we used a method developed by E. Brunner et al. (2001) by using statistical packets SAS V.8.02.

The SPSS 12.0 program was utilized for other analyses.

Because of outliers, the correlations between the parameter (TEWL) and ambient conditions (humidity, temperature) were checked using monotone correlation of Spearman.

For the evaluation of nominal data Chi-Quadrate-Test was used.

p value[8] <0.05 was considered significant. ≥0.05 p value <0.1 was considered as tendency. All analyses result from explorative sense.

Correlation coefficient (r) was considered as follows:

r=0 no correlation

r=0.5 middle correlation

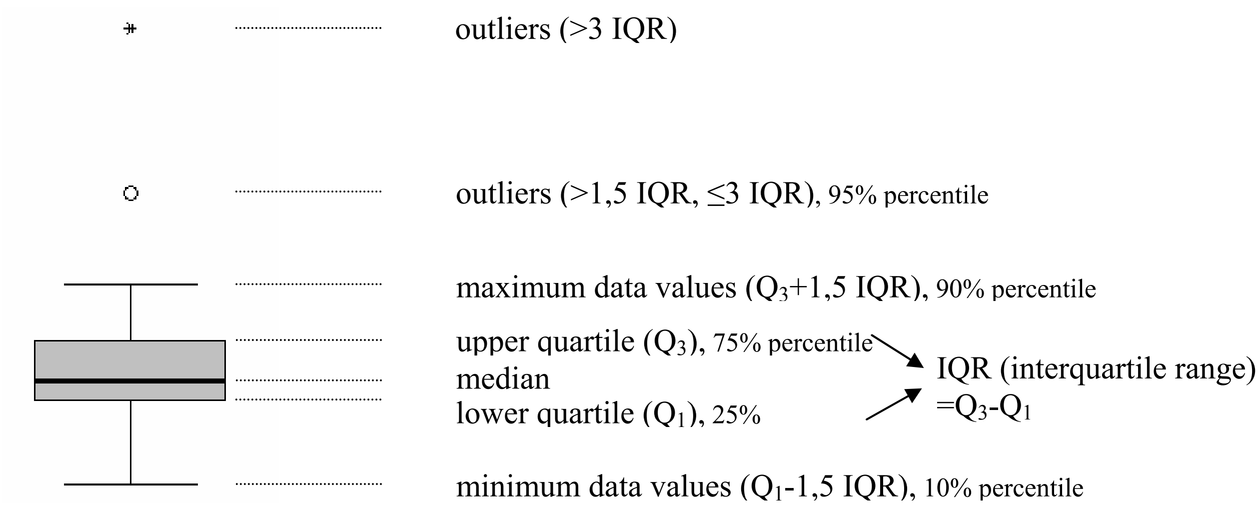
r=1 super/high correlation

By negative correlation coefficient (-r) exists inversely correlation.

By positive correlation coefficient (r) exists positive correlation.

For all figures with boxplots following description was hold (Fig.2.5).

Figure 2.5: Description of boxplot



In statistical analyses all data were included, although some outliers weren't presented on the boxplots.

Median (= 50% percentile)

---

8 In statistical hypothesis testing, the p-value is the probability of obtaining a result at least as "impressive" as that obtained, assuming the truth of the null hypothesis that the finding was the result of chance alone. The fact that p-values are based on this assumption is crucial to their correct interpretation.

In statistics, a median is a number separating the higher half of a sample, a population, or a probability distribution from the lower half. The median of a finite list of numbers can be found by arranging all the observations from the lowest value to the highest value and picking the middle one. If there is an even number of observations, one often takes the mean of the two middle values. At most, half the population has values less than the median and at most half have values greater than the median. If both groups contain less than half the population, then some of the population is exactly equal to the median.

Mean

In statistics, the arithmetic mean (or simply mean) of a list of numbers is the sum of all the members of the list divided by the number of items in the list.

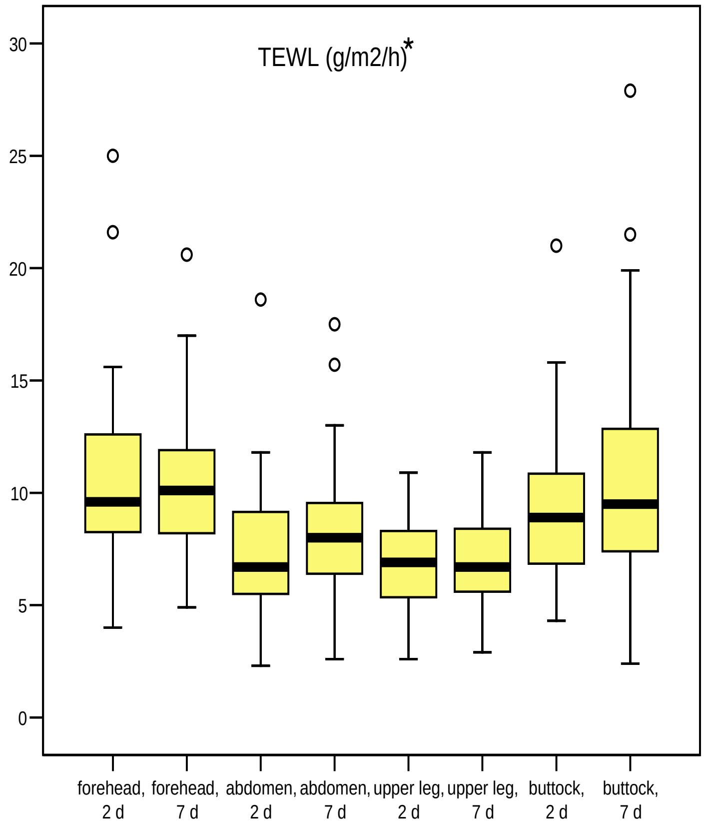
3. *Results*

3.1. Postnatal adaptation of the skin barrier from the 2nd to 7th day of life

The postnatal adaptation of skin barrier was analyzed during the first seven postnatal days for each physiological parameter separately. The differences between two single measurements, days 2nd and 7th, were compared using Wilcoxon Test, for both groups together, since both groups received the same skin care until the 7th day.

<u>TEWL</u>: As shown in Figure 3.1 differences in TEWL were not statistically significant at any anatomic location. TEWL showed a small tendency to increase on the abdomen ($p=0.081$) and buttock ($p=0.066$). Forehead and buttock had higher median values (>9.0 g/m^2/h) than did other examined sites (<8.0 g/m^2/h) (Atch. Tab.3.1).

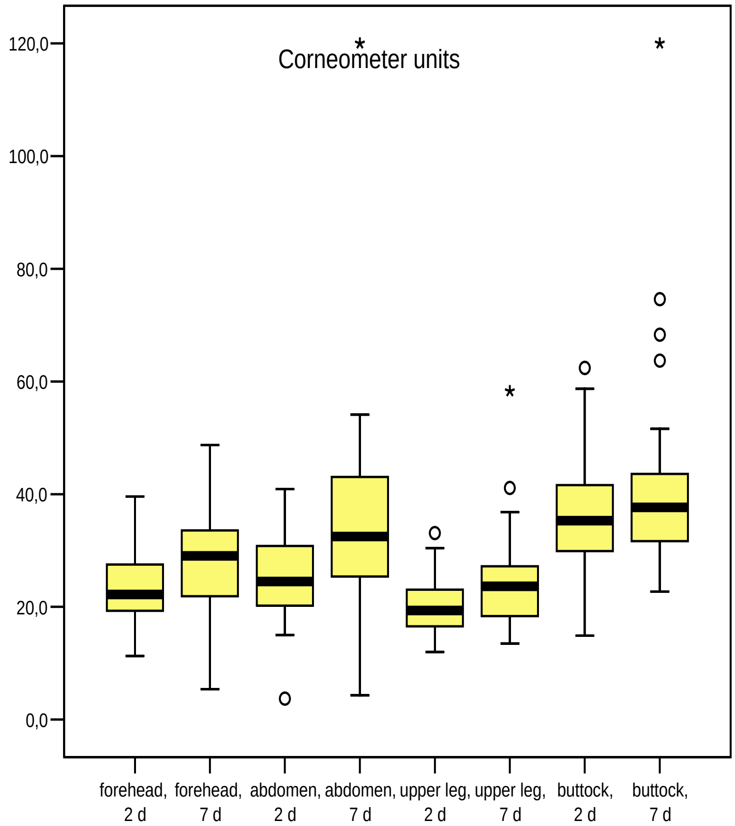
Figure 3.1: Postnatal development of TEWL in healthy full term newborn infants between the 2nd and the 7th day at different anatomic sites



n- number of subjects: n (forehead, buttock 2d/7d)=57/48, n (abdomen 2d/7d)=57/47, n (upper leg 2d/7d)=56/48. No significant differences between 2d and 7d for all body sites. Higher median values on the forehead and buttock (>9.0 g/m^2/h) in comparison to other examined sites (<8.0 g/m^2/h).

SCH: Analysis of SCH revealed a significant increase at most sites ($p<0.001$), except for the buttock (Fig.3.2).

Figure 3.2: Postnatal development of SCH in healthy full term newborn infants between the 2nd and the 7th day at different anatomic sites

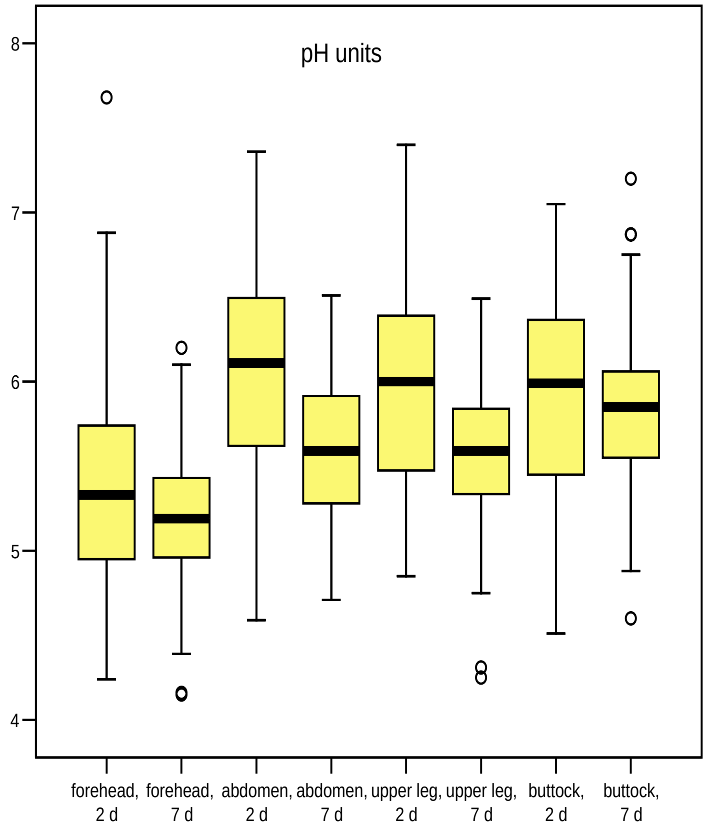


n (forehead, abdomen, buttock 2d/7d)=57/48, n (upper leg 2d/7d)=56/48. **Significant increase** of SCH from 2d to 7d on the forehead, abdomen and upper leg ($p<0.001$).

The biggest increase in skin hydration from the 2nd to 7th day was observed on the abdomen (+8.00 U) followed by the forehead (+6.85 U), the upper leg (+4.30 U) and the buttock (+2.35 U). The highest level of median values of SCH was marked on the buttock (35.30 and 37.65 U), followed by the abdomen (24.50 and 32.50 U) and the lower was found on the forehead (22.20 and 29.05 U), whereas on the upper leg the hydration value was the lowest (19.35 and 23.65 U) (Atch. Tab.3.2).

Skin pH: As shown in Figure 3.3, skin pH decreased significantly at most sites except for the buttock (forehead p=0.029, abdomen and upper leg p<0.001).

Figure 3.3: Postnatal development of skin pH in healthy full term newborn infants between the 2nd and the 7th day at different anatomic sites

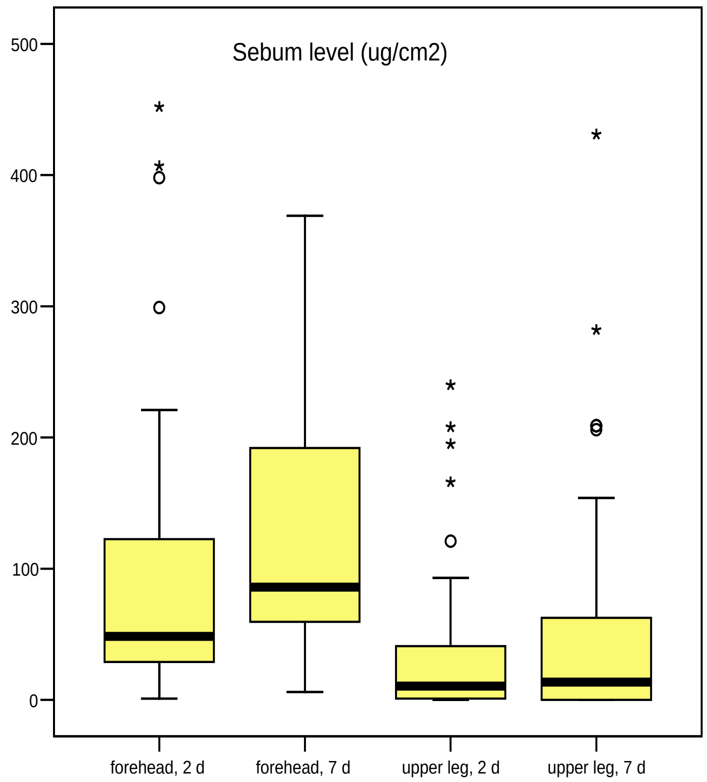


n (forehead, abdomen, upper leg, buttock 2d/7d)=56/47. **Significant decrease** of pH from 2d to 7d on the forehead (p=0.029), abdomen and upper leg (p<0.001).

The strongest decrease in skin pH was observed on the abdomen (-0.52 pH units) and upper leg (-0.40 pH units) followed by the buttock (-0.21 pH units) and the forehead (-0.14 pH units).
The highest level of median values of pH on the 2nd day was on the abdomen (6.11 pH units) and buttock (6.06 pH units), then on the upper leg (5.99 pH units) and on the forehead (5.33 pH units). At the end of the first week the highest level was observed on the buttock (5.85 pH units), then on the abdomen and upper leg (5.59 pH units), whereas on the forehead pH was the lowest (5.19 pH units). Except for the minimal changes between abdomen and buttock, the order of pH for anatomic sites remained remarkably stable from the 2nd to 7th day (Atch. Tab.3.3).

Sebum: As far as sebum level was concerned, there was a significant increase on the forehead (p=0.002); on the forehead the level of sebum was significantly higher than on the upper leg (Fig.3.4, Atch. Tab.3.4).

Figure 3.4: Postnatal development of sebum in healthy full term newborn infants between the 2nd and the 7th day at different anatomic sites



n (forehead, upper leg 2d/7d)=57/48. **Significant increase** of sebum from 2d to 7d on the forehead (p=0.002).

3.2. Effect of bathing on the skin barrier adaptation

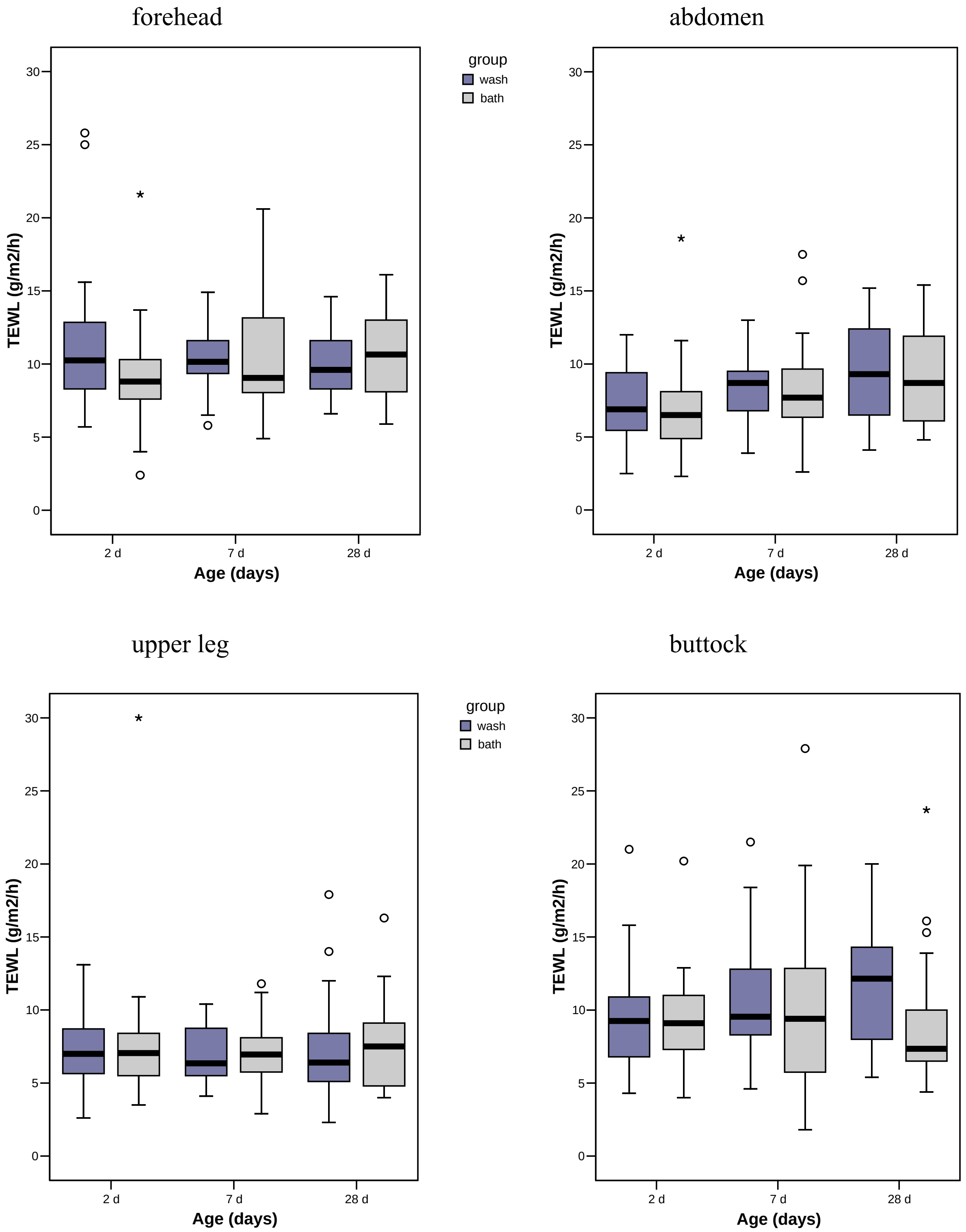
The influence of bathing on the skin barrier adaptation was compared to washing between the two groups ("b" vs. "w") using two different statistical methods: at first as single measurement points before and after three-weekly bathing (7^{th} day vs. 28^{th} day) using the Mann-Whitney U-Test, then as prospective analysis of dynamic changes from the 7^{th} to 28^{th} day using non parametric analysis of variance, eliminating influence of others factors.

