学位論文

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

（生後1年間における新生児・乳幼児の角層機能および成分の経時的変化）

堀　　仁 子

（　本間　大、藤井 瑞恵、野村 和加奈、菅野 恭子、林　時伸、
中村 英記、長屋　建、宮内 勇貴、藤村　努、堀田 光行、
高木　豊、北原　隆、竹馬 吉則、飯塚　一　）

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

Masako MINAMI-HORI[1], Masaru HONMA[1], Mizue FUJII[1], Wakana NOMURA[1], Kyoko KANNO[1]

Tokinaka HAYASHI[2], Eiki NAKAMURA[2], Ken NAGAYA[2]

Yuki MIYAUCHI[3], Tsutomu FUJIMURA[3], Mitsuyuki HOTTA[3], Yutaka TAKAGI[3], Takashi KITAHARA[3], Yoshinori TAKEMA[3]

Hajime IIZUKA[1]

1. Department of Dermatology, Asahikawa Medical University, Asahikawa, Japan
2. Department of Pediatrics, Asahikawa Medical University Asahikawa, Japan
3. Kao Corporation, Haga-Gun, Tochigi, Japan

Asahikawa , Hokkaido, Japan

Correspondence: Masako MINAMI-HORI, MD., Department of Dermatology, Asahikawa Medical University Midorigaoka Higashi 2-1-1-1, 078-8510, Asahikawa, Japan.

Tel: 81-166-68-2523 Fax: 81-166-68-2529

Email: masakoh@asahikawa-med.ac.jp

Short title: Developmental l alterations of skin properties during the first year.

Text word count 2736

Number of references 21

Number of figures 5

Abstract

Background: Although physical properties of neonatal-infantile stratum corneum (SC) change drastically after birth, precise developmental alterations of specific sites have not been fully elucidated.

Objective: To determine the longitudinal alterations of neonatal-infantile SC functions and components of upper thighs and diaper-covered buttocks during the first year of life. The data were compared with those of adults.

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

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

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

Introduction

The physical properties of neonatal-infantile skin drastically change after birth. Previous reports indicate that full-term neonates already show functionally mature SC at birth [1], and immature SC of preterm neonates become mature during the 1st month after birth [2-3]. Nevertheless, few reports exist, which show location-specific alterations during development specifically dermatitis-prone diaper-covered areas［4-5］We analyzed physical properties and various components of SC of 19 full-term neonates longitudinally during the 1st year of life and compared with those of their mothers. The analyzed areas were upper thighs and diaper-covered buttocks.

Alterations of physical properties and various components including total CERs and CER subtypes were also analyzed. To the best of our knowledge, this is the first report clarifying the physical properties and components of SC longitudinally at these specific locations using the same infants during the 1st year of development. Detailed analyses of infantile SC function of arms [3-12 months] have been described by Nikolovski et al [6].

Materials and Methods

Subjects and Study Design

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

Biophysical measurements were performed in a climate-controlled room and the babies were acclimated at temperature of 25℃ and relative air humidity of 40% for 20 minutes [7].

The measurements of neonates-infants were performed on two locations:

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

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

Instrumentation

Skin hydration (capacitance) and water sorption/desorption analyses were measured by by Corneometer® (CM825/Courage + Khazaka electronic GmbH, Germany). TEWL was measured, Tewameter® (TM300/Courage + Khazaka electronic GmbH, Germany). Analyses of NMF, ester binding sebum, and FFAs were performed by Attentuated total reflectance Fourier Transform Infrared (ATR-FTIR) spectrometer (Nicolet380, Thermo

Scientific, K.K, Japan) equipped with a fiber-optic probe coupler (FiberMate II, Systems Engineering Inc, Japan) and AgCl-AgBr polycrystalline infrared fiber-optic probe (PIR fiber probe, Systems Engineering Inc, Japan). These non-invasive measurements by the ATR-FTIR device, albeit superficial, showed more constant values than the previous measurements especially of NMF [8]. Normal-phase liquid chromatography-electrospray ionization-mass spectrometry (NPLC-ESI-MS) was used for the quantification of CER species [9], which can analyze as small as 2.5 cm × 6.0 cm tape-stripped SC. Tape-stripping was performed 3 times on the same sites and were analyzed for CERs, which was performed by Agilent 1100 Series LC/MSD single-quadrupole system equipped with an electrospray ionization source, ChemStation software, a 1100-well-plate autosampler (Agilent Technologies, Palo Alto, CA), and an Inertsil SIL 100A-3, 1.5mm i.d. × 150 mm column (GL Science, Tokyo, Japan) as was described previously [9-10].

Water sorption and retention capacity

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

Characterization of overall CER species in SC

Human SC CERs can be divided into 12 groups according to their fatty acid and sphingoid structures. The following CERs were analyzed by NPLC-ESI-MS. CER[NDS] stands for non-OH fatty acids [N] and dihydrosphingosines [DS]. CER[NS] stands for [N] and sphingosines [S]; CER[NH] for [N] and 6-hydroxy sphingosines [H]; CER[NP] for [N] and phytosphingosines [P], CER[ADS] stands for α-OH fatty acids [A] and [DS]; CER[AS] for [A] and [S]; CER [AH] for [A] and [H]; CER [AP] for [A] and [P]; CER [EOS] for ester-linked ω-OH fatty acids [EO] and [S]. CER [EOH] stands for [EO] and [H]; CER[EOP] for [EO] and [P]. CER[EODS] stands for [EO] and [DS], which was recently detected in human [12]. CER[EODS] was not measured in this study.

Results

Skin hydration and water holding capacity of upper inner thighs

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

Skin hydration and water holding capacity of diaper-covered buttocks

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

Transepidermal water loss (TEWL)

Neonatal TEWL of both upper thighs and buttocks was significantly lower than that of their mothers (Fig. 2A and 2B), suggesting more efficient barrier function of full-term neonates than that of adults. The infantile

TEWL of both upper thighs and buttocks rapidly increased during the 1st month, and were close to those of their mothers at 1 year. The TEWL of 5 age-matched adult female buttocks was similar to that of the mothers (data not shown).

Alterations of superficial SC components determined by ATR-FTIR Spectroscopy

ATR-FTIR provides the information of the first 1-2 microns of superficial SC components. The superficial NMF content of neonatal-infantile thighs and buttocks was relatively constant and comparable to that of their mothers (data not shown).

