

INVESTIGATIVE REPORT

Functional Characteristics of the Skin Surface of Children Approaching Puberty: Age and Seasonal Influences

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The aim of this study was to determine differences in the functional properties of the stratum corneum of children and adults, focusing on the influence of approaching puberty. Biophysical measurements were made of the stratum corneum of 32 healthy Japanese children aged 10–14 years and their mothers in summer and the following winter. The children showed significantly lower skin surface hydration. Stratum corneum barrier function, evaluated in terms of trans-epidermal water loss, was poorer on the forearm in the children than in the adults regardless of season. By contrast, the stratum corneum barrier of the cheek, which was better in the children, tended to become poorer when the children reached puberty. Although the immaturity of the cornified envelopes of the superficial corneocytes, which ratio increased significantly in winter, was not different from that of adults, the corneocytes were significantly smaller in the children, suggesting a more rapid turnover of the stratum corneum. The amount of skin surface lipid, which was measured only on the cheek, remained low until 13 years of age, but at 14 years of age it increased remarkably, approaching adult levels. We conclude that, until puberty, most functional characteristics of the skin of children remain distinct from those of adults. *Key words: children; puberty; skin surface hydration; skin surface lipids; trans-epidermal water loss.*

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Although the skin of children appears different from that of adults, few studies have been conducted on the skin surface properties of children. The skin of newborn babies normally has a greasy appearance due to the presence of vernix caseosa, but once this has been shed, the skin remains dry (1, 2). The water content of the stratum corneum (SC) of newborn's forearm skin, measured with a high-frequency conductance meter, approaches the adult level within 2 weeks after birth (3). With regard to the

skin of older infants, mildly elevated conductance values have been reported on the arm of those aged between 3 months and 6 years (4, 5), but significantly lower values have been reported on the face and trunk skin of children below 12 years of age (6). Besides these studies on infant skin, there is a lack of data about the skin of older children who have reached puberty.

The aim of this study was to investigate the skin surface properties of children approaching puberty, focusing on the differences from adult skin. Moreover, since SC functions show deterioration in the winter in adults over 20 years of age (7), we conducted the present study in both summer and winter in order to investigate the influence of seasonal change on the children's skin.

MATERIALS AND METHODS

Subjects

A total of 32 healthy Japanese children, age range 10–14 years, participated in the study. None of the children had any skin diseases requiring medical treatment. The subjects comprised 5 boys and 5 girls aged 10–11 years, 5 boys and 5 girls aged 12–13 years, and 6 boys and 6 girls aged 14 years. In addition, their mothers, age range 30–48 years (average age 40 ± 4 years), were examined as adult controls. Informed consent was obtained from the mothers in the presence of their children.

Biophysical measurements were performed in a climate-controlled room that was adjusted to 24°C and 50% relative air humidity. The measurements were carried out in July, then in February of the following year. The time of day measurements were taken, the measuring instruments, and the measuring personnel were identical for the summer and winter studies.

Clinical evaluation of the skin

Clinical assessment for dry skin was performed on the cheek, extensor forearm and flexor forearm by a dermatologist, based on the following scores: no sign (0 point), slight (1 point), marked (2 points), and severe scaling (3 points).

Measurements of biophysical parameters of the skin surface

All the biophysical parameters were measured 30 min after the faces and forearms of subjects had been washed with soap (D-Program Face Wash Soap, Shiseido Co., Tokyo, Japan). Non-invasive biophysical measurements of skin surface hydration and trans-epidermal water loss (TEWL), and collection of superficial SC for the analysis of cornified envelopes (CE) of corneocytes were performed on the cheek and flexor

aspect of the forearm in summer and winter. Corneocyte size was studied only in winter. Skin surface lipids were collected from the cheek adjacent to the ala nasi and from the centre of the forehead only in winter.

Skin surface hydration

The skin surface hydration state was assessed with a Skicon 200 (IBS, Hamamatsu-shi, Japan) and a Corneometer CM825 (Courage & Khazaka, Cologne, Germany). In the case of the Skicon 200, the mean of five values was calculated from seven measurements, excluding the maximal and minimum values, because Skicon measurements are very sensitive to the hydration state of the normal skin surface, and tend to show large variations (8). In the case of the Corneometer, we took the mean of five measured values.

To evaluate the correlation between the skin surface hydration state and clinically observable dry skin, the children were divided into two groups according to their clinical features, i.e. a group of children with dry skin whose clinical scores ranged from 1 to 3, and a group of children without dry skin whose score was 0. The averages of the skin surface hydration values of the two groups were calculated.