Comparison of skin parameters between groups "b" and "w" on the 7^{th} and 28^{th} day

TEWL: As shown in Figure 3.5, the difference between the two groups was statistically significant only on the 28^{th} day on the surface of the buttock (p=0.004). The newborns who were bathed had significant lower median values of TEWL on the buttock (7.35 $g/m^2/h$) than those who were washed (12.15 $g/m^2/h$) (Atch. Tab.3.5).

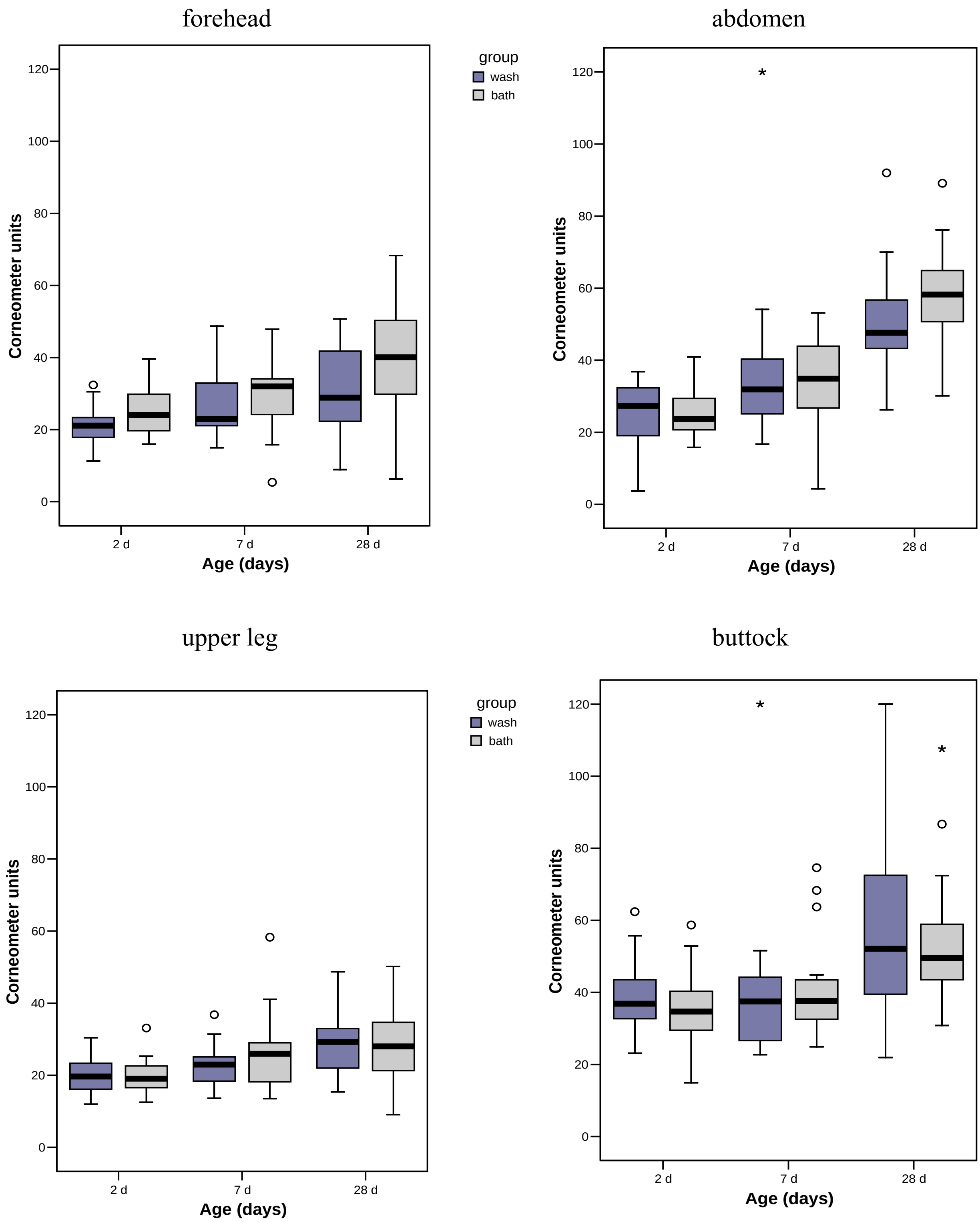
SCH: Significant differences between the two groups were observed on the abdomen (p=0.018) as well as on the forehead (p=0.032). SCH was significantly higher in group "b" on the abdomen (58.25 U) and the forehead (40.10 U) compared to group "w" (47.65 U abdomen; 28.85 U forehead) (Fig.3.6, Atch. Tab.3.6).

Figure 3.5: TEWL: comparison between groups “b” and “w” on the 2nd, 7th and 28th day



Group “b” n (forehead, buttock 2d/7d/28d)=29/24/22, n (abdomen 2d/7d/28d)=29/23/22, n (upper leg 2d/7d/28d)=28/24/22. **Group “w”** n (forehead, abdomen, upper leg and buttock 2d/7d/28d)=28/24/22. **Outliers,** non-presented on the boxplots, but included in statistical analysis: group “b”: forehead, 2d: 33 and 34, buttock, 2d: 32.6, 7d: 42.9 and 72.1. Group “w”: upper leg, 7d: 49.7, buttock, 7d: 42.0, 42.8, 28d: 49.3, 51.4, 57.2. **Significant lower** median value of TEWL in group ”b” on the buttock on the 28d (p=0.004).

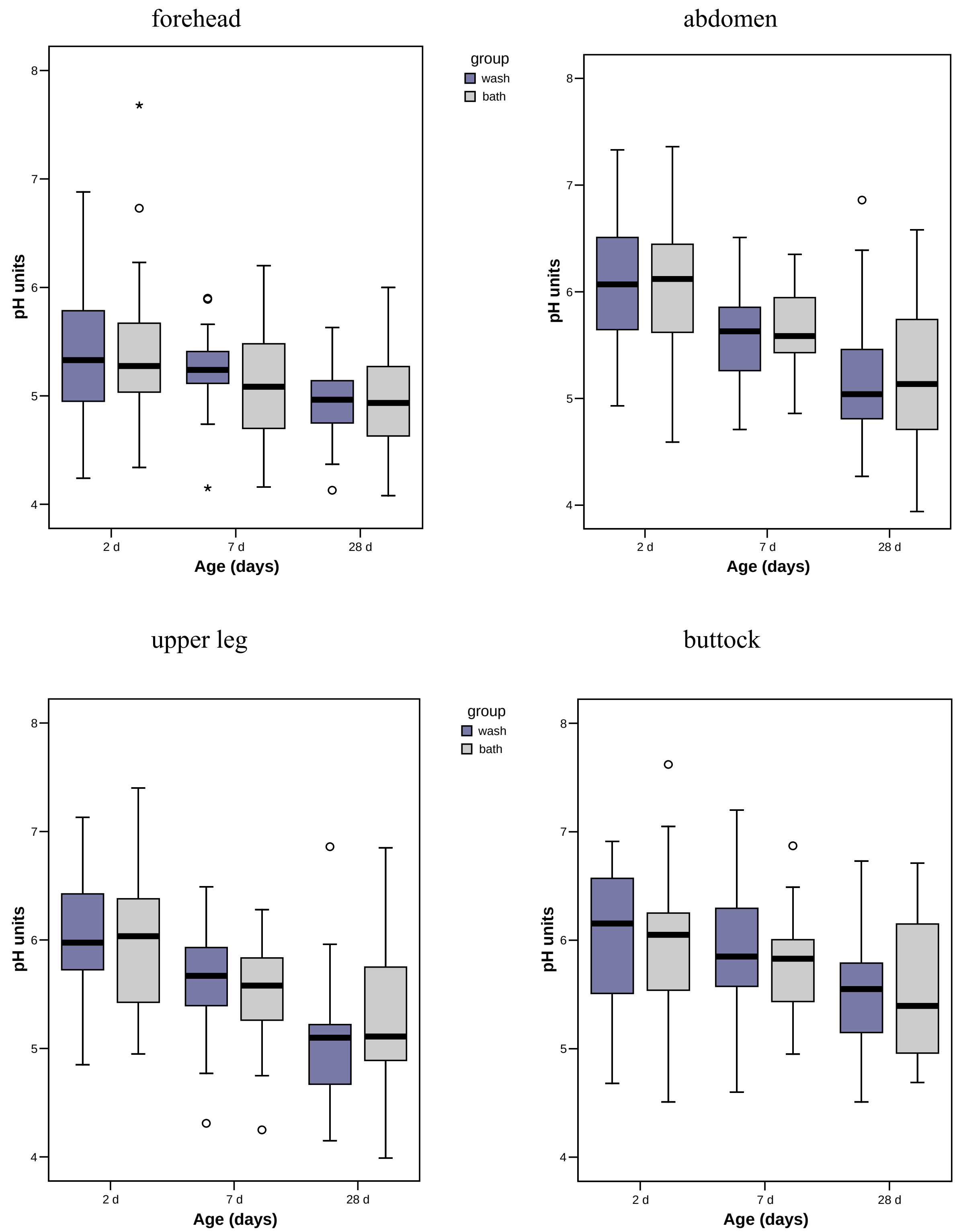
Figure 3.6: SCH: comparison between groups “b” and “w” on the 2nd, 7th and 28th day



Group “b” n (forehead, abdomen, buttock 2d/7d/28d)=29/24/22, n (upper leg 2d/7d/28d)=28/24/22. **Group “w”** n (forehead, abdomen, upper leg and buttock 2d/7d/28d)=28/24/22.
Significant higher median value of SCH in group ”b” on the forehead, 28d ($p=0.032$) and on the abdomen, 28d ($p=0.018$).

<u>Skin pH:</u> No significant differences were observed between the two groups at any of the four anatomic sites (Fig.3.7, Atch. Tab.3.7).

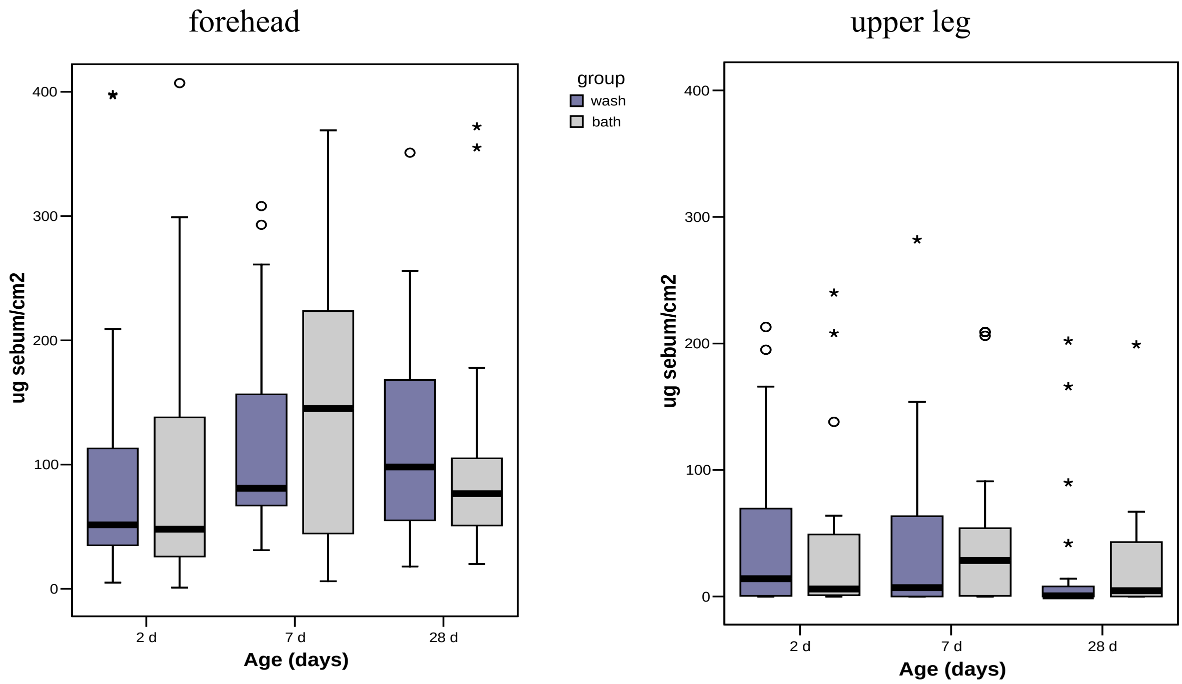
Figure 3.7: Skin pH: comparison between groups “b” and “w” on the 2nd, 7th and 28th day



Group “b” n (forehead, abdomen, upper leg and buttock 2d/7d/28d)=28/24/22. **Group “w”** n (forehead, abdomen, upper leg and buttock 2d/7d/28d)=28/23/22. No significant differences between the two groups at any anatomic site.

Sebum level: No significant differences between the two groups were noticed at any of the two anatomic sites (Fig.3.8, Atch. Tab.3.8).

Figure 3.8: Sebum level: comparison between groups “b” and “w” on the 2nd, 7th and 28th day



Group “b” n (forehead and upper leg 2d/7d/28d)=29/24/22. **Group “w”** n (forehead and upper leg 2d/7d/28d)=28/24/22. **Outlier**, non-presented on the boxplots, but included in statistical analysis: group “b”: forehead, 2d: 452.0. No significant differences between the two groups at any anatomic site.

Prospective analysis of dynamic changes in skin parameters of groups “b” and “w” from the 7th to 28th day with exclusion of the influence of measurements on the 2nd and 7th day

TEWL: Significant lower median value of TEWL was observed on the buttock ($p=0.001$) in group “b”.

SCH: Significant higher median values were noticed on the forehead ($p=0.022$) and abdomen ($p=0.006$) in group “b”.

For skin pH as well as for sebum value no significant differences were found between the two groups at any anatomic site.

Results of analysis of variance confirmed previous results of the Mann-Whitney U-Test.

The differences in TEWL and SCH between the two groups result from the influence of bathing on skin barrier. Others factors, which could falsify the results, were excluded.

3.3. Postnatal adaptation of the skin barrier from the 7th to 28th day analyzed in groups “b” and “w” separately

The measurements performed in the first week of life showed a significant increase of SCH and sebum as well as significant decrease of skin surface pH (chapter 3.1). These changes suggest that the adaptation process of the skin barrier takes place in healthy full-term newborns in extrauterine environment.

The aim of this chapter was to check if further development of the skin barrier from the 7th to 28th day takes place and if bathing or washing can influence this adaptation process.