Infants do not have active sebaceous glands except for the first month of life, when the sebaceous glands are still under the influence of the maternal androgens. Superficial ester binding sebum of neonatal upper thighs decreased during the 1st month after birth and thereafter and was relatively constant and similar to the values of their mothers during the 1st year of development (Fig. 3A). That of buttocks decreased during the 1st month after birth and remained lower than that of their mothers (Fig.3B). The total amount of superficial FFAs was relatively constant at both sites during the 1 year measurements (data not shown).

CERs analyses of SC

Total amount of CERs of both upper thighs and buttocks of full-term neonates was higher than those of their mothers and decreased during the 12 months towards the values of adults (Fig. 4A and B). The decrease of the diaper-covered buttocks was more rapid during the 1^{st} month after delivery than that of upper thighs (Fig. 4A and B).

Among the CERs subtypes neonatal CER[EOS] of upper thighs was slightly higher than that of their mothers, which transiently increased during the 1^{st} month and then decreased to the levels of the mothers during 12 months (Fig. 5A). ω-Hydroxy fatty acid-esterified CER[EOS, EOH, EOP] have been assumed to be associated with barrier function of SC [13]. Significant reduction in CER[EO] subclasses was observed in atopic dermatitis [14-15]. CER [EOS] of neonatal buttocks was much higher than that of their mothers and following the decrease during the 1^{st} month still remained high thereafter (Fig. 5B). Thus, contrary to the values of the upper thighs (Fig. 5A), the CER[EOS] of the neonatal-infantile buttocks was higher than that of their mothers throughout 1 year determination (Fig. 5B). CER[EOH + EOP] values showed essentially similar changes with that of CER[EOS] (Fig. 5A and B) showing higher values at buttocks than mothers. Among the CER subclasses ω-hydroxy fatty acid-esterified CERs showed mostly negative correlation with the SC barrier function (Fig. 5A). For example, CER[EOP] on the buttocks showed statistically significant negative correlation with TEWL in our regression analysis (p = 0.006). Other CER subclasses variably fluctuated and no consistent alterations were detected.

Discussion

In the present study we analyzed the physical properties and components of neonatal-infantile SC of upper thighs and diaper-covered buttocks longitudinally using the same infants during the 1st year of development; these values were compared with those of their mothers. Visscher et al [4] reported that the physical properties of diaper region is indistinguishable from non-diapered region at birth but exhibited differential behavior over the first 14 days, showing higher pH and hydration in the diaper-covered region. Giusti et al. also showed immaturity of infantile skin function including diaper-covered buttocks, which showed higher pH and hydration. These may lead to an increased permeability and a reduced capacity of defense against chemical and/or microbial damages in infants [5]. These preceding studies, however, were not performed longitudinally. To the best of our knowledge, our present study is the first of the analyses the neonatal-infantile SC function at these specific locations in a consecutive manner. The values of mothers 3 months after delivery were essentially similar to 5 age-matched control female values and also similar between upper thighs and buttocks enabling us to analyze the longitudinal location-related alterations of neonatal-infantile SC. The reason for the fluctuation of the mothers' data during the first 2 months after delivery, however, remains unknown. This could be related to marked hormonal alterations of the post-delivery mothers. No preceding report regarding this issue is available at present.

Our results indicate that considerable differences exist between the SC function of upper thighs and diaper-covered buttocks of neonates-infants during the 1st year development. These include neonatal skin hydration status (Fig. 1); the water content of neonatal thighs was lower than that of their mothers, while that of neonatal buttocks was similar to that of their mothers. Skin hydration of both thighs and buttocks rapidly increased during the 1st month after birth. At 1 year of age the water content of thighs was close to that of their mothers, while that of buttocks was significantly higher than that of their mothers. The increased hydration of infantile buttocks at 1 year might be related to the diaper covering effect, which was reflected by the increased TEWL at this location (Fig. 2 and vide infra).

The skin hydration status was associated with SC water sorption rates. The less hydrated SC of neonatal inner thighs showed more water sorption, while more hydrated neonatal buttocks showed less water sorption (Fig. 1). This indicates that neonatal-infantile SC hydration is not determined by the SC water-holding capacity *per se* but rather by the permeated water from underneath, which is reflected by the alterations of TEWL during the development. Slightly higher SC hydration of adult buttocks than upper thighs was also associated with less water sorption on this area suggesting that SC with less hydration show higher capacity to absorb the water.

Rapid increase in the skin hydration of both upper thighs and buttocks was noted during the 1st month. This was most-likely related to the decreased barrier function of these locations. Although the decreased TEWL does not necessarily mean more efficient barrier function such as the case in dehydration condition, it is usually assumed that the lower TEWL correlates with more efficient barrier function in the normal non-dehydrated condition. The barrier function of upper thighs and buttocks determined by TEWL was actually well established at the time of delivery, and then decreased during the development (Fig. 2). This decrease was associated with the increased SC water content (Fig. 1A and C) suggesting that the decreased barrier supplies the SC water from underneath. It is interesting to note that in atopic dermatitis showing impaired barrier function, the lesion usually develops after 1 month of age concomitant with the rapid increase in TEWL of at least 2 specific locations, upper thighs and diaper-covered buttocks (Fig. 2A,B).

Among the CER subclasses, ω-hydroxy fatty acid-esterified CERs such as CER[EOP], showed most marked and statistically significant negative correlation with the SC barrier function, for example, at buttocks. This was also negatively correlated with the SC water content. This suggests that as in adults the SC water content of neonates-infants is also correlated with the barrier function determined by the ω-hydroxy fatty acid-esterified CERs. Consistent with this, the content of CER[EOS] and CER[EOH＋EOP] was higher at infantile buttocks showing more efficient barrier function than adults (Fig. 2 and 5). It must be mentioned, however, that the alteration of total and specific CER subclasses did not completely determine the SC functions, which was typically manifested by the different time courses of CER levels during the 1st month after delivery; the decrease in CERs was much more marked in diaper-covered buttocks than upper inner thighs (Fig. 4 and 5) despite similar alteration patterns of SC water content (Fig. 1A and C) and TEWL (Fig. 2). Other SC components were not consistently associated with the SC function, either, except for the decreased ester binding sebum content at both locations (Fig. 3). The increased sebum of neonates might reflect the residual vernix caseosa, which is sebaceous and epidermal lipids combined with desquamation of fetal corneocytes, forming a natural fetal barrier film in late pregnancy [16]. The decrease in infantile ester binding sebum of buttocks was more conspicuous, which might be explained by the fact that the sebum would more easily be removed from the diaper-covered buttocks during daily nursing procedure.