TEWL

TEWL was measured with a Vapometer (Delfin Corporation, Kupio, Finland) because of the good correlation between Vapometer and Tewameter and the convenience of conducting measurements quickly at different anatomical body locations (9).

Ratio of immature cornified envelopes of superficial corneocytes

Superficial SC samples were collected by means of non-invasive tape stripping. The maturity of CEs was evaluated as described previously (10). Briefly, CEs were prepared from the SC samples by extensive boiling and washing in a dissociation buffer consisting of 2% sodium dodecyl sulphate (SDS)-20 mM

dithiothreitol (DTT)-5 mM ethylene diaminetetraacetic acid (EDTA)-0.1 M Tris-HCl (pH 8.5) to remove soluble substances. The CE suspension was spotted onto a slide glass, air-dried, and fixed in cold acetone (-30°C , 10 min). CEs were stained with anti-involucrin (1:100), followed by fluorescein isothiocyanate (FITC)-labelled anti-mouse immunoglobulin (Ig) (1:100) to evaluate the loss of antigenicity during maturation, and with Nile red (3 $\mu\text{g}/\text{ml}$) to assess their hydrophobicity. Fluorescence images were obtained using a fluorescence microscope (Olympus, Tokyo, Japan) equipped with a CCD (charge couple device) camera (SPOT, Diagnostic Instruments, MI, USA), and the ratio of involucrin-positive immature CEs was estimated by image analysis software (Win Roof ver.3.5, Mitani Corp., Fukui, Japan).

Corneocyte size

Superficial SC samples collected by non-invasive tape stripping were transferred to silicon-coated slide glass by sticking the tape onto the glass, and the tape was released from the slide glass by overnight incubation in xylene. The SC remained on the slide glass, and was subjected to haematoxylin and eosin staining. The corneocytes were imaged with a light microscope (Olympus, Tokyo, Japan). The projected area of 30 cells was estimated with an image analysis device and the mean value was calculated.

Skin surface lipids

A square (2×2 cm) of 5B filter paper (Toyo Roshi Kaishya, Tokyo, Japan) pre-treated with acetone was placed on the cheek under a 100 g weight for 1 min. Skin surface lipids absorbed in the filter paper were extracted with acetone, and analysed by means of gas chromatography (Agilent Technologies, Inc., CA, USA). Trimethylsilyl derivation was achieved in 50 μl of N,O-bis(trimethylsilyl)acetamide on a heat block at 105°C for 2 min. After cooling, 450 μl of cyclohexane was added, and 1 μl aliquots of this solution were subjected to gas chromatography. Total amount of fatty acid, squalene, cholesterol, wax ester and triglyceride was measured.

Table I. Comparison of biophysical parameters of the skin surface of children and adults

Parameter	Site	Season	Children	Adults	p-value
Skin surface hydration (Skicon 200, μS)	Cheek	Summer	20.0 \pm 17.2	58.5 \pm 61.8	<0.0001
		Winter	10.6 \pm 26.2	32.6 \pm 40.0	<0.0001
	Forearm	Summer	19.3 \pm 6.3	30.2 \pm 20.0	0.0016
		Winter	6.5 \pm 2.0	8.6 \pm 4.9	0.1000
Skin surface hydration (corneometer, au)	Cheek	Summer	45.7 \pm 8.4	56.3 \pm 8.8	<0.0001
		Winter	31.8 \pm 11.2	49.2 \pm 9.9	<0.0001
	Forearm	Summer	30.7 \pm 3.3	37.2 \pm 5.0	<0.0001
		Winter	27.7 \pm 3.2	31.2 \pm 5.0	0.0026
Transepidermal water loss ($\text{g}/\text{m}^2/\text{h}$)	Cheek	Summer	12.8 \pm 3.8	21.1 \pm 20.2	0.0010
		Winter	38.1 \pm 14.8	40.5 \pm 16.8	0.5360
	Forearm	Summer	8.4 \pm 3.8	6.7 \pm 6.2	0.0016
		Winter	13.9 \pm 4.1	11.8 \pm 4.3	0.0484
Ratio of immature cornified envelopes (%)	Cheek	Summer	45.6 \pm 28.4	42.9 \pm 23.2	0.6794
		Winter	58.4 \pm 25.0	58.5 \pm 25.9	0.9038
	Forearm	Summer	20.7 \pm 23.6	21.1 \pm 19.8	0.3755
		Winter	27.7 \pm 25.3	23.1 \pm 24.3	0.5728
Corneocyte surface area (μm^2)	Cheek	Winter	683.7 \pm 55.0	738.9 \pm 69.2	0.0008
	Forearm	Winter	953.7 \pm 81.7	1022.7 \pm 105.9	0.0049
Skin surface lipids (μg)	Cheek	Summer	76.3 \pm 98.3	132.0 \pm 68.1	<0.0001
		Winter	75.8 \pm 77.4	148.6 \pm 95.6	0.0001
	Forehead	Winter	74.5 \pm 54.7	125.2 \pm 88.1	0.0082