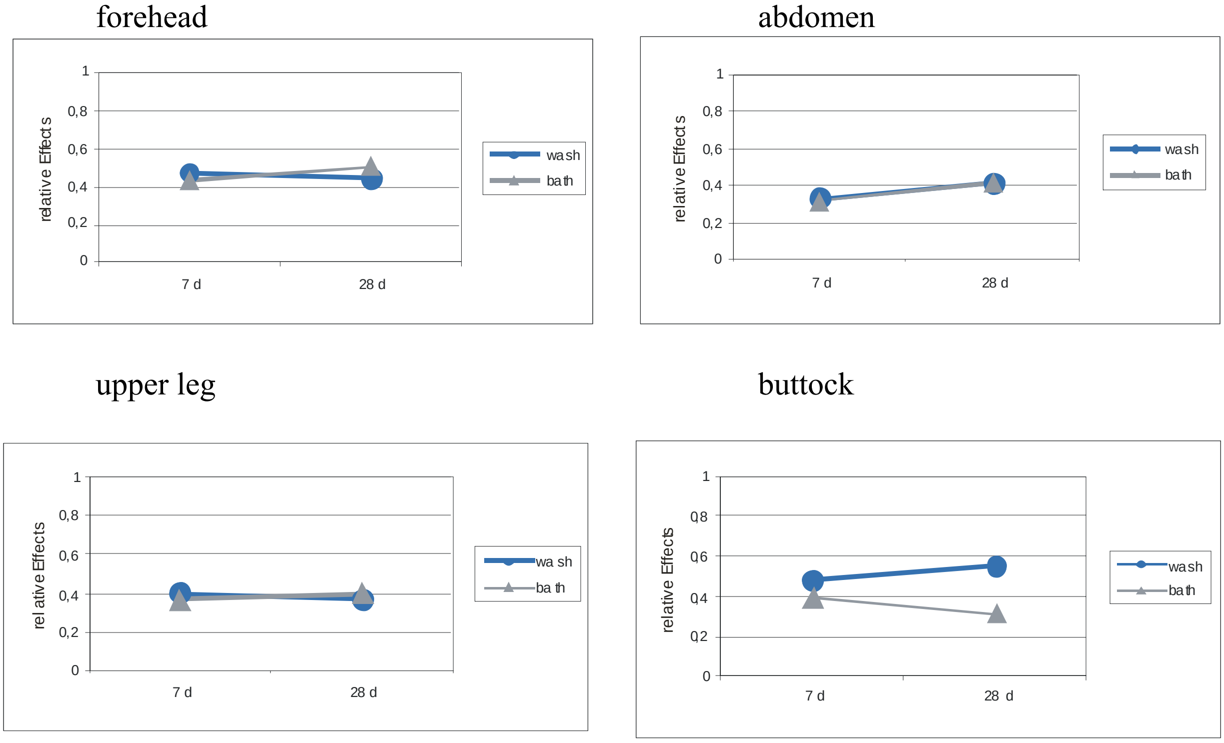
A prospective analysis of dynamic changes from the 7th to 28th day in each group and the effect of bathing on the skin barrier compared to washing was checked simultaneously using a non parametric analysis of variance. Using this non parametric analysis, influences of other factors, such as measurement on the 2nd day, were excluded.

The results were evaluated for each parameter according to the following scheme:

- adaptation process in each group with regards to changes from the 7th to 28th day
- comparison of adaptation process in group “b” to adaptation process in group “w” regarding the influence of bathing on the adaptation process

TEWL: As shown in Figure 3.9 the changes evaluated in each group were not significant, but the difference between the two groups was significant on the buttock (p=0.01). In group “w” in the gluteal region an increase of TEWL and in group “b” a decrease of TEWL were observed.

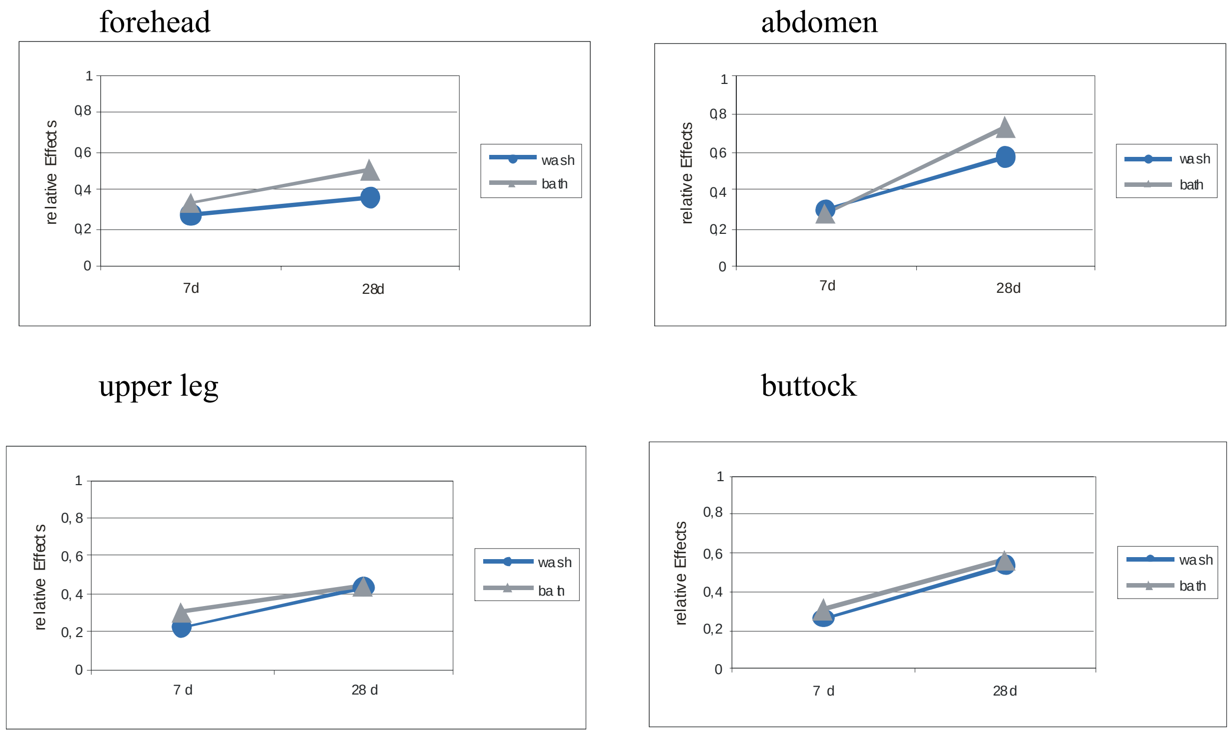
Figure 3.9: Prospective analysis of dynamic changes of TEWL from the 7th to 28th day at different anatomic sites in groups “b” and “w” analyzed separately



n (group “b”)=22, n (group ”w”)=22. No significant differences from 7d to 28d in either group at any site. **Significant difference** between the two groups on the buttock (p=0.01): higher values of TEWL in group “w” than in “b”. Increase of TEWL in gluteal region in group “w” and decrease in group “b”.

SCH: The significant changes between the 7th and the 28th day were as follows (Fig.3.10): in group “b” significant increase of SCH was noted on the forehead (p=0.019), abdomen and buttock (p<0.001), and in group “w” on the abdomen, upper leg and buttock (p<0.001). SCH values increased significantly on the abdomen in group “b” than in group “w” (p=0.026). Tendency to higher values of SCH on the forehead was observed in group “b” in comparison to group “w” (p=0.056).

Figure 3.10: Prospective analysis of dynamic changes of SCH from the 7th to 28th day at different anatomic sites in groups “b” and “w” analyzed separately



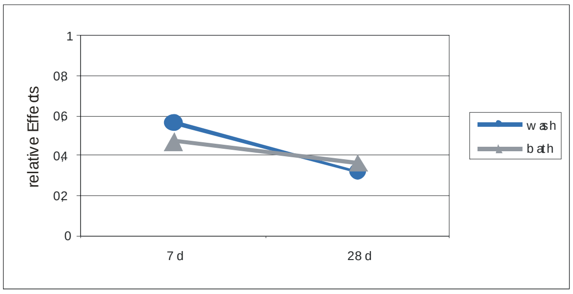
n (group “b”)=22, n (group ”w”)=22. **Group “b”:** significant increase of SCH on the forehead (p=0.019), abdomen and buttock (p<0.001). **Group “w”:** significant increase of SCH on the abdomen, upper leg and buttock (p<0.001). SCH values increased significantly on the abdomen in group “b” than in group “w” (p=0.026). Tendency to higher values of SCH on the forehead in group “b” in comparison to group “w” (p=0.056).

Skin pH: In group “w” significant decrease of skin surface pH was observed at all anatomic sites: on the abdomen (p=0.013), buttock (p=0.007), upper leg and forehead (p<0.001), whilst in group “b” on the abdomen (p=0.011) and upper leg only (p=0.025) (Fig.3.11).

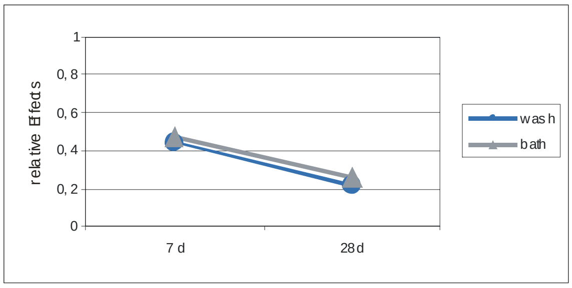
No significant differences in the adaptation process between the two groups were noted at any anatomic site.

Figure 3.11: Prospective analysis of dynamic changes of pH from the 7th to 28th day at different anatomic sites in groups “b” and “w” analyzed separately

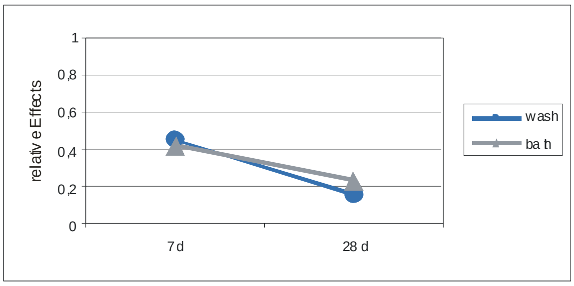
forehead



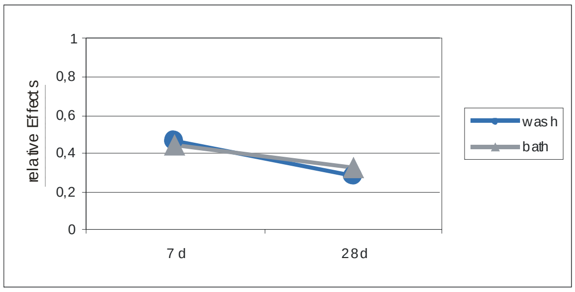
abdomen



upper leg



buttock



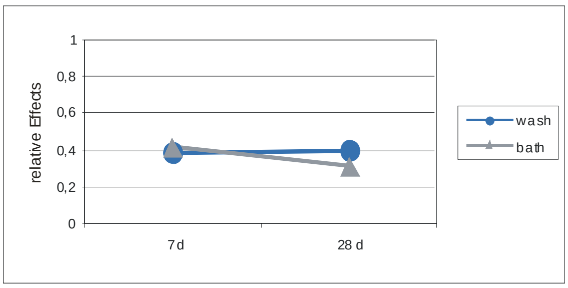
n (group “b”)=22, n (group ”w”)=22. **Group “b”:** significant decrease of pH on the abdomen (p=0.011) and on the upper leg (p=0.025). **Group “w”:** significant decrease of pH on the abdomen (p=0.013), buttock (p=0.007), upper leg and forehead (p<0.001). No significant differences in adaptation process between the two groups at any anatomic site.

Sebum:

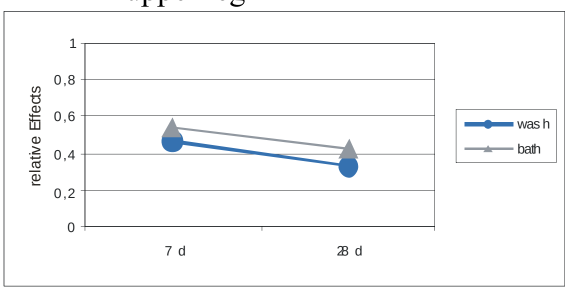
As shown in Fig.3.12 the changes evaluated in each group as well as the differences between the two groups were not significant at any anatomic site.

Figure 3.12: Prospective analysis of dynamic changes of sebum from the 7th to 28th day at different anatomic sites in groups “b” and “w” analyzed separately

forehead



upper leg



n (group “b”)=22, n (group ”w”)=22. No significant differences from 7d to 28d in either group at any site.
No significant differences in adaptation process between the two groups at any anatomic site.

3.4. Comparison of skin physiological parameters between male and female infants

Numbers of male and female newborns included in the study were 32 and 25, respectively. Differences between genders were compared on the 2nd, 7th and 28th day for each parameter separately using the Mann-Whitney U-Test and a prospective analysis of dynamic changes of skin surface pH from the 7th to 28th day was made using a non parametric analysis of variance. Using non parametric analysis, influences of other factors, such as measurement on the 2nd day, were excluded.

TEWL: Significant difference between male and female infants was observed only on the 2nd day on the buttock ($p=0.044$). Females had significantly higher median values (10.6 $g/m^2/h$) than males (8.45 $g/m^2/h$) (Tab.3.9).

Table 3.9: Values of TEWL (mean, median, minimum, maximum) measured on the 2nd, 7th and 28th day for girls and boys

TEWL ($g/m^2/h$) \ Location	Gender	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead, 2d	girl	25	10.09	2.46	7.00	16.00	8.25	9.90	12.55
	boy	32	11.89	7.63	2.00	34.00	7.65	9.25	13.02
forehead, 7d	girl	22	9.62	2.14	5.60	14.90	8.25	9.35	11.03
	boy	26	10.87	3.82	4.90	20.60	7.73	11.00	12.63
forehead, 28d	girl	20	10.33	2.29	6.00	14.60	8.50	9.95	12.00
	boy	24	10.40	2.81	5.90	16.10	7.93	10.35	12.93
abdomen, 2d	girl	25	7.18	2.41	2.50	11.80	5.20	7.10	9.15
	boy	32	7.15	3.19	2.30	18.60	5.23	6.50	8,60
abdomen, 7d	girl	21	8.46	1.85	4.90	11.20	7.25	8.60	10.05
	boy	26	8.18	3.39	2.60	17.50	6.00	7.50	9.53
abdomen, 28d	girl	20	9.69	3.32	4.80	15.40	6.43	9.60	12.48
	boy	24	8.92	3.11	4.10	13.40	6.43	8.65	11.70
upper leg, 2d	girl	25	8.00	4.98	3.00	30.00	5.50	7.20	8.65
	boy	31	7.11	2.43	2.60	13.10	5.20	6.90	8.60
upper leg, 7d	girl	22	6.88	1.87	3.50	10.40	5.90	6.65	8.63
	boy	26	8.45	8.71	2.90	49.70	5.33	6.75	8.35
upper leg, 28d	girl	20	6.81	2.35	4.00	12.00	4.43	6.75	8.40
	boy	24	8.00	3.89	2.30	17.90	5.23	6.75	9.65
buttock, 2d	girl	25	10.35	3.75	4.50	21.00	7.80	**10.60***	11.30
	boy	32	9.10	5.03	4.00	32.60	6.15	**8.45***	10.65
buttock, 7d	girl	22	16.34	16.50	1.80	72.10	7.43	9.70	18.77
	boy	26	10.75	7.78	2.40	42.90	6.43	9.40	12.25
buttock, 28d	girl	20	13.69	13.08	4.40	51.40	6.75	9.25	14,23
	boy	24	12.33	10.56	5.40	57.20	7.30	9.40	13.78

* significant difference between males and females ($p=0.044$)

SCH: Significant difference was observed on the buttock only on the 28th day (p=0.024). Females had a higher median value (56.05 U) than males (44.05 U), but with tendency to lower hydration on the forehead (p=0.056) and abdomen (p=0.097) (Tab.3.10).

Table 3.10: Values of SCH (mean, median, minimum, maximum) measured on the 2nd, 7th and 28th day for girls and boys

Location / SCH (corneometer units)	Gender	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead, 2d	girl	25	22.50	4.76	14.60	32.40	19.30	22.20	25.85
	boy	32	23.68	6.69	11.30	39.60	18.92	22.00	29.53
forehead, 7d	girl	22	27.80	8.69	15.00	48.70	20.38	29.10	33.00
	boy	26	28.55	9.44	5.40	47.90	22.35	28.70	35.98
forehead, 28d	girl	20	30.20	13.86	6.30	54.00	17.08	29.15	40.98
	boy	24	39.14	14.64	9.50	68.30	28.35	39.05	49.78
abdomen, 2d	girl	25	25.81	7.89	3.70	37.40	21.05	24.50	32.50
	boy	32	25.17	7.09	15.00	40.90	18.83	25.00	30.68
abdomen, 7d	girl	22	32.49	7.25	19.30	46.60	27.20	31.25	37.58
	boy	26	37.27	22.08	4.30	120.00	22.10	35.05	48.10
abdomen, 28d	girl	20	51.78	11.31	34.90	76.20	43.40	48.65	56.93
	boy	24	57.73	15.91	26.20	92.00	48.35	58.25	64.75
upper leg, 2d	girl	24	19.72	4.74	13.50	30.40	15.75	18.65	23.48
	boy	32	19.76	4.66	12.00	33.10	16.43	20.10	22.75
upper leg, 7d	girl	22	23.26	7,12	13.60	41.10	17.30	22.30	26.88
	boy	26	24.88	8.86	13.50	58.30	19.53	24.15	29.15
upper leg, 28d	girl	20	29.33	10.34	15.40	50.20	20.58	29.80	35.85
	boy	24	28.73	8.03	9.10	42.50	23.58	28.60	34.25
buttock, 2d	girl	25	39.58	11.66	23.10	62.40	28.60	37.50	48.70
	boy	32	34,86	8.10	14.90	52.70	31.90	34.45	40.30
buttock, 7d	girl	22	40.60	12.19	22.70	74.60	31.80	41.05	45,38
	boy	26	39.32	18.98	23.40	120.00	29.88	3.,40	43.43
buttock, 28d	girl	20	61.12	19.45	38.50	107.60	46,80	**56.05***	72.47
	boy	24	50.61	21.79	21.90	120.00	35.28	**44.05***	59.25

* significant difference between males and females (p=0.024)