The TEWL of upper thighs and buttocks of mothers (and age-matched female adults) were higher than the reported values such as adult forearms [17]. The less barrier function of inner thighs could be attributable to the thin SC at this location. However, the less barrier function of adult buttocks was an unexpected finding. This might be related to the persistent covering effect by underwear besides other cloths at this location. It has been known that occlusion alone affects and decreases skin barrier function, which was clearly demonstrated by plastic occlusion stress test [18]. Our unpublished observation also disclosed that wrap-covered skin for more than 24h shows increased TEWL for considerable time thereafter (manuscript in preparation).

Human SC must cope with the abrupt changes from amniotic fluid to arid environment just after birth. Previous reports clearly demonstrated the alteration of SC functions during the development [6, 19]. Our results indicate differences in the SC functions of diaper-covered buttocks compared with non-diaper-covered upper thighs suggesting a significance of location-specific skin care management during the neonatal-infantile development. Our results also indicate that the physical properties such as skin hydration and TEWL are by no means determined by single factors such as total and specific CERs, NMF, etc, but are the result of the overall effect of numerous SC components, which fluctuate during the 1st year of development.

Funding source

This study was supported in part by Kao Corporation, Japan, and a grant 21390323 from the Ministry of Education, Science, Sports and Culture of Japan.

Conflicts of Interest

TH, EN, KN, YM, TF, MH, YT, TK and YT are full-term employees of Kao Corp. M M-H, MH MF W-N and K-K and HI declare no conflict of Interest.

Acknowledgments

We would like to thank Ms. Haruko Toyoshima for helping our measurement.

Abbreviations

SC: stratum corneum; TEWL: transepidermal water loss; NMF: natural moisturizing factor; FFA: free fatty acids; ATR-FTIR: Attentuated total reflectance Fourier Transform Infrared spectrometer; CERs: ceramides; NPLS-ESI-MS: Normal-phase liquid chromatography- electrospray ionization-mass spectrometry; SDT: Sorption-Desorption Test;

References

[1] Barker N, Hadgraft J, Rutter N. Skin Permeability in the Newborn. J Invest Dermatol 1987;88: 409–11.

[2] Kalia YN, Nonato LB, Lund CH, Guy RH. Development of skin

barrier function in premature infants. J Invest Dermatol1998;111: 320-26.

[3] Mancini AJ. Skin. Pediatrics 2004;113:1114-19.

[4] Visscher MO, Chatterjee R, Munson KA, Pickens WL, Hoath SB. Changes in Diapered and Nondiapered Infant Skin Over thr First Month of life. Pediatr Dermatology 2000; 17: 45-51.

[5] Giusti F, Martella A, Bertoni L, Seidenari S. Skin barrier, hydration, and pH of the skin of infants under 2 years of age. Pediatr Dermatol 2001; 18: 93-96.

[6] Nikolovski J, Stamatas GN, Kollias N, Wiegand BC. Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life. J Invest Dermatol 2008;128: 1728-36.

[7] Saijo S, Tagami H. Dry Skin of Newborn Infants: Functional Analysis of the Stratum Corneum. Pediatr Dermatol1991; 8: 155-59.

[8] Takada S, Naito S, Sonoda J, Miyauchi Y. Non Invasive In vivo Measurement of Natural Moisturizing Factor Content in Stratum Corneum of Human Skin by Attenuated Total Reflection Infrared Spectroscopy. Spectrosc 2012;66: 26-32.

[9] Masukawa Y, Narita H, Shimizu E, Kondo N, Sugai Y, Oba T, et al. Characterization of overall ceramide species in human stratum corneum. J

Lipid Res 2008; 49: 1466-76.

[10] Masukawa Y, Narita H, Sato H, Naoe A, Kondo N, Sugai Y, et al. Comprehensive quantification of ceramide species in human stratum corneum. J Lipid Res 2009; 50: 1708-19.

[11] Tagami H, Kanamaru Y, Inoue K, Suehisa S, Inoue F, Iwatsuki K, et al. Water sorption-desorption test of the skin in vivo for functional assessment of the stratum corneum. J Invest Dermatol 1982; 78: 425-28.

[12] Van Smeden J, Hoppel L, van der Heijden R, Hankemeier T, Vreeken RJ, Bouwstra JA . LC/MS analysis of stratum corneum lipids: ceramide profiling and discovery. J Lipid Res 2011; 52: 1211-21.

[13] Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ,et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res 2012; 53:2755-66

[14] Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, et al. Lamellar lipid organization and ceramide composition in the stratum corneum of patients with atopic eczema. J Invest Dermatol 2011; 131: 2136-38.

[15] Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, et al . Changes in the ceramide profile of atopic dermatitis patients. J Invest Dematol 2010 ; 130: 2511-14.

[16] Visscher MO, Narendran V, PickensWl, Laruffa AA. Vernix caseosa in neonatal adaptation. J Perinatology 2005; 25: 444-46.

[17] Tagami H. Location- related differences in structure and function of the stratum corneum with special emphasis on those of facial skin. Int J Cosmet Sci 2008; 30: 413-34.

[18] Berardesca E, Maibach H. Monitaring the water holding capacity in visually non-irritated skin by plastic occlusion stress test. Clin Exp Dermatol 1990; 15: 107-10

[19] Hoeger PH, Enzmann CC. Skin physiology of the neonate and young infant: A prospective study of functional skin parameters during early infancy. Pediatr Dermatol 2002; 19: 256-62.

Figure legends

Figure1. Skin hydration and water sorption and desorption ratio (A.U.= Arbitrary units) of upper thighs and buttocks.