au: arbitrary units.

Statistically significant values ($p < 0.05$) are shown in bold text.

Testosterone concentration in the saliva

Salivary testosterone levels were measured by using commercially available testosterone assay kit, Testosterone Immunoassay (R&D Systems Inc., MN, USA).

Statistics

The significance of differences between children and adults was examined by using the F test, Student's *t*-test, Welch's *t*-test or Mann-Whitney *U* test (Table I). Similarly, the significance of differences between the summer and winter measurements was assessed by using the paired *t*-test and Wilcoxon signed-rank test (see Figs 2, 4–7). The results for the groups of children aged 10–11 years, 12–13 years and 14 years, and the adult group were compared using the Kruskal-Wallis test, one-factor analysis of variance (ANOVA) or the *post-hoc* test (see Figs 2, 4–7). Water content of the SC between children with and without dry skin on the cheek was compared using F test and Student's *t*-test (Fig. 3).

RESULTS

Clinical evaluation of skin dryness

Dry skin was seen in approximately one-third of the children in winter, whereas it was seen in only a few of their mothers, and then only on the extensor forearm. In the children, scaling was observed on the cheek and extensor forearm, but not on the flexor forearm (Fig. 1). However, in summer, none of the children showed any scaling.

Skin surface hydration

Both the skin surface hydration values measured with the Skicon 200 and those with the Corneometer were higher in summer than in winter, although the former was more sensitive to seasonal differences. The measured values obtained with the two instruments corresponded well with each other. They tended to be higher on the cheek than on the forearm. The skin surface hydration state of the children was significantly lower than that of the adults in both seasons (Table I, Fig. 2). The skin hydration state of the children showing scaling was evidently lower than that in the children

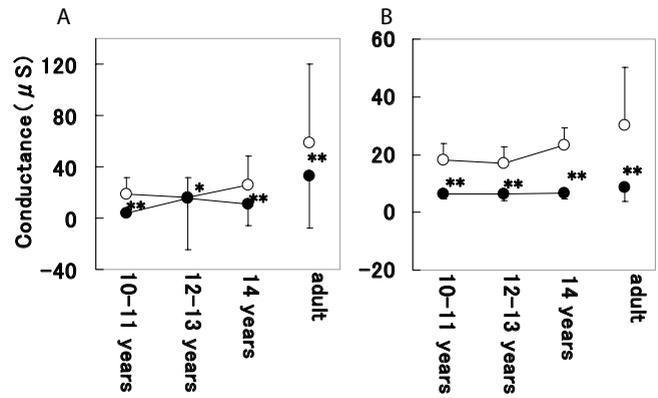


Fig. 2. Comparison of the skin surface hydration state of children and adults. The skin surface hydration state of (A) the cheek and (B) the flexor forearm was measured with a Skicon-200, as described in Materials and Methods. Open circles and closed circles represent the values measured in summer and winter, respectively. Data are presented as the mean ± standard deviation. * ($p < 0.05$) and ** ($p < 0.01$) indicate statistically significant differences between summer and winter.

without scaling, and there appeared to be a close correlation between the clinically assessed state and the water content of SC (Fig. 3).

TEWL

TEWL values were markedly different depending on the season and on the location on the body. They were higher in winter than in summer, and much higher on the cheek than on the forearm. The TEWL values measured on the flexor forearm of the children were significantly higher than those of the adults in both seasons. However, on the cheek, the children's TEWL values were significantly lower than those of the adults in summer, but not in winter. TEWL of the children tended to become lower on the forearm and higher on the cheek with increasing age (Table I, Fig. 4).

Ratio of immature cornified envelopes

The ratio of immature CEs in the children was similar to that of the adults, except for the group of 14 year-olds, whose ratio was higher than those of other groups.