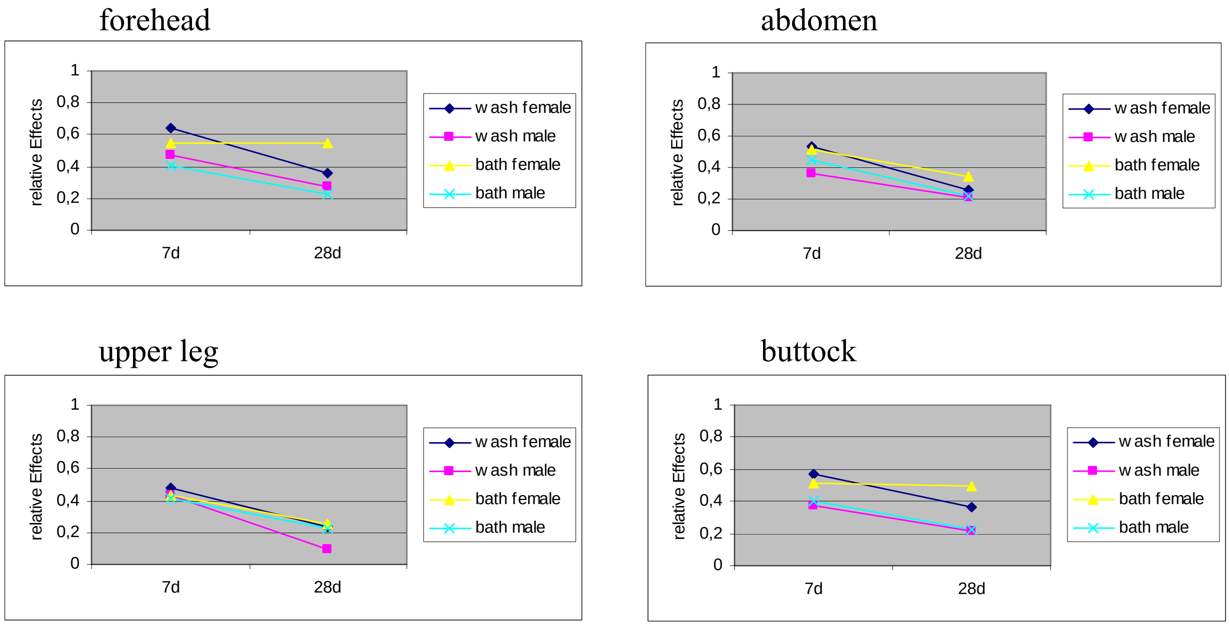
Skin pH: Significantly higher median values were noted in females than males on the 7th day on the forehead (p=0.040, 5.24 vs. 5.04) and abdomen (p=0.023, 5.69 vs. 5.49) as well as on the 28th day on the forehead (p=0.021, 5.05 vs. 4.91) and buttock (p=0.016, 5.74 vs. 5.22).
Differences in dynamic changes of skin surface pH from the 7th to 28th day were observed in each group on the forehead and buttock (p=0.002). Male infants had significantly lower pH than female and pH decreased significantly in male than in female infants (Fig.3.13, Tab.3.11).

Table 3.11: Values of skin pH (mean, median, minimum, maximum) measured on the 2nd, 7th and 28th day for girls and boys

Location / Skin surface pH (pH units)	Gender	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead, 2d	girl	24	5.40	.61	4.24	6.88	5.00	5.27	5.75
	boy	32	5.38	.70	4.31	7.68	4.94	5.33	5.75
forehead, 7d	girl	21	5.31	.41	4.16	6.20	5.17	**5.24***	5.54
	boy	26	5.08	.44	4.15	6.10	4.73	**5.04***	5.37
forehead, 28d	girl	20	5.14	.43	4.46	6,00	4.78	**5.05***	5.39
	boy	24	4.80	.38	4.08	5.63	4.48	**4.91***	5,11
abdomen, 2d	girl	24	6.07	.67	4.59	7.33	5.61	6.13	6.52
	boy	32	6.03	.56	4.94	7.36	5.65	6.08	6.49
abdomen, 7d	girl	21	5.74	.39	4.86	6.51	5.52	**5.69***	6.04
	boy	26	5.47	.41	4.71	6.35	5.15	**5.49***	5.82
abdomen, 28d	girl	20	5.36	.76	4.12	6.86	4.81	5.08	6.13
	boy	24	5.10	.59	3.94	6.48	4.64	5.09	5.28
upper leg, 2d	girl	24	6.03	.62	4.85	7.13	5.52	6.05	6.51
	boy	32	5.97	.59	4.95	7.40	5.47	5.98	6.29
upper leg, 7d	girl	21	5.66	.54	4.31	6.49	5.37	5.63	6.07
	boy	26	5.48	.44	4.25	6.07	5.28	5.54	5.82
upper leg, 28d	girl	20	5.32	.69	4.18	6.86	4.92	5.18	5.80
	boy	24	5.01	.54	3.99	5.99	4.58	5.04	5.34
buttock, 2d	girl	24	6.02	.60	4.68	7.04	5.48	6.14	6.52
	boy	32	6.03	.65	4.51	7.62	5.53	6.02	6.32
buttock, 7d	girl	21	5.99	.42	5.29	7.20	5.67	5.95	6.25
	boy	26	5.71	.60	4.60	6.87	5.26	5.65	6.06
buttock, 28d	girl	20	5.76	.56	4.69	6.73	5,36	**5.74***	6.14
	boy	24	5.33	.52	4.51	6.64	4.94	**5.22***	5.71

* significant differences between males and females: 7d on the forehead (p=0.040) and abdomen (p=0.023), 28d on the forehead (p=0.021) and buttock (p=0.016)

Fig. 3.13: Dynamic changes of skin pH from the 7th to 28th day in relation to sex and skin care regime ("b" vs. "w")



n ("b" females)=9, n ("w" females)=11, n ("b" males)=13, n ("w" males)=11. **Significantly** higher median values of pH in females on the forehead and buttock ($p=0.002$)

Sebum: Significant difference between genders was noted only on the forehead ($p=0.025$) on the 2nd day. Female infants had lower median values (38.0 µg/cm^2) than male infants (67.0 µg/cm^2) (Tab 3.12).

Table 3.12: Values of sebum (mean, median, minimum, maximum) measured on the 2nd, 7th and 28th day for girls and boy

Location / Sebum level (µg/cm^2)	Gender	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	girl	25	67.72	90.04	1.00	452.00	21.00	**38.00***	77.00
	boy	32	120.69	119.22	5.00	407.00	36.25	**67.00***	179.75
forehead, 7d	girl	22	137.00	107.93	6.00	365.00	59.25	104.00	206.25
	boy	26	129.27	95.05	31.00	369.00	58.75	83.00	190.75
forehead, 28d	girl	20	123.50	83.27	20.00	355.00	57.50	98.50	165.75
	boy	24	108.79	98.28	18.00	372.00	38.00	76.50	160.75
upper leg, 2d	girl	25	47.36	61.32	.00	240.00	1.00	28.00	59.00
	boy	32	34.63	63.90	.00	213.00	.00	3.00	29.75
upper leg, 7d	girl	22	56.77	82.47	.00	282.00	1.00	13.50	91.50
	boy	26	49.27	92.89	.00	431.00	.00	15.00	50.00
upper leg, 28d	girl	20	31.40	62.87	.00	202.00	.00	.00	44.00
	boy	24	21.00	37.39	.00	166.00	.00	2.50	35.00

* significant difference between males and females ($p=0.025$)

3.5. Comparison of skin physiological parameters between Caucasian and non-Caucasian infants

In this study 50 Caucasian infants, whose both parents were Caucasians, and 7 non-Caucasian infants, at least one of whose parents came from Latin America, Asia or North Africa, were included.

Differences in TEWL, SCH, pH and sebum level were compared on the 2nd day using the Mann-Whitney U-Test. Significant differences were observed for TEWL on the upper leg (p=0.040) and for pH on the forehead (p=0.002). Caucasian infants had significantly higher median values of TEWL on the upper leg (7.5 $g/m^2/h$) than non-Caucasian infants (6.2 $g/m^2/h$) and significantly lower pH values on the forehead (5.25 pH units) compared with non-Caucasian (5.81 pH units) (Tab.3.13 a,b,c,d).

Table 3.13: Comparison of skin parameters between Caucasian and non-Caucasian infants at different anatomic sites

a: Values of TEWL (mean, median, minimum, maximum)

Location / TEWL ($g/m^2/h$)	Ethnicity	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead	Caucasian	50	11.56	6.19	2.00	34.00	8.23	9.75	13.05
	non-Caucasian	7	7.80	2.21	4.00	11.00	5.90	8.20	9.10
abdomen	Caucasian	50	7.10	2.93	2.30	18.60	5.15	6.60	8.80
	non-Caucasian	7	7.59	2.31	4.20	10.10	5.50	7.90	10.00
upper leg	Caucasian	49	7.76	3.95	2.60	30.00	5.55	**7.50***	8.75
	non-Caucasian	7	5.73	1.17	4.10	7.20	4.60	**6.20***	6.80
buttock	Caucasian	50	9.29	3.43	4.00	21.00	6.98	9.00	11.00
	non-Caucasian	7	12.21	9.25	4.30	32.60	8.70	9.50	10.90

* significant difference between Caucasian and non-Caucasian infants (p=0.040)

b: Values of SCH (mean, median, minimum, maximum)

Location / SCH (corneometer units)	Ethnicity	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead	Caucasian	50	23.45	5.82	14.60	39.60	19.23	22.20	27.75
	non-Caucasian	7	21.17	6.58	11.30	31.60	16.30	21.00	26,10
abdomen	Caucasian	50	25.43	7.44	3.70	40.90	20.10	24.35	31.15
	non-Caucasian	7	25.64	7.59	15.00	35.90	18.60	26.10	32.50
upper leg	Caucasian	49	19.94	4.51	12.00	33.10	17.20	19.60	23.05
	non-Caucasian	7	18.37	5.76	12.50	27.00	13.20	15.20	24.30
buttock	Caucasian	50	36.65	10.05	14.90	62.40	29.35	35.25	42.33
	non-Caucasian	7	38.91	10.18	26.70	55.70	32.50	36.50	49.80

c: Values of skin pH (mean, median, minimum, maximum)

Location / Skin surface pH (pH units)	Ethnicity	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead	Caucasian	49	5.32	.66	4.24	7.68	4.93	**5.25***	5.57
	non-Caucasian	7	5.91	.36	5.40	6.44	5.70	**5.81***	6.28
abdomen	Caucasian	49	6.02	.63	4.59	7.36	5.60	6.11	6.49
	non-Caucasian	7	6.23	.38	5.69	6.63	5.94	6.19	6.62
upper leg	Caucasian	49	5.97	.63	4.85	7.40	5.45	5.97	6.46
	non-Caucasian	7	6.14	.24	5.88	6.52	5.95	6.03	6.33
buttock	Caucasian	49	5.98	.64	4.51	7.62	5.45	6.05	6.37
	non-Caucasian	7	6.35	.46	5.60	6.83	5.98	6.33	6.82

* significant difference between Caucasian and non-Caucasian infants (p=0.002)

d: Values of sebum level (mean, median, minimum, maximum)

Location / Sebum level (µg/cm^2)	Ethnicity	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead	Caucasian	50	101.66	115.91	1.00	452.00	27.00	48.50	129.00
	non-Caucasian	7	67.43	38.82	24.00	143.00	40.00	61.00	83.00
upper leg	Caucasian	50	34.86	57.32	.00	240.00	.00	7.50	50.25
	non-Caucasian	7	78.43	87.98	.00	208.00	13.00	28.00	195.00

3.6. Correlation between skin physiological parameters and week of gestation

29 infants were born from 37 to 40 weeks of gestation and 28 infants were born after full 40 to 42 weeks of gestation. 55 of newborns were AGA, with a birth weight between 10th and 90th percentile (eutrophic newborns) and 2 were LGA, with birth weight above 90th percentile (hypertrophic newborns). No significant correlation was found between newborns born before and after 40 week of gestation (Tab 3.14 a,b,c,d) or between AGA and LGA.

Table 3.14: Correlation between week of gestation and skin parameters (2d)

a: TEWL

	forehead	abdomen	upper leg	buttock
Coefficient of correlation	0.00	-0.122	-0.183	0.138
p-value	1.00	0.365	0.177	0.306
n	57	57	56	57

b: SCH

	forehead	abdomen	upper leg	buttock
Coefficient of correlation	0.132	-0.169	-0.248	-0.94
p-value	0.328	0.209	0.65	0.89
n	57	57	56	57

c: Skin pH

	forehead	abdomen	upper leg	buttock
Coefficient of correlation	0.119	0.940	0.194	0.110
p-value	0.381	0.489	0.152	0.421
n	56	56	56	56

d: Sebum level

	forehead	upper leg
Coefficient of correlation	-0.320	0.970
p-value	0.811	0.472
n	57	57

3.7. Skin physiological parameters in relation to mode of birth

Delivery was vaginal in 41, by caesarean section in 12, and by forceps or vacuum extraction in 4 infants. No significant influence of the mode of birth was observed on any skin physiological parameter using Kruskal Wallis Test (2d).

3.8. Correlation between TEWL and room conditions

In the course of this study the measurements were performed in three different room conditions for each investigation. From 1st to 3rd measurement RH increased and RT decreased. The changes of environment conditions are presented in Table 3.15.

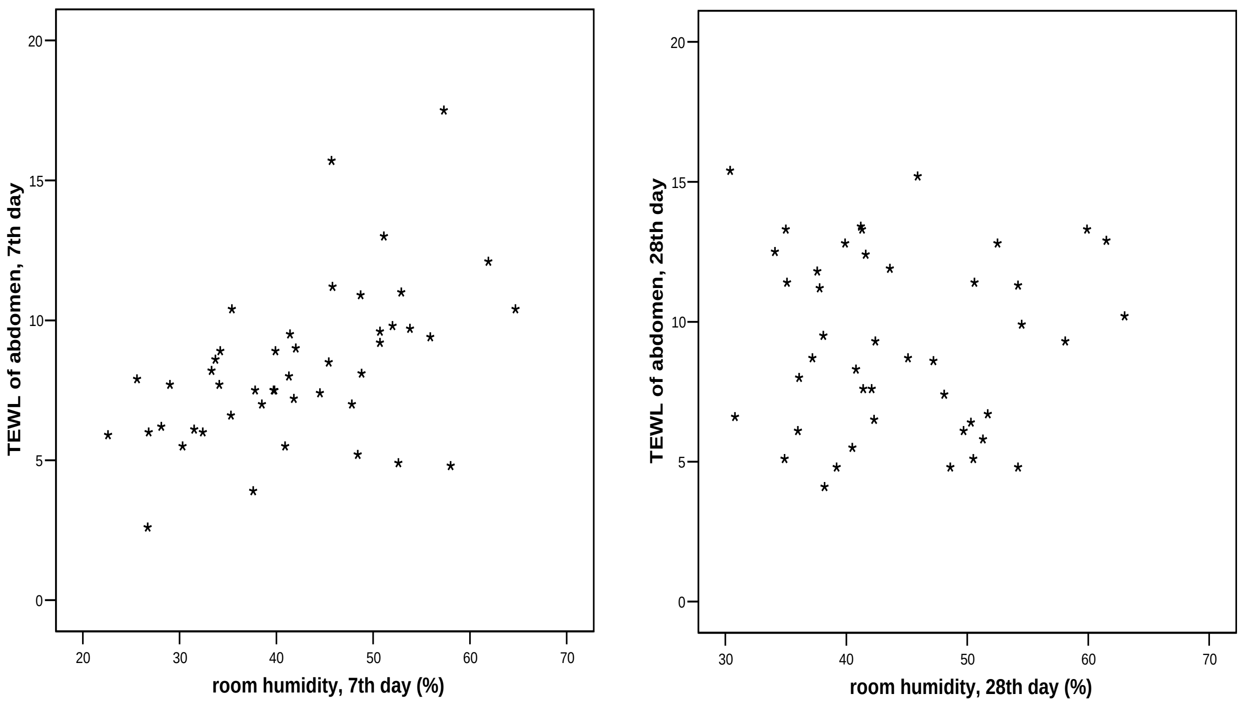
Table 3.15: Median values of ambient humidity and temperature measured on the 2nd, 7th and 28th day

Measurement points (day)	2d	7d	28d
RH, Median (25%; 75%), %	29.7 (23.8; 36.3)	41.3 (33.8; 50.7)	42.2 (37.8; 50.5)
RT, Median (25%; 75%), °C	27.4 (26.0; 29.9)	24.7 (23.0; 26.1)	22.8 (21.9; 24.6)

These changes of room conditions have resulted from using the warming lamp during the measurements on the 2nd day. The measurements on the 7th and 28th day were performed usually without using the warming lamp any more. During each investigation ambient conditions (RH and RT) were monitored using a special external sensor.

Ambient humidity: Positive correlation by significant p-values between TEWL and RH was found on the 2nd day on the abdomen (r=0.421) and on the 7th day on the abdomen (r=0.519) as well as on the upper leg (r=0.309). On the 28th day on the abdomen and upper leg as well as on the 2nd day on the upper leg p-values weren't significant (Fig.3.14, Tab 3.16).

Figure 3.14: Correlation between TEWL and RH on the abdomen on the 7th and 28th day



Positive correlation (r=0.519) between TEWL and room humidity measured on 7d (p<0.001). Higher RH correlated with higher values of TEWL. No correlation between TEWL and RH was measured on 28d.