(A) Longitudinal alterations of skin hydration of neonatal-infantile upper thighs during 12 months development. (*P<0.05 **P<0.01 ***P<0.001 compared to mothers) ○: Infants ●: Mothers

(B) Water holding capacity of upper thighs determined by Sorption-Desorption Test. (*P<0.05 **P<0.01 ***P<0.001 compared to mothers). ○: Water sorption ratio of infants ●: Water sorption ratio of mothers △; Water desorption ratio of infants ▲: Water desorption ratio of mothers

(C) Developmental alterations of skin hydration of neonatal-infantile buttocks during 12 months development. (*P<0.05 **P<0.01 ***P<0.001 compared to mothers)

○: Infants ●: Mothers

(D) Water holding capacity of buttocks determined by

Sorption-Desorption Test. (*$P<0.05$ **$P<0.01$ ***$P<0.001$ compared to mothers) ○: Water sorption ratio of infants ●: Water sorption ratio of mothers △; Water desorption ratio of infants ▲: Water desorption ratio of mothers

Figure 2. TEWL values

(A) Longitudinal alterations of TEWL of upper thighs during 12 months development. **$P<0.01$ ***$P<0.001$ compared to mothers.) ○: Infants ●: Mothers

(B) Longitudinal alteration of TEWL of buttocks during 12 months development. ***$P<0.001$ compared to mothers.) ○: Infants ●: Mothers

Figure 3. Ester binding sebum content.

(A) Ester binding sebum content of upper thighs. (*$P<0.05$ compared to mothers) ○: Infants ●: Mothers

(B) Ester binding sebum content of buttocks. (*$P<0.05$ **$P<0.01$ ***$P<0.001$ compared to mothers) ○: Infants ●: Mothers

Figure 4. Developmental alterations of total amount of CERs (ng/ μg protein)

(A) Total amount of CERs of upper thighs. (*P<0.05 **P<0.01 ***P<0.001 compared to mothers) ○: Infants ●: Mothers

(B) Total amount of CERs of buttocks. (***P<0.001 compared to mothers) ○: Infants ●: Mothers

Figure 5. Developmental alterations of the amount of specific CER subclasses (ng/ μg protein)

(A) CER[EOS] and CER[EOH+EOP] of upper thighs.

(*P<0.05 **P<0.01 ***P<0.001 compared to mothers)

○: CER[EOS] of Infants ●: CER[EOS] of mothers △: CER[EOH+EOP] of Infants ▲: CER[EOH+EOP] of mothers

(B) CER[EOS] and CER[EOH+EOP] of buttocks. (*P<0.05 **P<0.01 ***P<0.001 compared to mothers) ○: CER[EOS] of Infants ●: CER[EOS] of mothers △: CER[EOH+EOP] of Infants ▲: CER[EOH+EOP] of mothers