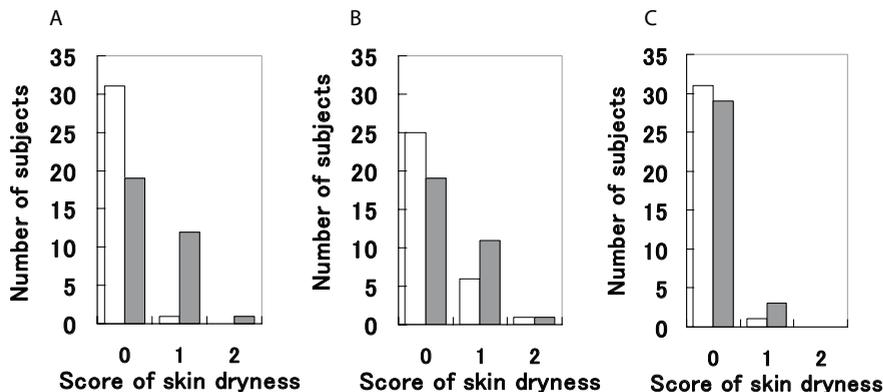


Fig. 1. Grade of dry skin observed in winter. Clinical assessment of dry skin was performed on (A) the cheek, (B) the extensor forearm, and (C) the flexor forearm in children (solid columns) and adults (open columns). The scoring system was: absent (0 points), slight (1 point), marked (2 points), and severe (3 points). There were no subjects with severely dry skin.

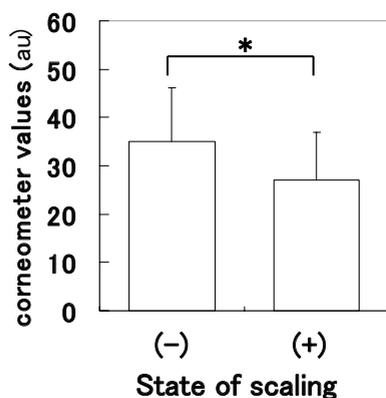


Fig. 3. Comparison of water content of the SC of children with and without dry skin on the cheek. Clinical assessment of dry skin was performed on the cheek. The scoring system was: absent (0 points), slight (1 point), marked (2 points) and severe (3 points). The children were divided into two groups according to their clinical features; those with dry skin whose clinical assessment scores ranged from 1 to 3, and those without dry skin (score 0). Then the averages of the skin surface hydration values of the 2 groups were calculated. (-) indicates “group of children with dry skin”. (+) indicates “group of children without dry skin”. Data presented are the mean ± standard deviation. *($p < 0.05$) indicates a statistically significant difference. Au: arbitrary units.

Ratios of immature CEs were higher on the cheek than on the flexor forearm. On both sites, the ratio of immature CEs became elevated in winter, compared with summer (Table I, Fig. 5).

Corneocyte size

Corneocyte size on the cheek was smaller than on the flexor forearm, suggesting that the SC turnover time is shorter in the former than in the latter. They were smaller in the children than in the adults on both sites, suggesting that the SC turnover in children, even including those aged 14 years, is faster than that of adults (Table I, Fig. 6). Because SC was collected only in

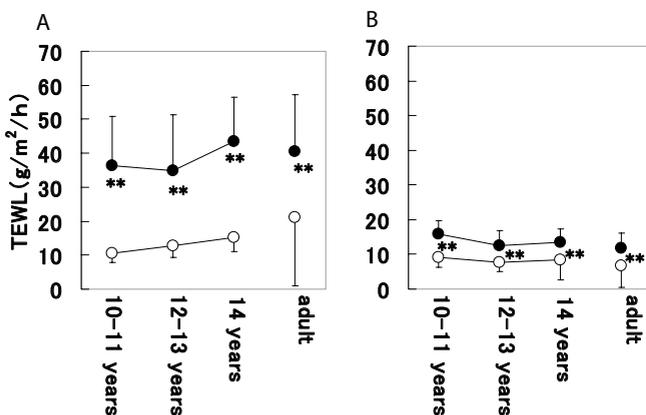


Fig. 4. Comparison of trans-epidermal water loss (TEWL) between the children and adults. TEWL was measured with a vapometer on (A) the cheek and (B) the flexor forearm. Open circles and closed circles represent the values measured in summer and winter, respectively. Data are presented as the mean ± standard deviation. **($p < 0.01$) indicate statistically significant differences between summer and winter.