Table 3.16: Correlation between ambient humidity and TEWL measured on the 2nd, 7th and 28th day

2d	abdomen	upper leg
Coefficient of correlation	**0.421***	0.198
p-value	0.001	0.147
n	56	55

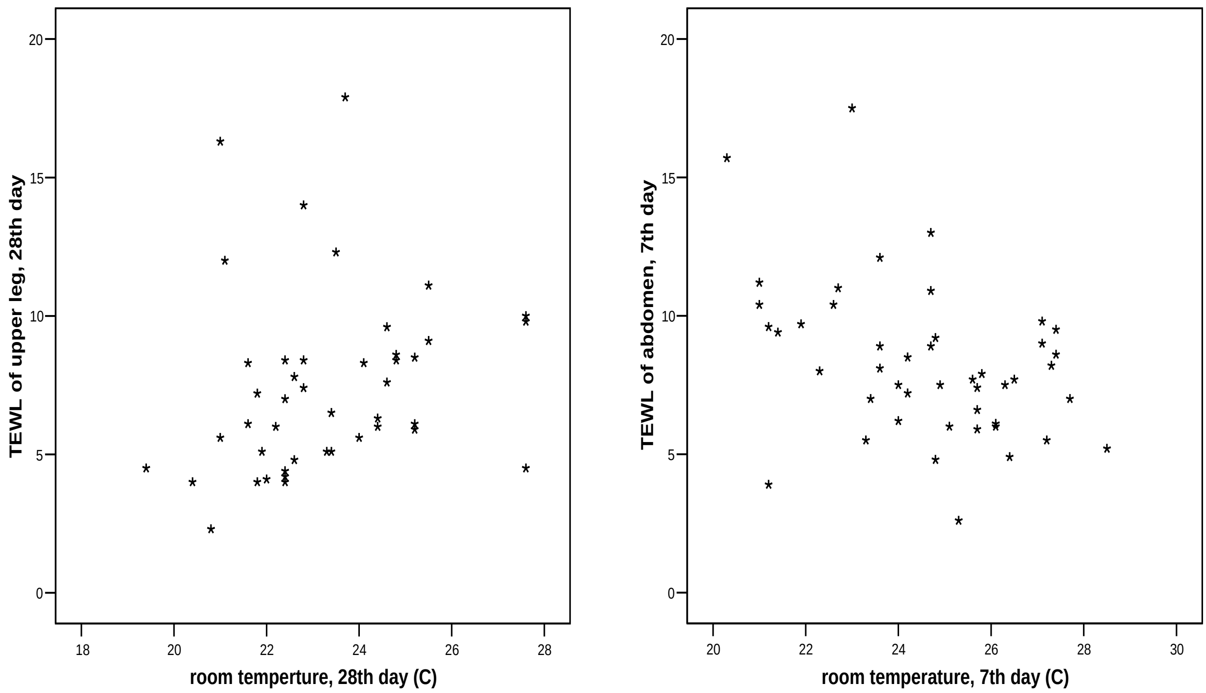
7d	abdomen	upper leg
Coefficient of correlation	**0.519***	**0.309***
p-value	0.000	0.035
n	46	47

28d	abdomen	upper leg
Coefficient of correlation	-0.028	-0.184
p-value	0.859	0.231
n	44	44

*positive correlation by significant p-values

Room temperature: Positive correlation by significant p-values between TEWL and RT was observed on the 2nd day on the abdomen (r=0.337) and on the 28th day on the upper leg (r=0.400). The negative correlation was observed on the 7th day on the abdomen (r=-0.408). P values weren't significant on the upper leg on the 2nd and 7th day and on the abdomen on the 28th day (Fig.3.15, Tab 3.17).

Figure 3.15: Correlation between TEWL and RT on the upper leg on the 28th day and on the abdomen on the 7th day



Positive correlation (r=0.400) between TEWL measured on the upper leg on 28d and RT (p=0.007). The higher the RT, the higher the value of TEWL. Negative correlation (r=-0.408) between TEWL measured on the abdomen on 7d and RT (p=0.005). The higher the RT, the lower the value of TEWL.

Table 3.17: Correlation between ambient temperature and TEWL during the measurements on the 2nd, 7th and 28th day

2d	abdomen	upper leg
Coefficient of correlation	**0.337***	-0.103
p-value	0.011	0.453
n	56	55

7d	abdomen	upper leg
Coefficient of correlation	**-0.408** °	-0.102
p-value	0.005	0.494
n	46	47

28d	abdomen	upper leg
Coefficient of correlation	0.265	**0.400***
p-value	0.083	0.007
n	44	44

*positive correlation by significant p-values; °negative correlation by significant p-values

3.9. Changes of skin condition during the study period

In the course of the study, the following changes in skin conditions were observed in 32 infants (56.1%).

- skin dryness: n=18 (31.6%)
- diaper dermatitis or diaper rash (erythema): n=7 (12.3%) (Fig.3.16)
- acne neonatorum: n=6 (10.5%)
- icterus: n=7 (12.3%)
- others: one of the following changes: naevus flammeus, heamangioma, seborrhoeic eczema or pityrosporum ovale: n=5 (8.8%)

Newborn infants with diaper dermatitis had a tendency to have a higher SCH and pH (p=0.062 and p=0.053 respectively for SCH and for pH) on the buttock on the 28th day. However, no statistically significant differences in median values of skin parameters were observed between newborns with and without diaper dermatitis (Tab.3.18-3.20).

Figure 3.16: Diaper dermatitis of a 28-day-old female newborn receiving washing with a wet cotton wool cloth (a) and diaper erythema of a 7-day-old female newborn receiving washing (b)

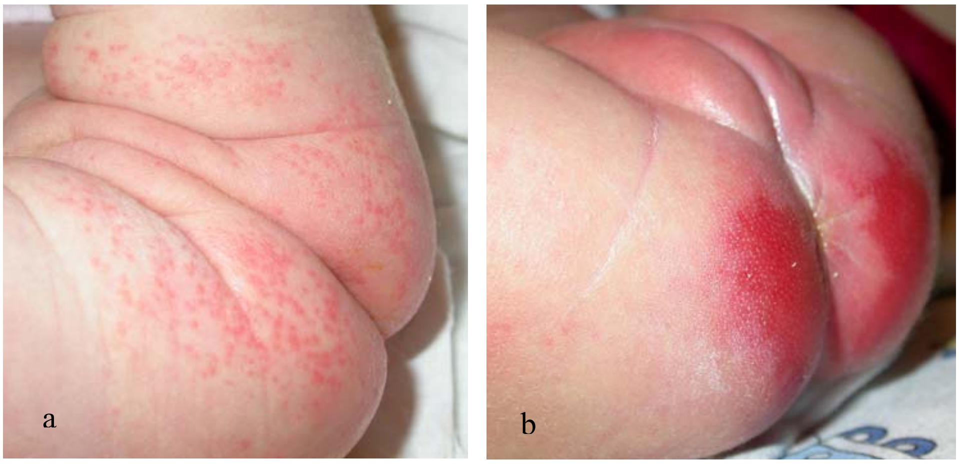


Table 3.18: Comparison of TEWL on the buttock between infants with (1) and without diaper dermatitis (0) on the 2nd and 28th day

Diaper dermatitis			TEWL, 2d	TEWL, 28d
0	n		50	37
	mean		9.33	11.62
	SD		3.37	8.86
	min		4.00	4.40
	max		21.00	57.20
	percentile	25	7.18	7.20
	percentile (median)	50	8.95	9.40
	percentile	75	11.00	14.05
1	n		7	7
	mean		11.96	20.00
	SD		9.51	20.80
	min		4.50	5.20
	max		32.60	51.40
	percentile	25	4.90	7.30
	percentile (median)	50	10.70	9.00
	percentile	75	10.90	49.30

p=0.712 p=0.900

0- without diaper dermatitis
1- with diaper dermatitis

Table 3.19: Comparison of SCH on the buttock between infants with (1) and without diaper dermatitis (0) on the 2nd and 28th day

Diaper dermatitis			SCH, 2d	SCH, 28d
0	n		50	37
	mean		36.61	53.16
	SD		9.35	20.91
	min		16.60	21.90
	max		62.40	120.00
	percentile	25	29.80	39.15
	percentile (median)	50	34.95	49.20
	percentile	75	41.60	60.90
1	n		7	7
	mean		39.16	67.14
	SD		14.63	20.09
	min		14.90	48.70
	max		55.70	107.40
	percentile	25	27.60	49.30
	percentile (median)	50	37.20	67.20
	percentile	75	52.70	72.40
			p=0.438	**p=0.062**

0- without diaper dermatitis
1- with diaper dermatitis

Table 3.20: Comparison of pH on the buttock between infants with (1) and without diaper dermatitis (0) on the 2nd and 28th day

Diaper dermatitis			pH, 2d	pH, 28d
0	n		49	37
	mean		5.98	5.45
	SD		.63	.55
	min		4.51	4.51
	max		7.62	6.73
	percentile	25	5.49	4.98
	percentile (median)	50	6.05	5.32
	percentile	75	6.37	5.79
1	n		7	7
	mean		6.34	5.92
	SD		.56	.60
	min		5.41	4.99
	max		7.05	6.71
	percentile	25	5.97	5.57
	percentile (median)	50	6.33	5.82
	percentile	75	6.77	6.64
			p=0.161	**p=0.053**

0- without diaper dermatitis
1- with diaper dermatitis

No significant differences were noted in the frequency of diaper dermatitis between male and female newborns (p=0.687), between groups “b” and “w” (p=1) as well as between newborns who were breast-fed all the time and those who were not constantly breast-fed (p=1) (Tab.3.21-3.23).

Table 3.21: Frequency of diaper dermatitis (0=without, 1=with diaper dermatitis) between boys and girls

Diaper dermatitis		Gender		Total
		girl	boy	
0	n	21	29	50
	% of gender	84.0%	90.6%	87.7%
1	n	4	3	7
	% of gender	16.0%	9.4%	12.3%
Total	n	25	32	57
	% of gender	100.0%	100.0%	100.0%

0- without diaper dermatitis
1- with diaper dermatitis
p=0.687

Table 3.22: Frequency of diaper dermatitis (0=without, 1=with diaper dermatitis) between groups “w” and “b”

Diaper dermatitis		Group		Total
		wash	bath	
0	n	25	25	50
	% of group	89.3%	86.2%	87.7%
1	n	3	4	7
	% of group	10.7%	13.8%	12.3%
Total	n	28	29	57
	% of group	100.0%	100.0%	100.0%

0- without diaper dermatitis
1- with diaper dermatitis
p=1

Table 3.23: Frequency of diaper dermatitis (0=without, 1=with diaper dermatitis) between newborn who were breast-fed all the time and those who were not constantly breast-fed

Diaper dermatitis		were breast-fed all time	were not constantly breast-fed	Total
0	n	43	7	50
	% of breastfeed	87.8%	87.5%	87.7%
1	n	6	1	7
	% of breastfeed	12.2%	12.5%	12.3%
Total	n	49	8	57
	% of breastfeed	100.0%	100.0%	100.0%

0- without diaper dermatitis
1- with diaper dermatitis
p=1

No significant differences were observed in sebum level on the 28th day between infants with and without acne (p=0.329) (Tab.3.24).

Table 3.24: Comparison of sebum level on the forehead between infants with (1) and without acne (0)

Acne neonatorum			sebum level, 2d	sebum level, 28d
0	n		51	39
	mean		92.35	110.41
	SD		101.73	86.05
	min		1.00	18.00
	max		452.00	355.00
	percentile	25	32.00	44.00
	percentile (median)	50	49.00	83.00
	percentile	75	126.00	159.00
1	n		6	5
	mean		140.83	155.00
	SD		170.00	128.49
	min		10.00	55.00
	max		407.00	372.00
	percentile	25	12.25	66.00
	percentile (median)	50	58.00	103.00
	percentile	75	326.00	270.00
			p=0.949	p=0.329

0- without acne
1- with acne

Neonatal skin condition score

Among this study population the skin condition score ranged from 3 to 5 points. Nobody had a score higher than 5. No statistically significant difference in NSCS between newborns in group “b” and “w” was observed. Most of the tested anatomic sites had a score of 3 (Fig.2.3) which corresponds to normal skin without sign of dryness, erythema or excoriation (Tab.3.25).

Table 3.25: Frequency of perfect score (=3) on 2nd, 7th and 28th day between group “b” and “w” at different anatomic sites

Site	2d	7d	28d
Abdomen			
wash	92.9%	70.8%	86.3%
bath	86.2%	58.3%	95.4%
Hand			
wash	89.3%	87.5%	90.9%
bath	89.7%	83.0%	95.4%
Foot			
wash	89.3%	75.0%	81.8%
bath	82.8%	75.0%	90.9%

3.10 Transient neonatal hair loss

During the first four weeks of life entire scalp was covered by hair in all newborns. None of the newborn developed an alopecic patch during the study period.

4. *Discussion*

Although basal epidermal barrier function in term infants suffices to ensure survival, recent studies suggest that the newborn skin barrier function is not as robust or resilient as that in children or adults. In fact, neonatal skin displays a well-known bias to develop dermatitis and microbial infections and to percutaneous absorption of toxic agents. Thus, newborn skin must undergo further postnatal developmental adjustments to achieve an optimal function in the dry ex-utero environment (Behne 2003). This suggests that barrier stabilization may be dependent on achieving a balance between different skin physiological parameters regarding skin barrier function (Chiou 2004). For indicating functional maturation of neonatal skin, physiological parameters such as TEWL, SCH, skin pH and sebum level have been investigated separately in different studies (Hoeger and Enzmann 2002, Yosipovitch 2000, Giusti 2001, Fluhr 2000, Saijo 1991, Fox 1998, Visscher 2002, Okah 1995, Rutter 1979, Harpin 1983, Agache 1980). These four are considered to be the main parameters characterizing the barrier function in children and adults and were all evaluated for the first time simultaneously in the present study. However, at least three key questions remain: (i) which parameter reflects most of all the functional maturation of neonatal skin, (ii) is one single parameter sufficient to evaluate skin surface characteristics of human newborn infants and (iii) does the water exposure during the bathing or washing influence this cutaneous adaptation process.

So far, differences between washing and bathing were usually analyzed based on measurement of rectal or axillary temperature, duration of healing of umbilical cord and bacterial colonization rate (Henningson 1981, Hylen 1983, Nako 2000, Bryanton 2004).

Although previous studies revealed a better effect on the condition of the newborns by bathing with clear water, compared to washing, which is characterized by a lower loss of body temperature, as well as a higher satisfaction of infants and parents who prefer bathing to washing, many controversies are in existence as to whether healthy full-term newborn infants should be bathed during the first weeks after birth. In previous studies bathing or washing was usually performed once or twice, and measurements were taken within hours or minutes after skin care.

This is the first study comparing skin function parameters between two parallel randomized groups “w” and “b”, prospectively, over a period of 4 weeks giving at least a 12-hour difference between skin care and standardized measurement. In view of the dynamics of growth during early infancy, a prospective analysis rather than a single measurement appeared to be appropriate for the assessment of skin function parameters.

The purposes of the present study were:

1. To gain an insight into postnatal adaptation of skin barrier to a new environment.
2. To investigate generally the effect of bathing in comparison to washing with tap water on the skin barrier within the first 28 days of life.

Changes in four skin parameters, such as TEWL, SCH, pH, sebum level were prospectively monitored among full-term healthy newborn infants during the first four weeks of life. In order to assess possible correlations, different body sites were analyzed. In addition, among these variables, the sex, ethnicity, gestational age and type of delivery of the infants were taken into account.

Because of regional differences, the measurements of these four parameters were performed on four specially selected anatomical sites: abdomen, upper leg, and buttock as occluded areas, and the forehead as non-occluded area. The choice of these anatomical sites was guided by two considerations: firstly, abdomen and upper leg appeared to be most neutral and non-sensitive to external influences and, secondly, diaper rash was previously associated with increased skin wetness and higher skin pH. It is well documented that female newborns present with diaper dermatitis much earlier than males and have a higher degree of infection by this skin disease. While for girls the vulva region is more afflicted, diaper dermatitis often spreads to the interior thighs of boys (Wollina 2005). Therefore, the aim of this study was to check the properties of the skin barrier in this special region, both in female and male newborns separately in order to minimize the influences of occlusion and exposure to urine and feces.

The results of the present study were analyzed in comparison to previous studies, and are discussed with respect to each parameter in subgroups below. The changes in skin parameter between the 2^{nd} and the 7^{th} day for both groups were compared together and, subsequently, each group was compared separately from the 7^{th} to 28^{th} day.

4.1. Postnatal adaptation of TEWL and SCH

By the time of birth, human term newborns have developed mechanisms for successful transition from an aquatic to an extrauterine environment. In the skin, epidermal-based mechanisms confer maturational advantages on the developing fetus during late gestation. The development of a hydrophobic layer, the VC, in the last trimester of gestation, and the formation of the SC as the result of terminal differentiation of epidermal keratinocytes lead to the formation of a relatively water-impermeable barrier. This barrier hinders the movement of water through the skin, rendering the skin surface dry (Okah 1995). A less efficient barrier seems to be reflected by a higher TEWL value (>10 $g/m^2/h$) and lower SCH (<50 units) (Ertel 2003).

In the current literature, it has been reported that generally term newborns present with most of the anatomical sites equal or a lower TEWL (less than 10 $g/m^2/h$) and reduced SCH compared to older infants and adults. However, so far, normal ranges of SCH have not been defined in neonates. Interestingly, the lower values of TEWL were observed usually in healthy term newborn infants only on day 1, whereas, in the older infants (mean age: 14.3 months) no differences in comparison to adults have been reported (Cunico 1977, Wilson and Maibach 1982, Giusti 2001, Yosipovitch 2000). However, there are some exceptions regarding regional differences.