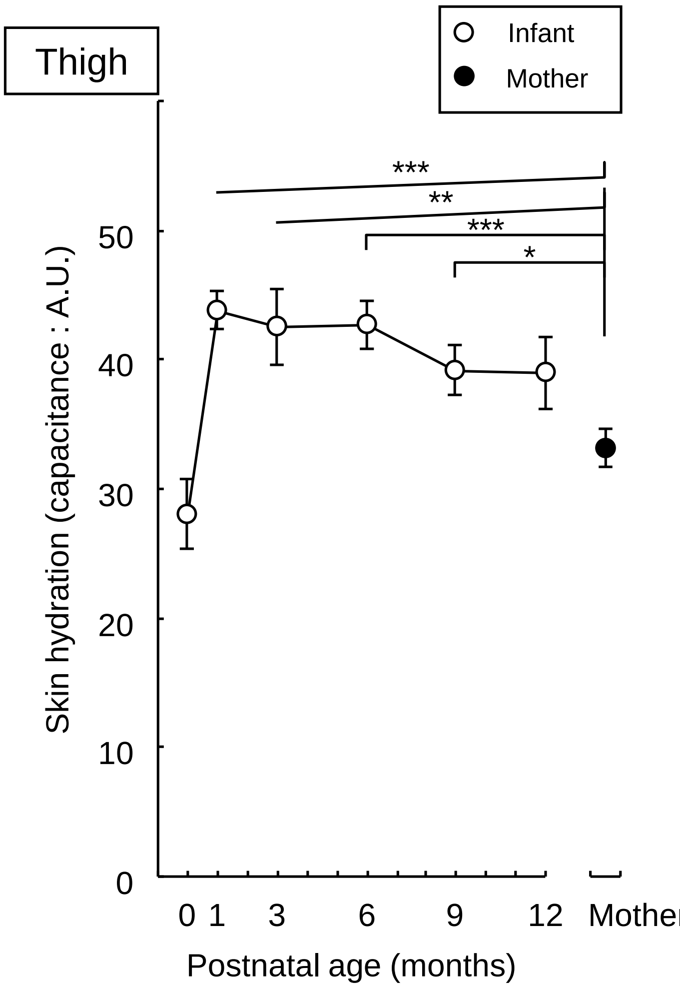

Figure 1A

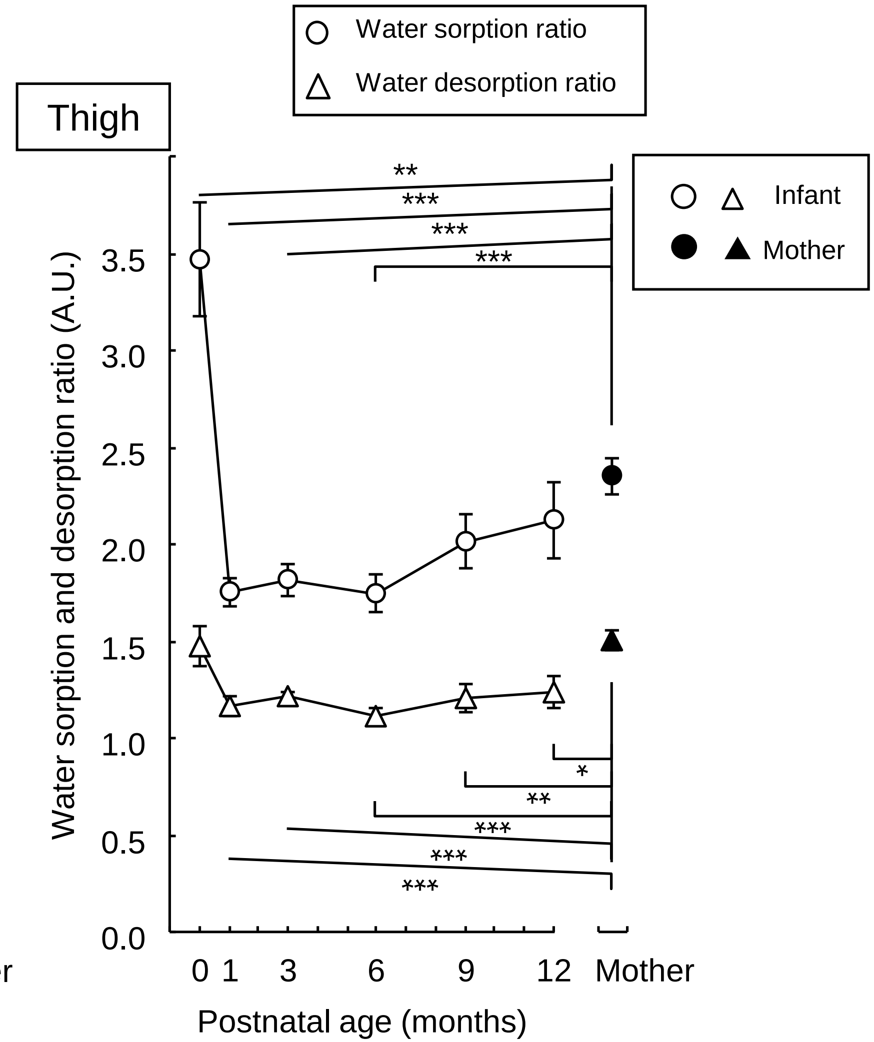

Figure 1B

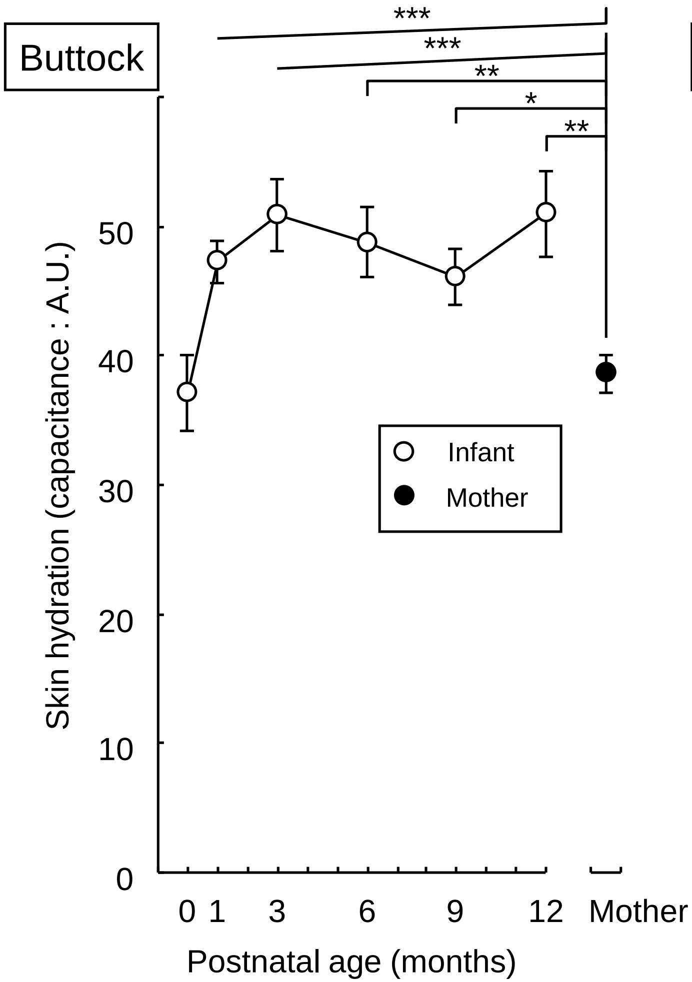

Figure 1C

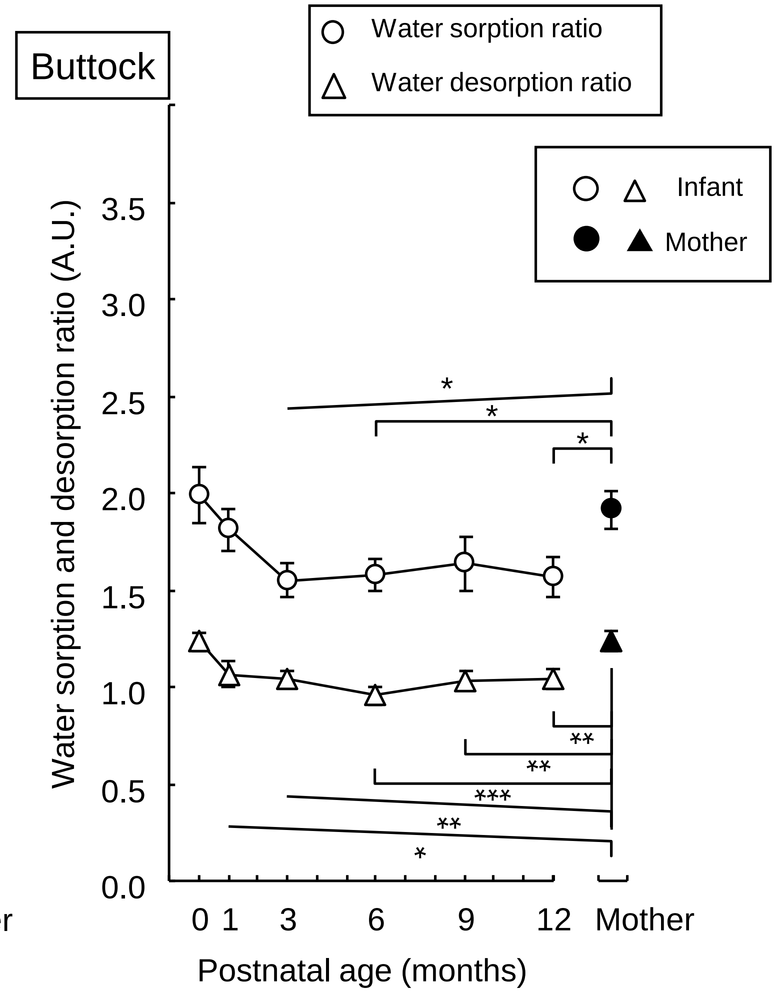

Figure 1D

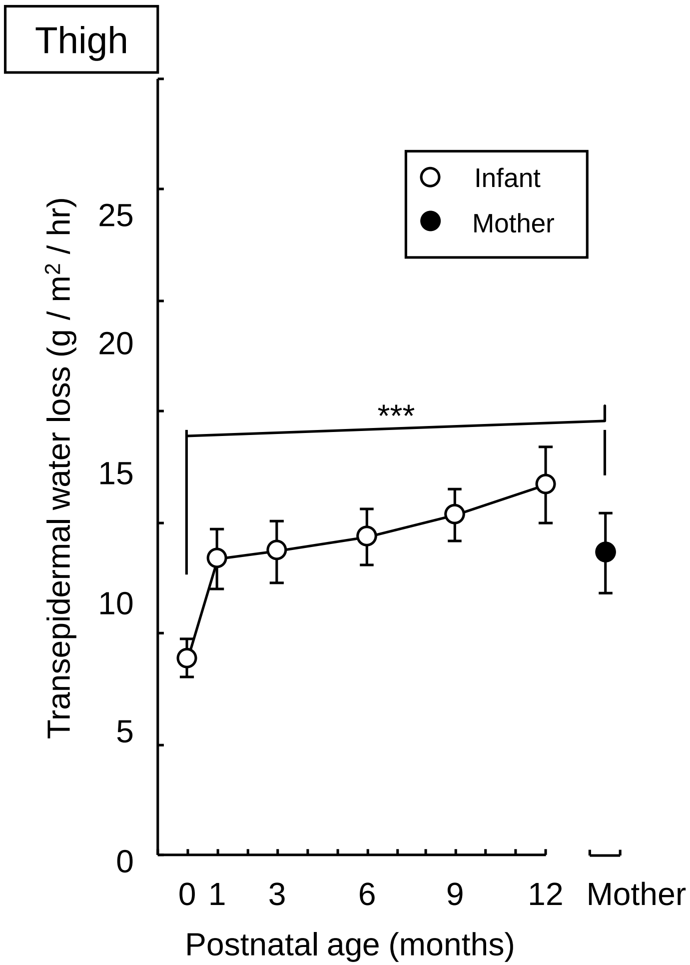

Figure 2A

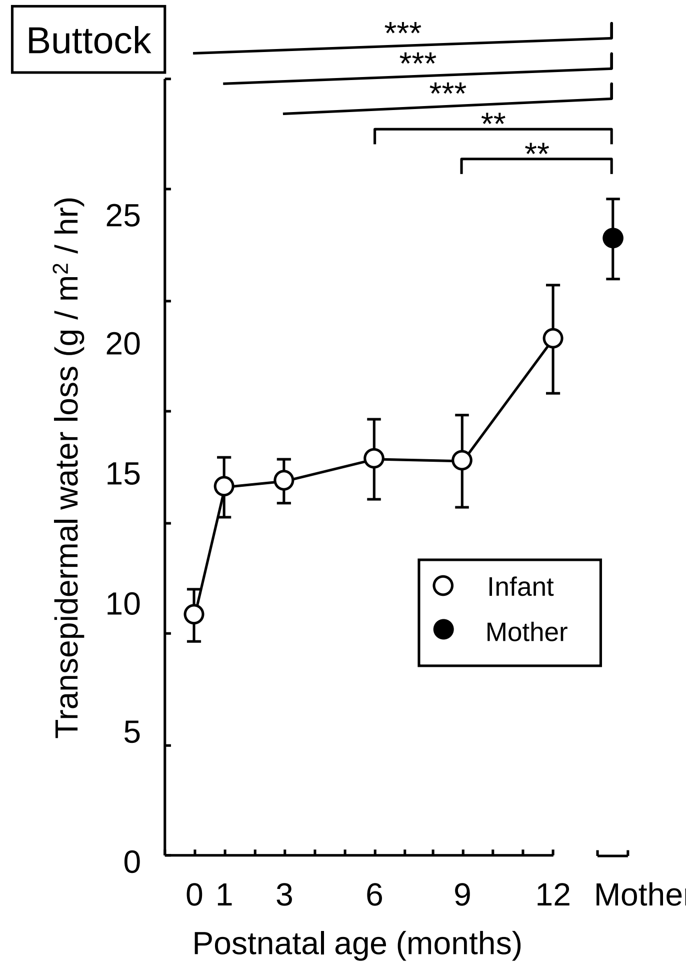

Figure 2B