According to Yosipovitch's data, the median values of TEWL of these results ranged from 6.60 to 9.30 $g/m^2/h$ on the 2^{nd} day on measured test sites. No significant differences in TEWL between the 2^{nd} and the 7^{th} day, performed for all infants of both groups, were found (Fig.3.1), nor were any significant differences observed between days 7 and 28 when each group was analyzed separately (Fig.3.9).

In a study by Rutter and Hull (1979) it was observed that in the first 4 hours of life the TEWL was relatively high and subsequently decreased, suggesting that the surface of the skin was drying out. These findings, although limited to certain anatomical regions (soles, palms and forearms), were confirmed by Yosipovitch (2000). In neonates, the TEWL was significantly higher on soles, palms and forearms on the 1^{st} day than on the 2^{nd} day. However, on the forehead, back, abdomen and inguinal region, no significant differences were observed between days 1 and 2. Reduction of TEWL during the first 4 hours of life observed in most of the anatomic sites by Rutter and Hull (1979) and during the 24 hours of life observed on the soles, palms and forearms by Yosipovitch (2000) may reflect a rapid adaptation to extrauterine life in neonates in specific body areas.

In the present study, TEWL was measured initially on the 2^{nd} day and remained remarkably stable on the forehead, abdomen, upper leg and buttock during the first 4 weeks of life. Based on previous results confirmed by this study, it could be assumed that the barrier function in healthy term infants must be stable starting on day 2 after birth. These data are concordant with values cited in a current book of pediatric dermatology, 6-8 g/m^2/h (Rutter 2000).

Under standardized measurement conditions, stable values of TEWL were observed, whereas, hydration of SC increased significantly from the 2nd to 7th day on all anatomic sites, except for the buttock (Fig.3.2). Within each group, “w” and “b”, from the 7th to 28th day an increase was observed on the abdomen and buttock, also an increase in SCH was noted on the forehead in group “b” and on the upper leg in group “w” (Fig.3.10).

Increase of corneal layer hydration on all tests sites was proportionate to age, as previously reported (Hoeger and Enzmann 2002). However, in comparison to the study of Hoeger and Enzmann (2002), these results showed lower values of the SCH on all anatomic sites during the first four weeks. On the 7th day, the median value of SCH was around 30 units, whereas Hoeger and Enzmann (2002) found median values of about 70 units already on the 3rd day, using the same technique. At the end of the present study, on the 28th day, the range of SCH, as in older children or adults (60-80 units) was not achieved. The lower values in the present study could be the result of the differences in the tested areas and the washing of newborns directly after birth. This finding provided support for another clinical observation, namely that newborns have dry skin (Yosipovitch 2000, Saijo 1991). Based on SCH, the adaptation process of skin barrier seems to be in a state of flux in healthy full-term newborns during the first four weeks after birth, however, TEWL remains stable.

The present study also showed another important finding: *regional differences in TEWL and SCH* were demonstrated especially on the buttock and forehead (Tab.3.1-3.2).

The higher value of SCH observed on the buttock compared to other anatomical regions (Tab.3.6) was in agreement with previous results (Hoeger and Enzmann 2002) and most likely induced by diaper occlusion. After occlusion, water might be prevented from evaporating from the upper epidermis and excessive local hydration of SC can be generated on the buttock playing an important role in the development of diaper dermatitis. In order to minimize the occlusion effect of diapered skin, each measurement was performed 5-10 minutes after removing the diaper, when the skin was relatively dry. Also a high value of TEWL was found in the diaper area on the 2nd as well as on the 7th day (Tab.3.1), and after dividing into two groups, only group “w” showed high TEWL values on the buttock in comparison to group “b”, where the TEWL value was lowest (Tab.3.5). This could be due to the beneficial effect of bathing in the gluteal region and relevant for prevention of diaper dermatitis. However, other studies should be performed to further clarify this point.

Similar to the buttock, higher values of TEWL and SCH were also present on the forehead in this study population. This phenomenon can possibly be attributed to background thermal sweating on this site. It is well documented that the intensity of thermal sweating is much higher on the forehead than on other skin sites. Moreover, on the forehead the sweat glands appear first and then spread down to the trunk and limbs (Rutter 2003). Analogical to present results, a further study showed the highest TEWL and SCH on the palms, forearms and inguinal region (Yosipovitch, 2000). These could be explained by emotional sweating on the palms and occlusion in the inguinal area.

These findings are very interesting as they underscore the immature barrier function in newborns. Low hydration values of SC and high TEWL have so far been well documented in *pathologic skin conditions*, such as atopic dermatitis and psoriasis (Chiou 2004). Moreover, a significant decrease in hydration of SC, both in eczematous as well as in unaffected areas has been described in atopic skin (Giusti 1995, Berardesca 1990). In the present study population, none of the newborns had atopic dermatitis or psoriasis; however, all infants presented low hydration values of SC similar to values documented in the literature on pathologic skin condition. This can indicate that skin barrier in full-term newborn infants, directly after birth, is insufficient.

Decreased SCH, observed in term newborn infants, could be the response to environmental changes that take place after birth, i.e., transition from a heated, water-filled container to an expansive, cold, dry environment and reflect the imperfection of SC.

On the other hand, because of a rapid adaptation of TEWL, some authors suppose that newborns have an effective barrier function against water and excellent epidermal barrier integrity at birth (Saijo 1991, Yosipovitch 2000, Rutter 1979, Harpin 1983, Hammarlund and Sedin 1979, Wilson 1980, Sedin 1985). In general, some authors describe a comparable barrier function in infants and adults, but in newborns the barrier function might undergo a postnatal process of either adaptation or maturation (Ertel 2003). This is now also evident from present investigations showing that at least in the first 4 weeks the SC barrier is not completely mature.

4.2. Postnatal adaptation of skin pH

Full-term mammalian infants are born with a neutral skin surface pH, which normalizes to acidic values over the first few postnatal days to weeks, depending on the species (Behne 2003).

At birth, during the first 24 hours, term neonates exhibit a neutral or alkaline skin pH of 6.2-7.5 (Behrendt 1958, Beare 1960, Yosipovitch 2000). The time needed for the development of an acidic SC and the forming of a competent epidermal barrier in term newborns is open to debate. It can range from several weeks up to 24 months (Behrendt 1958, Beare 1960, Behrendt 1971, Hoeger and Enzmann 2002, Mauro 2003).

The median values of skin pH of this study ranged from 5.33 to 6.11 on the 2nd day after birth, depending on the test sites.

Dynamic changes of skin pH observed during the complete study period were as follows: between the 2nd and the 7th day pH declined rapidly on all anatomic sites, especially on the abdomen and upper leg (Fig.3.3). Between the 7th and the 28th day reduction of pH was slow, but significant on the abdomen and upper leg within each group and on the forehead and buttock in group "w" (Fig.3.11). A pH range of 4.9- 5.55 achieved at week four (Tab.3.7) corresponds to pH values of older children and adults, which is in agreement with the Hoeger and Enzmann time period (5.0-5.5) or the analysis of Courage and Khazaka (4.5-5.5).

Decrease of skin pH on all test sites was proportionate to age as previously reported (Hoeger and Enzmann 2002) and reflects the postnatal adaptation of skin barrier to a new environment. These results showed that babies develop an acidic SC, the so-called "acid mantle", within 4 weeks after birth.

The acidification process affected all anatomic sites assessed; however the *differences between tested areas were noted.* During the study period, the lowest value of pH that was observed on the forehead was 4.9 on the 28th day (Tab.3.7). This result confirmed the findings of Hoeger and Enzmann (2002), who performed the measurements at 3 days, 4 weeks and 12 weeks of age on four different body sites (frontal area, cheek, volar forearm and gluteal surface), finding the same lowest pH value (pH 4.9) on the forehead on the 4th and 12th week, as in this study. According to the studies of Emery (1991), Priestly (1996) and Hoeger and Enzmann (2002), the highest value of pH was found on the buttock.

The differences between occluded and non-occluded areas suggest an effect of the environment on the skin surface pH. Intertriginous areas and diapered skin tend to have a higher pH than open skin (Fox 1998). Present results show the highest pH on the buttock and the lowest on the forehead. Significantly higher skin pH in the diaper region can be caused by exposure to urine, feces and bacterial colonization (Visscher 2000, Priestly 1996, Emery 1991).

The differences between anatomic sites could also result from a different skin surface composition, such as the amount of sebum, density and activity of sweat glands or the extent of skin desquamation delivering filaggrin breakdown products (Parra 2003).

It is well documented that the activity of sebaceous gland is much greater on the forehead than on other skin sites. The high level of sebum observed in the present study on the forehead may artificially affect the "apparent pH" measurement on this body site. Consequently, pH on the forehead is significantly lower compared to other body sites (Tab.3.4). Also, the high density of eccrine sweat glands found in newborns on the forehead may influence the skin surface pH, which may explain why the pH is lowest on the forehead. The significance of eccrine sweat glands is described in chapter 4.1.

It can be assumed that a generally higher pH at birth may be related to exposure to amniotic fluid, pH 7.15 or to the VC, pH 7.4 (Boening 1967, Prott 1976). On the other hand, skin pH in newborn infants may reflect not the pH of the SC, but the pH of the amniotic fluid or vernix. At birth, the skin is covered with differing amounts of vernix- white grease formed mainly by fatty degeneration of epidermal cells that protect the skin in utero from the amniotic fluid, blood and maternal bodily fluids (Medves 2001). However, previous readings taken after careful cleansing of the test sites using clear water and dehydrated alcohol were as high as before removal of apparent remains of vernix (Behrendt 1958). These results can suggest that the acidification process is not related to vernix clearance and the pH measured on the skin surface does not reflect the pH of the vernix. To avoid measurement inaccuracy, all newborns in the present study were washed about 2.5 hours after delivery and standardized skin care regime during the entire study period was used.

Another important factor accounting for increased skin pH after delivery is the absence of normal flora. Skin colonization takes place through contact with the environment. A close correlation exists between bacterial colonization and skin pH. At birth, the skin is not colonized with microorganisms. Only bacterial contamination has been found in newborns, which stemmed from vaginal contact during delivery or contact to personnel during cesarean section. This contamination is temporary and probably does not influence the skin pH (Medves 2001). Colonization of the skin with normal skin flora increases over time and is first achieved after about 4 days, before skin pH reaches the acid value.

So far, it has not been proved that the time needed for skin colonization in newborns born by caesarean section (delivered through a sterile field) differs from that of newborns born by vaginal delivery (Medves 2001).

To the best of our knowledge, only one study has been published regarding the relationship between skin physiological parameters, including TEWL, SCH and pH, and mode of delivery. Therefore, it was interesting to check for a possible relation in this study. However, none of the tested variables (TEWL, SCH, pH, sebum) were found to be related to the mode of the delivery, which is in agreement with earlier reports (Yosipovitch 2000).

To sum up: the decrease of pH observed during the neonatal period can reflect the maturation of the skin barrier. According to present results, the skin of newborns achieves an acidic pH during the first 4 weeks of life. The previous incongruity about time needed to development of an acidic SC could result from the lack of a standardized skin care regime, analysis of a single measurement instead of a prospective analysis of dynamic changes and analysis of different anatomic sites in each project.

To explain the acidification processes, both exogenous and endogenous mechanisms have been proposed (Mauro 2003). Endogenous mechanisms are probably related to postnatal maturation of enzyme systems. Neonate skin has been reported to have lower levels of enzyme activity than adult skin, and adult levels are not reached until the age of 6 to 12 months (Yosipovitch 2000). These enzyme systems are involved in the synthesis of many components, such as lactic acid from eccrine sweat, free fatty acids generated from metabolism of sebaceous gland lipids and by-products of bacterial metabolism from colonizing bacteria (Öhman 1998, Mauro 2003). The components described in the foregoing cover the surface of skin in different anatomic regions and belong to the exogenous mechanisms. Possibly other factors can create surface conditions that prevent the establishment of physiological acidification; however, maturation of enzyme systems, and consequently, the physiological skin flora are relevant factors responsible for maintenance of the delicate pH balance.

4.3. Postnatal adaptation of sebum level

Sebum is mainly composed of squalene, wax esters, cholesterol esters and triglycerides. It is proposed to be a waterproof barrier, an immunological modulator and a precursor for sphingolipids, which are essential for the barrier function (Dowing 1987, Metze 1988, Thody 1989). The development of the sebaceous glands is closely related to the differentiation of hair follicles and epidermis (Zouboulis 2003). Genetic and hormonal factors cause individual differences in sebaceous lipid *composition* (Zouboulis 2003). It is well documented that the sebum of newborn infants and children contains different lipids compared to that of adults (Chiou 2004). Also, the postnatal *glandular activity and consequent amount* of produced sebum, is changed in the course of life.

At birth, term neonates present a reduced level of sebum compared to adults. A strong increase in sebum excretion occurring a few hours after birth achieves in the first week of life the same magnitude of sebum level as in young adults (Agache 1980, Henderson 2000). According to the analysis from Courage and Khazaka (2002), 100-200 μg/cm^2 lipids can be recovered on the forehead of adults, whilst on the trunk and legs scarcely 6 μg/cm^2.

In the present study, sebum level increased significantly on the forehead from the 2nd to the 7th day (Fig.3.4.), whilst between the 7th and the 28th day it remained stable (Fig.3.12). The median value reached 86 μg/cm^2 on the forehead and 13.50 μg/cm^{2}on the upper leg on the 7th day. During the first year of life, the level of sebum decreases, most notably in the postnatal period (Agache 1980, Pöschl 1991). A very low level, approximately 10 μg/cm^2 and often below 0.5 μg/cm^2, remains constant from 6 months to the prepubertal period and correlates well with low levels of gonadal and adrenal androgens (Zouboulis 2003). A new rise takes place at about the age of 9 years with adrenarche and continues up to the age of 17 years, when the adult level is reached again. It has been suggested that the endocrine environment correlates and may influence the sebaceous gland development in puberty (Zouboulis 2003).

A strong increase in sebum excretion, occurring a few hours after birth and comparable to that of adults, can be achieved due to the influence of the hormonal environment of the mother on the sebaceous gland of the newborns. It suggests that androgenic stimulus for sebum secretion occurs before birth (Agache 1980, Henderson 2000).

The effect of androgens on sebaceous cell proliferation and differentiation is dependent on the origin of the sebaceous glands from *different skin areas*. Because of the increased sensitivity of facial sebaceous glands to androgens, the level of sebum on the forehead is the highest.

Human sebaceous glands are present in all areas of the skin, except for the palms and soles, and only sparsely on the dorsal surfaces of the hand and foot (Zouboulis 2003).

The significant difference in sebum values between male and female infants confirms the key role of androgens in sebum production. Higher values of sebum in males can be explained by the influence of testosterone.

A strong increase in sebum excretion occurring during the first week of the newborn’s life, confirmed by present results, can reflect the adaptation of the skin barrier. Although at about 7 days, the neonate achieves the glandular activity similar to that observed in adults, it changes in the course of life.

The role of sebum is important in the pathogenesis of acne (Chiou 2004). While high levels of sebum linoleate found in young children may protect them from comedonal acne, high levels of dehydroepiandrosterone found immediately after birth and in the adrenarche, may be responsible for acne infantum and prepubertal acne. Acne neonatorum, which is present at birth or appears 2-4 weeks after birth, is not uncommon, with a prevalence of approximately 20% in newborns (Zouboulis 2003). In the present study, acne neonatorum was observed in 10.5% of newborns.

Interestingly, no significant difference in sebum level between newborns with and without acne was noted (Tab.3.24).

On the other hand, sebaceous lipids can be reduced in *atopic patients* compared with those of normal controls (Sator 2003). However, this is not always the rule (Zouboulis 2003).

4.4. Influence of bathing on the skin barrier adaptation

One of the functions of the initial bath is the removal of blood, maternal bodily fluids and vernix from the skin surface. However, the significance of VC for the skin barrier in neonates after birth has not been completely explained.

Traditionally, bathing involves exposure to water and various types of cleansing agents. Both water and cleanser, singly and in combination, can influence the skin in a dynamic fashion. The effect of clear water exposure and its influence on the skin function parameters, compared between two parallel randomized groups "w" and "b" from birth to the age of 28 days, were investigated in this study.

These results showed significantly that a beneficial effect of bathing twice weekly with clear water seems to exist within the first month of life for the TEWL, on the buttock and for the SCH on the abdomen and forehead. Newborns, who were bathed, had a significantly lower TEWL on the buttock than those who were washed (Fig.3.5) and significantly higher SCH on the forehead and on the abdomen (Fig.3.6). SCH increased more significantly in group "b" than in group "w". Interestingly, bathing markedly altered the biophysical properties of both skin regions, exposed and non-exposed to the water. This phenomenon can be possibly attributed to the enhancement of activity of thermal sweating on the forehead in response to changes in air temperature during regular bathing. So far, beneficial effect of bathing with clear water has been proved by Visscher (2002). His study showed a decrease in erythema, dryness, skin friction, rate of moisture accumulation, and water-holding capacity immediately (2 minutes) and 15 minutes after single bathing for the diapered, as well as non-diapered skin in healthy infants aged 3-6 months. However, contrary to present results this positive effect of bathing was not reflected in the changes of TEWL and SCH. In the Visscher study for the non-diapered skin immediately after bathing, TEWL and SCH were significantly higher than before the bath. In contrast, the diapered skin had similar TEWL after diaper removal and bathing. Also, 15 minutes after bathing, TEWL and SCH were not significantly different from TEWL and SCH before bathing for either non-diapered or diapered sites.

This difference is believed to be the result of a rapid release of water from the skin surface.

Analysis of pH and sebum values revealed no differences between groups “w” and “b” (Fig.3.7, Fig.3.8), suggesting that the beneficial effect of bathing is limited to TEWL and SCH.