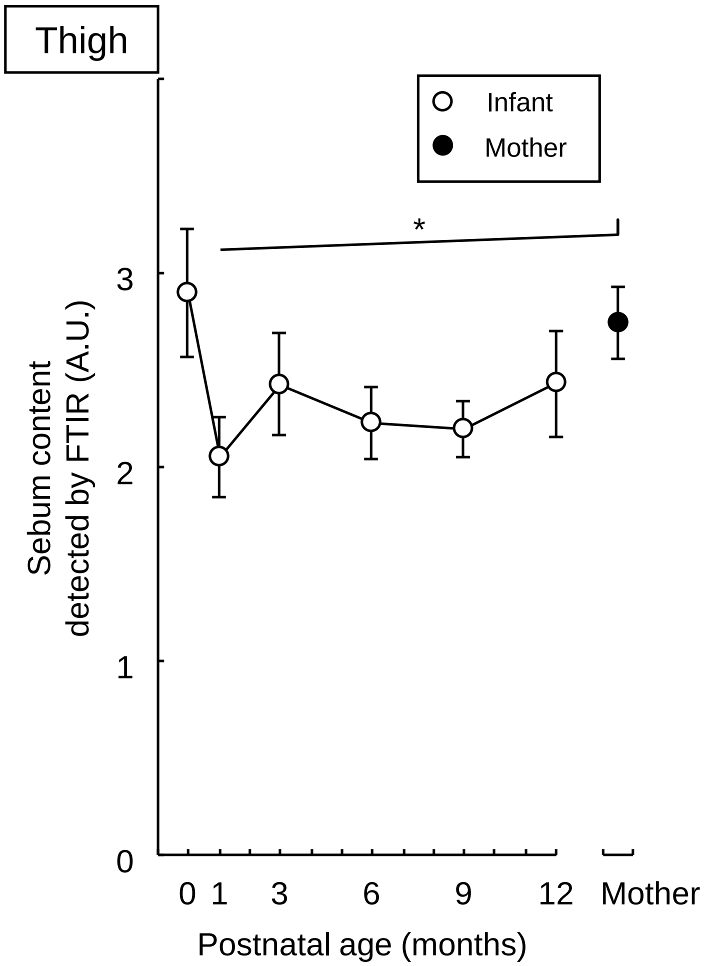

Figure 3A

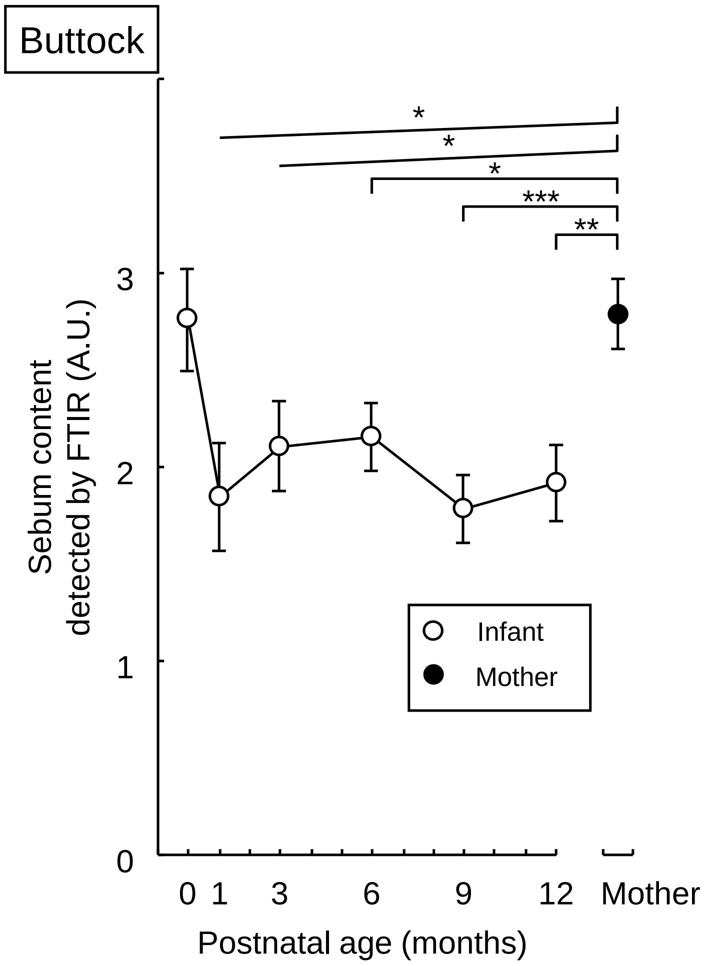

Figure 3B

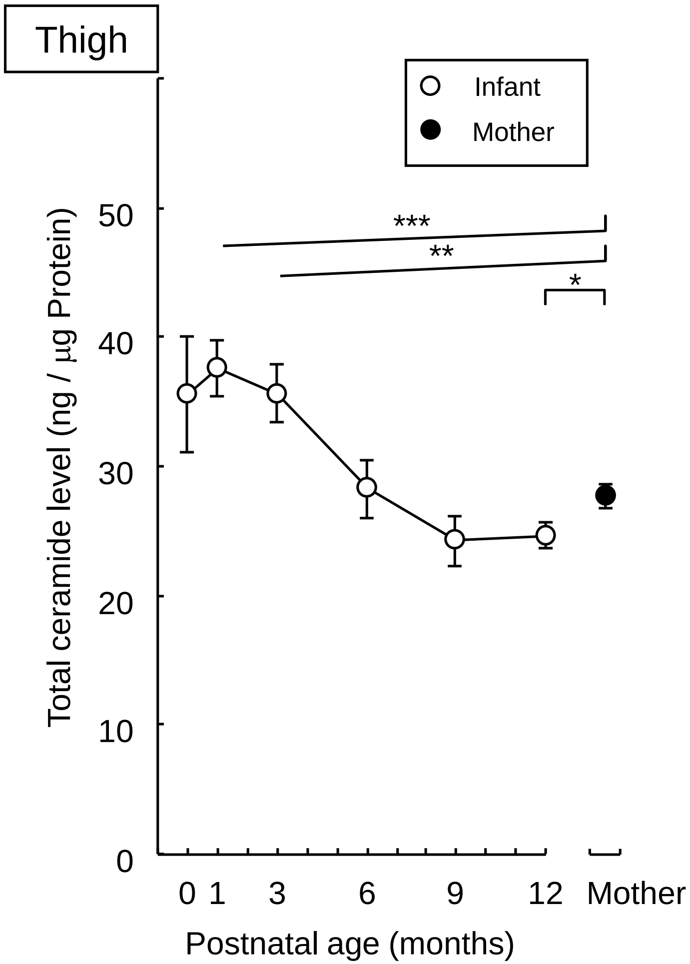

Figure 4A