So far, the short-term effect of bathing and various types of cleansing agents on the skin barrier in neonates was documented by Gfatter (1997). It has been shown that cleansing with water alone (pH 7.8-8.2), synthetic liquid cleanser (pH 5.5), synthetic bar cleanser (pH 5.5), and fatty acid soap (pH 9.5) changes the skin surface pH and sebum level 10 minutes after using the products (Gfatter 1997). Interestingly, even normal tap water, used 10 minutes before measurement, increases skin surface pH and lowers the values of sebum level. The increase of the skin pH irritates the physiological protective “acid mantle”, changes the compositions of the cutaneous bacterial flora and the activity of enzymes in the upper epidermis, which have an acid pH optimum (Gfatter 1997). However, the most pronounced influence on the skin is exerted by the alkaline soap rather than water alone.

Since NMF consists of water-soluble materials, routine bathing might be expected to alter the NMF content of the SC. The results of Visscher (2002) suggest that freshwater bathing removes water-soluble amino acids, i.e., NMF, thereby reducing the amount of secondary water bound in the skin. Skin surface morphology is influenced by the water content of the SC, desquamation process and epidermal expression of keratins and proteins (Sato 2000, Pierard 1991, Engelke 1997). Disorder in skin hydration disrupts the lamellar structure of the SC, altering tensile properties of the skin, increasing its frictional coefficient and permeability and promoting microbial growth and leading to maceration and dermatoses (Warner 2003, Visscher 2003, Willis 1973, Okah 1995, Giusti 2001).

To sum up: appropriate hydration of keratinocytes is essential for maturation and optimal maintenance of physiological properties of the skin barrier. This reflects the need for the development of skin care regimen adapted in the future to improve skin barrier after birth and to better protect the newborn. Results of the present study have confirmed the beneficial effect of bathing the neonate with clear water. However, for the first time, in order to evaluate the influence of bathing on the skin barrier, all four skin function parameters, TEWL, SCH, pH and sebum, were taken into consideration and also for the first time, two parallel randomized groups, “w” and “b”, were compared, prospectively over a period of 4 weeks.

Significant difference in TEWL and SCH between both groups and limited to certain anatomical regions can suggest that among all physiological parameters, TEWL and SCH are susceptible to regular bathing with clear water. Moreover, the biophysical properties of diapered skin differ from those of other anatomical regions.

4.5. Differences in skin physiologic parameters between male and female newborns

Differences in skin barrier properties between male and female gender are the subject of much controversy. It is well documented that, e.g., acne neonatorum shows a male predominance, whilst diaper dermatitis a female one. Therefore, it can be assumed that female skin properties differ from male ones. Thus, to be able to detect physiological variations there is an important need also for future studies to carefully control and standardize skin care.

The present study has shown, for the first time, significant differences between female and male genders in TEWL, SCH, pH and sebum. So far, differences between genders were only reported in skin pH and sebum. Interestingly, the differences detected in the present study were limited to the buttock and the forehead and only once the difference between genders was detected on the abdomen.

On day 2, females showed higher values of TEWL on the buttock (Tab.3.9) and on the 28th day higher values of SCH than in males (Tab.3.10).

Until day 7, no significant difference in pH values between male and female infants at any test site could be observed. However, from the 7th day reduction of pH occurred more quickly in male than in female infants, and pH values of male infants were significantly lower (Fig.3.13). These results are highly interesting, because so far differences in skin pH between male and female genders were documented in adults and children (Blank 1939a, Courage and Khazaka 2002). Male children and male adults have lower values of skin pH in comparison to females and pH determination in females has a wider range than those in males. However, it has not been proved when these differences of skin pH between male and female begin. For the first time, based on present results, it can be assumed that the process starts as early as the 7th day of life.

Interestingly, a relationship between male and female pH was established by Fox in preterm newborns born with a mean gestational age of 29 weeks and a weight of less than 1500 g. Preterm male newborns showed significantly higher pH values at birth than females (1998). Up to now, the use of postnatal change in skin pH as maturational marker in preterm infants was unknown.

A possible factor accounting for a difference between male and female skin physiology could be a different hormonal environment and consequently different sebum excretion. Directly after birth, the levels in females are lower than in males, but a high increase takes place between the days 3 and 6, followed by a fall, bringing the levels below that of the males (Zouboulis 2003). In the present study, on the forehead, female infants showed on day 2 significantly lower values of sebum (median 38.0) than male (median 67.0). Interestingly, on the 7th as well as on the 28th day, the differences between genders were no longer significant (Tab.3.12). There is no doubt that androgens play a major role in controlling sebaceous gland activity, but other hormones are also directly or indirectly involved.

In summary it can be said that the results of the present study are highly interesting. However, perhaps the differences between genders, detected for the first time in TEWL and SCH, can result from the use of standardized skin care procedures, contrary to previous studies (Hoeger and Enzmann 2002, Yosipovitch 2000, Giusti 2001).

Because children with diaper dermatitis tended to display higher values of SCH and pH on the buttock, it is important to take special care of the skin in this anatomic region, in order to minimize the influences of occlusion and exposure to urine and feces.

In the present study, newborns with diaper dermatitis showed a tendency to have a higher SCH (Tab.3.19) and pH (Tab.3.20) on the buttock than infants without diaper dermatitis. However, the difference in the frequency of diaper dermatitis between male and female newborns was not significant (Tab.3.21). According to results concerning differences between the genders presented above, there is a higher risk of developing diaper dermatitis in female infants.

4.6. Differences in skin physiological parameters between Caucasian and non-Caucasian newborns

Marked differences were observed for TEWL on the upper leg and for pH on the forehead. Caucasian infants had significantly higher values of TEWL on the upper leg (Tab.3.13 a) and significantly lower pH values on the forehead (Tab.3.13 c) than non-Caucasian infants. No significant racial differences in SCH and sebum production in four investigated body sites over a period of 28 days were found.

Because these differences affected only two anatomic sites, the hypothesis that skin surface pH and TEWL differ between races in general could not be verified. However, it has previously been published that term black infants have a more mature barrier than white infants. Racial differences have also been examined in the adult population, although these results were conflicting. In general, the trend is that black individuals have a more efficient, water-impermeable barrier (Okah 1995).

Because in the present study black infants were not included (in the group of non-Caucasian were newborns from Latin America, Asia and North Africa); the number of all newborns was 57, these results should be confirmed by further research in a large group.

4.7. Correlation between skin physiological parameters and week of gestation

Fifty-five full-term newborn AGA infants and two full-term LGA infants from 37 completed weeks of gestation, aged less than 48 hours were included in the study.

No significant correlation between gestational age and skin physiological parameters (TEWL, SCH, pH, sebum) (Tab.3.14 a-d) or between AGA and LGA among full-term newborn infants were found.

Differences in skin barrier function occur between pre-and full-term newborns, but not within full-term infants, with one exception. Significant difference in TEWL within full-term infants was observed between eutrophic and hypotrophic newborns only: full-term SGA infants showed lower TEWL than full-term AGA (Hammarlund and Sedin 1980/1983). Because in this study SGA newborns were not included, this thesis could not be checked.

A strong correlation is evident between gestational age and SCH, as well as between gestational age and TEWL (Okah 1995, Sedin 1983). Preterm infants born at less than 30 weeks of gestational age have statistically significantly greater SCH on the first day of life as compared with infants born after more than 30 weeks of gestational age (Okah 1995). Also TEWL in a one-day-old neonate at 25 weeks of gestational age is 15 times greater than that of a term infant (i.e., approximately 90 $g/m^2/h$). Probably, higher SCH as well as higher TEWL in preterm infants in comparison to term newborns results from a lack of mature skin barrier, absence of VC or diminished vernix production. Exogenous lipids of vernix cause the surface to be hydrophobic and less permeable to water. Excessive skin hydration in preterm infants is associated with pronounced permeability (Okah 1995).

4.8. Correlation between TEWL and room conditions

Among all four skin physiological parameters, TEWL is the most sensitive to be influenced by different ambient factors, such as: body temperature, activity, phototherapy, ambient temperature and humidity. Thus, in the present study the influenced environmental conditions on TEWL values were checked.

Moreover, humidity and temperature measured on the 2^{nd}, 7^{th} and 28^{th} day ranged from 29.7 to 42.2% and from 27.4 to 22.8° C (Tab.3.15).

TEWL and ambient humidity showed a positive correlation in 50% of investigated newborns (Fig.3.14, Tab.3.16). Between TEWL and ambient temperature- in half of the cases- two positive correlations and one negative were checked (Fig.3.15, Tab.3.17).

Present results are distinguishable from previous findings, where inversely linear relation between evaporation rate and relative humidity at a constant ambient temperature was found: Evaporation rate was much higher at low humidity (20%) than at high (60%) humidity (Hammarlund 1977). However, measurements were performed in infants on their first day after birth. At this time, the fluctuation of TEWL is at its highest. The test zone was the intrascapular skin area and the infants were often asleep when the measurements were performed, in contrast to the present study, when the infants ware awake, but quiet and calm without much spontaneous motoric activity.

Relationship between TEWL and room conditions affected only half of the cases, it could be hypothesized that environmental humidity and temperature did not influence the TEWL.

TEWL in newborn infants depends mainly on the gestational age at birth, which determines the permeability of the skin to water, but also on the others factors, such as postnatal age, state of activity, body temperature, activity of eccrine glands, and birth weight. The environmental conditions constitute other factors influencing TEWL. The most important being ambient humidity and temperature. TEWL is the principal factor in the loss of body water early after birth and has a substantial impact on postnatal fluid and heat balance (Sedin 2003).

4.9. Transient neonatal hair loss

For many years the aetiology of neonatal occipital alopecia has been reported to be friction, caused by the newborn's sleeping position. A recent study showed that neonatal occipital alopecia is not always occipital and no relationship exists to the sleeping position. Cutrone and Grimalt (2005) included TNHL in common transient neonatal dermatoses related to the physiology of hair-shaft shedding.

The lanugo hairs in the first pelage of the occiput remain in anagen until near term, at which time they abruptly change into telogen. These occipital telogen hairs are generally shed approximately 8 to 12 weeks later until they are replaced by the newly emergent anagen hair growth. This accounts for the decreased density of hair normally seen in this area in 2-to-3-month-old infants (Cutrone 2005). In the present study, in all newborns the entire scalp was covered with hair. The phenomenon of TNHL was not observed during the first four weeks of life.

5. *Summary*

Ultrastructural studies have shown that the epidermis of full-term infants born after 40 weeks of gestation is morphologically indistinguishable from that of adults. It was therefore assumed that the biophysical properties are similar as well (Yosipovitch 2000).

The present study investigated skin physiology in neonates, especially the barrier function during the first 4 weeks of life and the influence of bathing and washing.

The following important results could be obtained: significant differences in skin barrier function were shown to be dependent on postnatal age, gender and anatomical region. In addition, a beneficial effect of bathing twice weekly with clear water compared to washing with tap water on neonatal skin during the first four weeks of life was evident.

Thus, the anatomical and morphological appearance of full-term newborn skin correlates with the functional maturation only partly. This discrepancy affects the skin barrier function located in the outermost layer of the epidermis, in the SC.

The values of skin physiological parameters in healthy full-term newborns infants obtained in this study differ from adult values cited in the literature (Pinnagoda 1990, Hoeger and Enzmann 2002) and in product information (Courage und Khazaka 2002). The results of the present study showed that a healthy full-term newborn infant has lower SCH (19.3-35.3 vs. >60 units) and sebum (<50 vs. 100-220 $\mu g/cm^2$), and higher pH (up to 6.2 vs. 4.5-5.5 units) on day 2 after birth. Interestingly, already on day 2, values of TEWL are comparable to adults ($<10 g/m^2/h$).

Normally, the functional maturation of the SC begins in the third trimester, at around 24 weeks (Holbrook 2000), whereas, the skin barrier of preterm newborns matures only during the first 10-14 postnatal days, whilst in preterm newborns born before 23 weeks of gestation this process can last up until 8 weeks. However, the adaptation process of skin barrier to extrauterine life in full-term newborns is not completely understood. Thus, the aims of this study were to prospectively monitor the changes in different skin parameters, determine when the SC matures and reaches full barrier function and to investigate the effect of bathing with clear water on skin barrier during the first four weeks of life.

57 healthy, full-term newborns (32 boys and 25 girls) aged less than 48 hours were randomly assigned to 2 groups: “b” (bath) and “w” (wash). Group “b” (n=29) received a bath twice weekly with clear water and group “w” (n=28) was washed with a wet cotton wool cloth twice weekly, starting after the 7th day of life in both groups. TEWL, skin pH, sebum production and SCH were measured at day 2, day 7 and day 28 under standardized conditions on the forehead, abdomen,

upper leg and buttock using a non-invasive Multi Probe Adapter System MPA® connected with four probes: tewameter, corneometer, pH-meter and sebumeter.

The statistical analysis of the results obtained in both groups showed a significant increase in SCH and sebum as well as a significant decrease in pH values during the first four weeks of life. These findings indicate that **in healthy full-term newborn infants, directly after birth, cutaneous adaptation or maturation process takes place and is not completely finished at the end of the fourth week.**

The time needed for normalization of skin parameters depends on the different skin physiological parameters (TEWL, SCH, pH and sebum). Up until day 7, sebum and up until day 28 pH reached values comparable to those in older children and adults. In contrast, SCH did not reach the standard values obtained in adults even at the end of the fourth week. Interestingly, during the whole study period TEWL was remarkably stable, confirming previous findings that the adaptation process for TEWL only takes place during the first four postnatal hours.

It seems that all skin physiological parameters, such as TEWL, SCH, pH and sebum should be used as a marker to evaluate the adaptation process of skin to extrauterine life in healthy full-term newborn infants. So far, TEWL was the main parameter used for evaluation of skin barrier effectiveness, perhaps because of the marked differences in TEWL between pre- and full-term newborns and physiological and pathological skin conditions. Until now, TEWL has been a highly important parameter for the evaluation of skin barrier in pathological states, but it is also a parameter which needs highly standardized measurement conditions in order to provide reliable and reproducible results.

Comparing parameters between genders, significantly higher transepidermal water loss and higher hydration of SC was found for the first time on the buttock in females. The present study also proved that the differentiation process of pH between full-term newborn females and males begins on day 7 after birth and lasts at least until day 28.

Among all four anatomical regions measured in the present study, the values of skin parameters deviated mostly on the buttock by contrast with other anatomical regions. This study confirms the assumption that the buttock is the most sensitive region in a newborn, so special skin care in this region is recommended. However, the exact type and schedule of skin care should be confirmed in further research studies.

This is the first study comparing skin function parameters between healthy term newborns investigating on the one hand, the stratum corneum maturation and on the other hand, the influence of washing and bathing. The study results demonstrated that bathing with clear water leads to a better skin barrier function than washing a newborn: specifically, a lower transepidermal water loss on the buttock and a higher hydration of the stratum corneum on the forehead and abdomen were noticed. Moreover, healthy full-term newborn infants can be bathed with clear water from the 7^{th} day of life without negative influence on the newborn's general condition and skin adaptation process.

6. *Zusammenfassung*

Ultrastrukturelle Studien haben gezeigt, dass die Epidermis der Neugeborenen nach 40-wöchiger Gestationszeit morphologisch ist von der Epidermis der Erwachsenen nicht zu unterscheiden. Es wurde daher vermutet, dass die biophysikalischen Eigenschaften der Hautbarriere direkt nach der Geburt vollständig entwickelt sind (Yosipovitch 2000).

In dieser Studie wurden die Hautphysiologie der Neugeborenen, besonders die Funktion der Hautbarriere während der ersten 4 Lebenswochen und der Einfluss von Waschen und Baden untersucht.

Die Studie ergab folgende wichtige Ergebnisse: Es zeigten sich signifikante, von postnatalem Alter, Geschlecht und Körperregion abhängige Unterschiede in der Funktion der Hautbarriere und ein positiver Einfluss auf die Hautbarriere durch 2-mal wöchentliches Baden mit klarem Wasser, im Vergleich zum Waschen, während der ersten 4 postnatalen Wochen.

Dies erlaubt die Schlussfolgerung, dass die anatomische und morphologische Reife der Reifgeborenen nur zum Teil mit der funktionellen Reife des Hautorgans korreliert. Diese Diskrepanz betrifft die Barrierefunktion, die in der obersten Schicht der Epidermis, SC, lokalisiert ist.

Die in dieser Studie erhaltenen Werte hautphysiologischer Parameter gesunder Reifgeborener unterscheiden sich von Erwachsenen-Werten, die in der Literatur (Pinnagoda 1990, Hoeger and Enzmann 2002) und in der Gerätegebrauchsanweisung zitiert sind (Courage und Khazaka 2002). Die Studienergebnisse zeigten, dass der gesunde Reifgeborene einen niedrigeren SCH (19.3-35.3 vs. >60 units) und Sebumgehalt (<50 vs. 100-220 $\mu g/cm^2$), und einen höheren pH-Wert (up to 6.2 vs. 4.5-5.5 units) am 2. Tag nach der Geburt hat. Interessanterweise, war der TEWL schon am 2. Tag nach der Geburt vergleichbar mit dem TEWL Erwachsener (<10$g/m^2/h$).

In der Regel, beginnt die funktionelle Reifung des Stratum corneum im dritten Trimester, ca. in der 24. SSW (Holbrook 2000), indessen bei Frühgeborenen reift die Barrierefunktion nur während der ersten 10-14 Tage postnatal, während bei sehr unreifen Frühgeborenen, die vor der 23. SSW geboren sind, dieser Prozess sogar bis zu 8 Wochen dauern kann. Unklar ist bis dato, inwieweit sich die Hautbarriere von reifen Neugeborenen an die neue Umgebung ex utero anpasst.