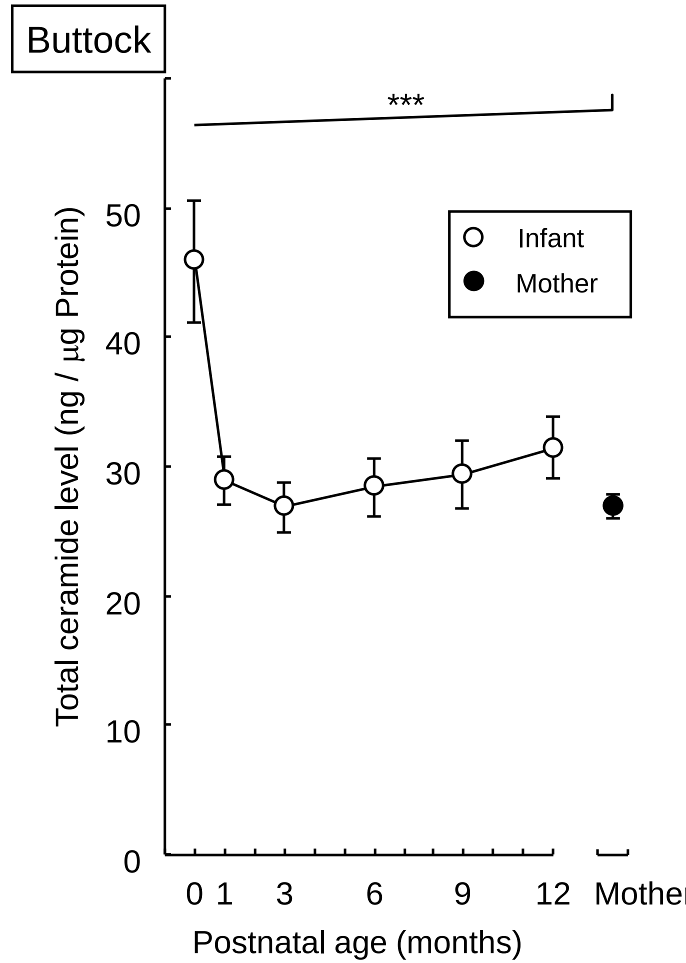

Figure 4B

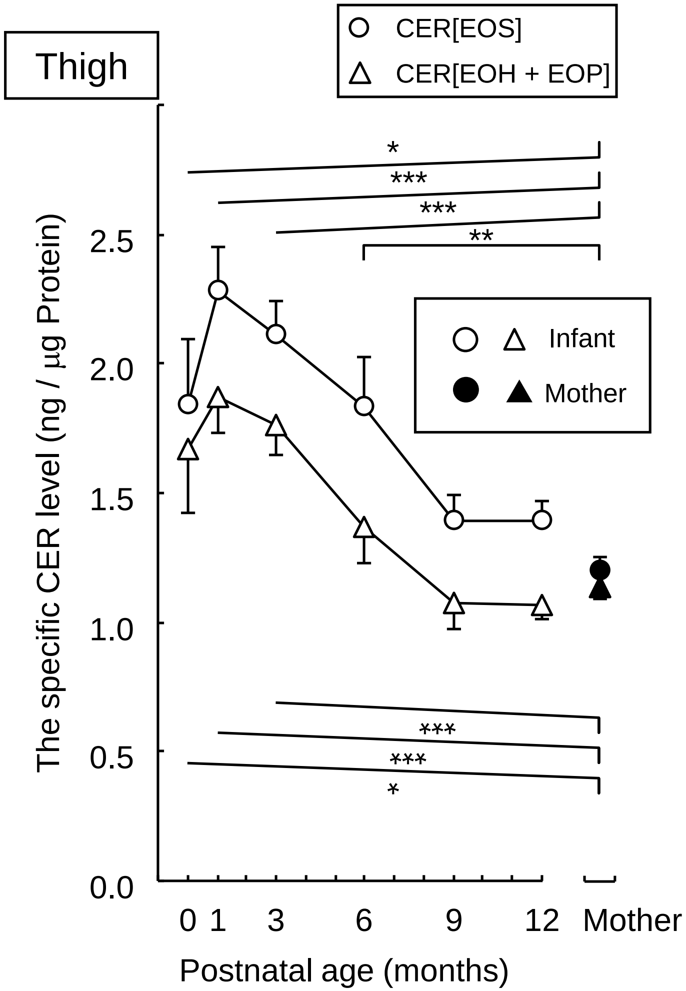

Figure 5A

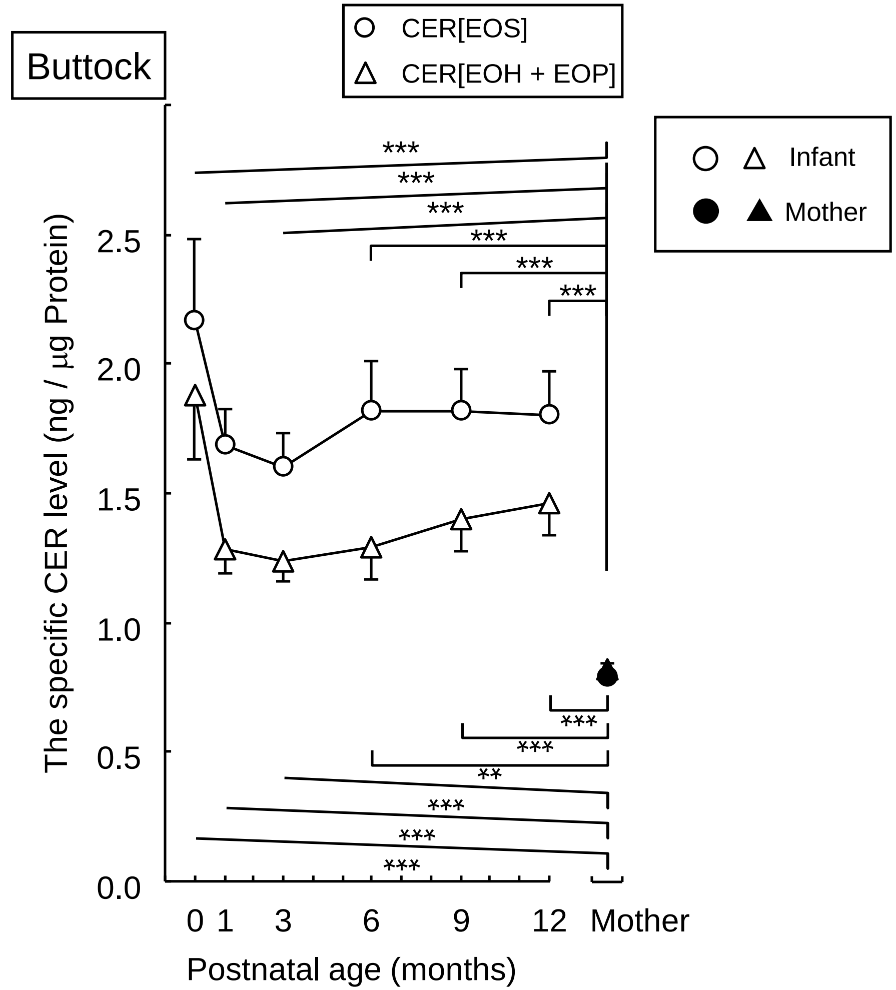

Figure 5B