Die Ziele dieser Studie waren folgende: prospektives Monitoring der Änderungen der Hautfunktionsparameter; Bestimmung, wann das SC reift und die volle Barrierefunktion erreicht;

Untersuchung des Einflusses von Baden mit klarem Wasser auf die Hautbarriere von Neugeborenen während der ersten 4 Wochen.

57 gesunde Neugeborene mit vollendetem Geburtsalter nach der 37. SSW (32 Jungen und 25 Mädchen) wurden in weniger als 48 Stunden randomisiert und in 2 Gruppen eingeteilt. In Gruppe „b“ (n=29) wurde die Hautpflege mittels 2-mal wöchentlichem Baden in klarem Wasser und in Gruppe „w“ (n=28) mittels 2-mal wöchentlichem Waschen mit einem mit klarem Wasser getränkten Waschlappen durchgeführt. Diese Hautpflege wurde am 7. Tag nach der Geburt in beiden Gruppen begonnen.

Als Messparameter wurden der TEWL, der pH-Wert, der Sebumgehalt der Haut und die SCH am 2., 7. und 28. Tag nach der Geburt an der Stirn, dem Abdomen, am seitlichen Oberschenkel und am Gesäß nicht-invasiv unter einheitlichem Hautpflegekonzept mittels des Multi Probe Adapters Systems MPA® gemessen.
Die statistische Analyse der Ergebnisse zeigte in beiden Gruppen sowohl einen signifikanten Anstieg der SCH und des Sebumgehaltes als auch einen signifikanten Abfall des pH-Wertes während der ersten 4 Lebenswochen. Diese Ergebnisse weisen hin, dass **bei gesunden Reifgeborenen direkt nach der Geburt ein Adaptionsprozess oder Reifungsprozess der Haut stattfindet, der am Ende der vierten Woche nicht vollständig abgeschlossen ist.**
Die Zeit für die Normalisierung der Hautfunktion hängt von den verschiedenen physiologischen Hautparametern ab (TEWL, dem pH-Wert, dem Sebumgehalt und der SCH). Der Sebumgehalt erreicht bis zum 7. Tag, der pH-Wert bis zum 28. Tag die Werte, die mit denen der Erwachsenen und älteren Kinder vergleichbar sind. Im Gegensatz dazu erreicht die SCH bis zum Ende der 4. Woche das Niveau, das mit dem Niveau der Erwachsenen vergleichbar ist, nicht. Im Verlauf der Studienperiode zeigte der TEWL keinen signifikanten Unterschied, ein Hinweis darauf, dass der Adaptionsprozess des TEWL nur während der ersten 4 postnatalen Stunden stattfindet.
Man kann vermuten, dass alle Hautfunktionsparameter wie TEWL, SCH, pH und Sebum benutzt werden sollten, um den Adaptionsprozess der Hautbarriere an das extrauterine Milieu bei Reifgeborenen zu beurteilen. Bisher wurde der TEWL als Hauptparameter für die Evaluation der Effektivität der Hautbarriere benutzt, vielleicht wegen der signifikanten Unterschiede zwischen den Früh- und Reifgeborenen und zwischen den physiologischen und pathologischen Hautzuständen. Weiterhin bleibt der TEWL besonders wichtiger Parameter für die Evaluation der Hautbarriere bei pathologischen Zuständen. Auch ist es ein Parameter, der nur unter hoch einheitlichen Betriebsbedingungen sichere und reproduzierbare Ergebnisse führt.

Diese Studie zeigte erstmalig einen signifikanten geschlechterunabhängigen Unterschied in der Hautphysiologie. Im Bezug auf das Geschlecht waren die Werte des TEWL und der SCH bei den weiblichen Neugeborenen am Gesäß signifikant höher als bei den männlichen.
Der pH-Wert fiel bei den männlichen stärker als bei den weiblichen Neugeborenen im Verlauf der ersten 4 Wochen ab. Anhand dieser Studie wurde festgestellt, dass der Differenzierungsprozess in pH zwischen den weiblichen und den männlichen Neugeborenen am 7. postnatalen Tag beginnt und mindestens bis zum 28. Tag dauert.
Unter allen Körperregionen, die in dieser Studie gemessen wurden, zeigten die Werte am Gesäß die größte Schwankung, was darauf hinweist, dass das Gesäß eine sehr sensible Körperregion ist, die eine spezielle Hautpflege braucht. Jedoch sollte der genaue Typ und das Schema der Hautpflege durch weitere zukünftige Studien festgestellt werden.
Anhand des signifikant niedrigen TEWL am Gesäß und der signifikant höheren SCH in der Gruppe „b" an Abdomen und Stirn wurde ein positiver Einfluss auf die Hautbarriere der Neugeborenen durch Baden verglichen zu Waschen mit klarem Wasser festgestellt.

Aktuelle Ergebnisse deuten darauf hin, dass die gesunden Reifneugeborenen ab dem 7. Lebenstag ohne negativen Einfluss auf Kondition und Hautadaption gebadet werden können.

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Attachment

Table 3.1: Values of TEWL (mean, median, minimum, maximum) measured on the 2nd and 7th day

Location / TEWL ($g/m^2/h$)	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	57	11.10	5.97	2.00	34.00	8.00	9.30	12.60
forehead, 7d	48	10.30	3.19	4.90	20.60	8.15	9.95	11.90
abdomen, 2d	57	7.16	2.85	2.30	18.60	5.25	6.60	9.10
abdomen, 7d	47	8.31	2.78	2.60	17.50	6.20	8.00	9.60
upper leg, 2d	56	7.51	3.77	2.60	30.00	5.50	7.05	8.57
upper leg, 7d	48	7.73	6.52	2.90	49.70	5.45	6.70	8.45
buttock, 2d	57	9.65	4.52	4.00	32.60	7.15	9.10	10.95
buttock, 7d	48	13.31	12.72	1.80	72.10	7.23	9.50	12.93

Table 3.2: Values of SCH (mean, median, minimum, maximum) measured on the2nd and 7th day

Location / SCH (corneometer units)	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	57	23.17	5.90	11.30	39.60	19.15	**22.20***	27.55
forehead, 7d	48	28.21	9.02	5.40	48.70	21.80	**29.05***	33.58
abdomen, 2d	57	25.45	7.39	3.70	40.90	20.00	**24.50***	31.50
abdomen, 7d	48	35.08	16.99	4.30	120.00	25.25	**32.50***	43.13
upper leg, 2d	56	19.74	4.65	12.00	33.10	16.43	**19.35***	23.13
upper leg, 7d	48	24.14	8.07	13.50	58.30	18.18	**23.65***	27.45
buttock, 2d	57	36.93	10.01	14.90	62.40	29.70	35.30	43.05
buttock, 7d	48	39.91	16.08	22.70	120.00	31.58	37.65	43.65

* significant differences between 2d and 7d ($p<0.001$)

Table 3.3: Values of skin pH (mean, median, minimum, maximum) measured on the 2nd and 7th day

Location / Skin surface pH (pH units)	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	56	5.39	.66	4.24	7.68	4.97	**5.33***	5.75
forehead, 7d	47	5.18	.44	4.15	6.20	4.96	**5.19***	5.43
abdomen, 2d	56	6.05	.60	4.59	7.36	5.63	**6.11***	6.50
abdomen, 7d	47	5.59	.42	4.71	6.51	5.27	**5.59***	5.93
upper leg, 2d	56	5.99	.60	4.85	7.40	5.51	**5.99***	6.41
upper leg, 7d	47	5.56	.49	4.25	6.49	5.33	**5.59***	5.85
buttock, 2d	56	6.02	.63	4.51	7.62	5.51	6.06	6.47
buttock, 7d	47	5.83	.54	4.60	7.20	5.54	5.85	6.06

* significant differences between 2d and 7d, forehead ($p=0.029$), abdomen and upper leg ($p<0.001$)

Table 3.4: Values of sebum (mean, median, minimum, maximum) measured on the 2nd and 7th day

Location / Sebum level (µg/cm²)	n	mean	SD	min	max	Percentile		
						25	50 (median)	75
forehead, 2d	57	97.46	109.76	1.00	452.00	29.00	**49.00***	126.00
forehead, 7d	48	132.81	100.12	6.00	369.00	59.25	**86.00***	194.50
upper leg, 2d	57	40.21	62.55	.00	240.00	.50	12.00	54.50
upper leg, 7d	48	52.71	87.42	.00	431.00	.00	13.50	62.75

* significant difference between 2d and 7d (p=0.002)

Table 3.5: Values of TEWL (mean, median, minimum, maximum) measured on the 2nd,7th and 28th day for group "b" and "w"

Location / TEWL (g/m²/h)	Group	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead, 2d	wash	28	11.28	4.74	6.00	26.00	8.25	10.25	12.93
	bath	29	10.93	7.04	2.00	34.00	7.45	8.80	11.40
forehead, 7d	wash	24	10.18	2.17	5.80	14.90	9.27	10.15	11.70
	bath	24	10.42	4.01	4.90	20.60	8.03	9.05	13.68
forehead, 28d	wash	22	10.15	2.37	6.60	14.60	8.23	9.60	11.75
	bath	22	10.58	2.78	5.90	16.10	8.10	10.65	13.00
abdomen, 2d	wash	28	7.38	2.49	2.50	12.00	5.43	6.90	9.50
	bath	29	6.95	3.19	2.30	18.60	4.75	6.50	8.20
abdomen, 7d	wash	24	8.27	2.09	3.90	13.00	6.50	8.70	9.55
	bath	23	8.35	3.41	2.60	17.50	6.10	7.70	9.80
abdomen, 28d	wash	22	9.50	3.15	4.10	15.20	6.48	9.30	12.50
	bath	22	9.04	3.30	4.80	15.40	5.85	8.70	12.05
upper leg, 2d	wash	28	7.18	2.49	2.60	13.10	5.48	7.00	8.75
	bath	28	7.84	4.75	3.50	30.00	5.50	7.05	8.45
upper leg, 7d	wash	24	8.52	8.95	4.10	49.70	5.45	6.35	8.82
	bath	24	6.95	2.32	2.90	11.80	5.58	6.95	8.20
upper leg, 28d	wash	22	7.44	3.59	2.30	17.90	5.10	6.40	8.40
	bath	22	7.48	3.07	4.00	16.30	4.72	7.50	9.23
buttock, 2d	wash	28	9.44	3.48	4.30	21.00	6.60	9.25	10.90
	bath	29	9.86	5.39	4.00	32.60	7.20	9.10	11.00
buttock, 7d	wash	24	13.15	9.86	4.60	42.80	8.30	9.55	13.00
	bath	24	13.47	15.27	1.80	72.10	5.68	9.40	12.93
buttock, 28d	wash	22	16.81	15.02	5.40	57.20	7.95	**12.15***	14.68
	bath	22	9.09	4.58	4.40	23.70	6.38	**7.35***	10,35

* significant difference between groups (p=0.004)

Table 3.6: Values of SCH (mean, median, minimum, maximum) measured on the 2nd,7th and 28th day for group "b" and "w"

Location / SCH (corneometer units)	Group	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	wash	28	21.09	4.69	11.30	32.40	17.83	21.10	23.38
	bath	29	25.17	6.32	16.00	39.60	19.50	24.10	30.50
forehead, 7d	wash	24	26.48	8.97	15.00	48.70	20.90	22.95	33.13
	bath	24	29.94	8.91	5.40	47.90	23.80	32.00	34.35
forehead, 28d	wash	22	30.56	11.90	8.90	50.70	21.88	**28.85***	41.83
	bath	22	39.60	16.30	6.30	68.30	29.50	**40.10***	51.18
abdomen, 2d	wash	28	25.56	8.30	3.70	36.80	18.83	27.30	32.42
	bath	29	25.35	6.54	15.80	40.90	20.45	23.70	29.50
abdomen, 7d	wash	24	36.48	20.49	16.70	120.00	25.10	31.90	41.18
	bath	24	33.68	12.88	4.30	53.10	26.20	34.90	44.25
abdomen, 28d	wash	22	50.86	14.30	26.20	92.00	43.20	**47.65***	58.13
	bath	22	59.19	13.06	30.10	89.10	50.60	**58.25***	66.78
upper leg, 2d	wash	28	19.83	4.95	12.00	30.40	15.68	19.65	23.43
	bath	28	19.65	4.43	12.50	33.10	16.43	19.05	22.75
upper leg, 7d	wash	24	22.68	5.55	13.60	36.80	18.18	22.95	25.40
	bath	24	25.60	9.88	13.50	58.30	17.65	25.95	29.50
upper leg, 28d	wash	22	29.24	8.77	15.40	48.70	21.70	29.25	33.75
	bath	22	28.77	9.51	9.10	50.20	21.15	28.00	35.23
buttock, 2d	wash	28	38.23	10.09	23.10	62.40	32.60	36.85	44.45
	bath	29	35.67	9.94	14.90	58.70	28.85	34.70	42.40
buttock, 7d	wash	24	39.25	19.51	22.70	120.00	26.18	37.50	44.45
	bath	24	40.57	12.11	24.90	74.60	32.53	37.65	43.48
buttock, 28d	wash	22	57.11	24.55	21.90	120.00	38.65	52.10	72.85
	bath	22	53.67	17.62	30.80	107.60	42.48	49.55	59.33

* significant differences between groups, forehead (p=0.032), abdomen (p=0.018)

Table 3.7: Values of skin pH (mean, median, minimum, maximum) measured on the 2nd,7th and 28th day for group “b” and “w”

Skin surface pH (pH units) / Location	Group	n	mean	SD	min	max	Percentile		
							25	50 (median)	75
forehead, 2d	wash	28	5.40	.64	4.24	6.88	4.94	5.33	5.80
	bath	28	5.38	.68	4.34	7.68	5.03	5.28	5.69
forehead, 7d	wash	23	5.24	.37	4.15	5.90	5.10	5.24	5.43
	bath	24	5.13	.50	4.16	6.20	4.70	5.09	5.49
forehead, 28d	wash	22	4.92	.34	4.13	5.63	4.72	4.97	5.15
	bath	22	4.99	.52	4.08	6.00	4.59	4.94	5.31
abdomen, 2d	wash	28	6.07	.56	4.93	7.33	5.64	6.07	6.52
	bath	28	6.02	.65	4.59	7.36	5.61	6.12	6.46
abdomen, 7d	wash	23	5.56	.44	4.71	6.51	5.25	5.63	5.90
	bath	24	5.62	.40	4.86	6.35	5.42	5.59	5.95
abdomen, 28d	wash	22	5.20	.63	4.27	6.86	4.79	5.04	5.51
	bath	22	5.23	.73	3.94	6.58	4.70	5.14	5.79
upper leg, 2d	wash	28	6.00	.57	4.85	7.13	5.65	5.98	6.46
	bath	28	5.99	.64	4.95	7.40	5.39	6.04	6.40
upper leg, 7d	wash	23	5.62	.51	4.31	6.49	5.39	5.67	6.03
	bath	24	5.51	.48	4.25	6.28	5.24	5.58	5.84
upper leg, 28d	wash	22	5.09	.58	4.15	6.86	4.66	5.10	5.24
	bath	22	5.21	.67	3.99	6.85	4.89	5.11	5.76
buttock, 2d	wash	28	6.05	.60	4.68	6.91	5.51	6.16	6.59
	bath	28	6.00	.66	4.51	7.62	5.51	6.05	6.26
buttock, 7d	wash	23	5.91	.63	4.60	7.20	5.56	5.85	6.31
	bath	24	5.76	.45	4.95	6.87	5.40	5.83	6.02
buttock, 28d	wash	22	5.49	.49	4.51	6.73	5.13	5.55	5.79
	bath	22	5.57	.66	4.69	6.71	4.95	5.40	6.17

Table 3.8: Values of sebum level (mean, median, minimum, maximum) measured on the 2^{nd},7^{th} and 28^{th} day for group "b" and "w"

Location / Sebum level (µg/cm^2)	Group	n	mean	SD	min	max	Percentile 25	Percentile 50 (median)	Percentile 75
forehead, 2d	wash	28	91.64	100.51	5.00	398.00	35.00	51.50	11.00
	bath	29	103.07	119.52	1.00	452.00	24.00	48.00	14.00
forehead, 7d	wash	24	117.29	76.68	31.00	308.00	66.50	81.00	157.75
	bath	24	148.33	118.75	6.00	369.00	43.25	145.00	228.75
forehead, 28d	wash	22	126.77	90.12	18.00	351.00	50.50	98.00	173.00
	bath	22	104.18	92.61	20.00	372.00	49.25	76.50	113.25
upper leg, 2d	wash	28	46.43	64.59	.00	213.00	.25	14.00	75.75
	bath	29	34.21	61.05	.00	240.00	.50	6.00	51.50
upper leg, 7d	wash	24	57.33	105.06	.00	431.00	.00	7.00	63.75
	bath	24	48.08	67.33	.00	209.00	.25	28.50	58.00
upper leg, 28d	wash	22	24.32	55.86	.00	202.00	.00	.50	9.50
	bath	22	27.14	45.16	.00	199.00	.00	4.50	45.75

Curriculum vitae

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Affirmation

„Ich, Anna Mleczko, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: Klinische Untersuchung der hautphysiologischen Parameter und des Einflusses vom Baden mit klarem Wasser auf die Hautbarriere von Neugeborenen vom 1. Lebenstag bis zur 4. Lebenswoche selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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