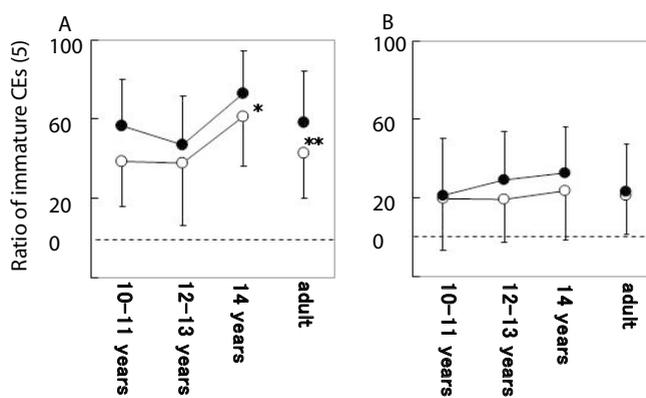


Fig. 5. Comparison of the ratio of immature cornified envelopes (CEs) of children and adults. CEs were collected on (A) the cheek and (B) the flexor forearm, and their maturity was evaluated as described in Materials and Methods. Open and closed circles represent the values measured in summer and winter, respectively. Data presented are the mean ± standard deviation. *($p < 0.05$) and **($p < 0.01$) indicate statistically significant differences between summer and winter.

winter, we could not determine whether the corneocyte size exhibits seasonal change.

Skin surface lipids

In contrast to other functional parameters, there was no seasonal change in the amount of skin surface lipids on the cheek. In the children, it tended to remain low until age 13 years, but began to increase rapidly at the age of 14 years, quickly reaching adult levels on both cheek and forehead (Table I, Fig. 7).

Absolute values of skin surface lipid components and the ratios of wax esters, fatty acids, squalene and cholesterol in the children’s skin were similar to, or lower than those in adult skin. The absolute values of triglycerides in the children’s skin were lower than those in the adult skin, but the ratio of triglycerides in the children’s skin

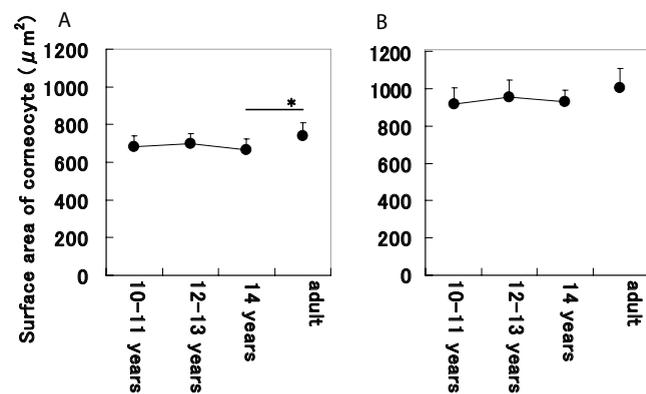


Fig. 6. Comparison of corneocyte size in children and adults in winter. The stratum corneum samples were collected on (A) the cheek and (B) the flexor forearm, and corneocyte size was estimated as described in Materials and Methods. Data presented are the mean ± standard deviation. *($p < 0.05$) indicates statistically significant differences between groups of children of different ages and adults.

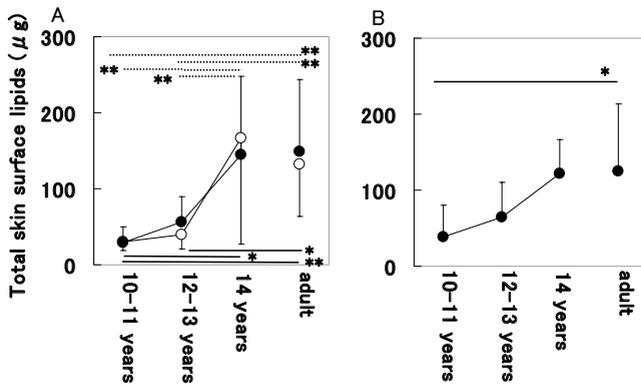


Fig. 7. Comparison of skin surface lipids of children and adults. Lipids were collected from (A) the cheek and (B) the forehead for gas chromatographic analysis as described in Materials and Methods. Open and closed circles represent the values measured in summer and winter, respectively. Data presented are the mean \pm standard deviation. * ($p < 0.05$) and ** ($p < 0.01$) indicate statistically significant differences between children of different age groups and adults in summer (solid line) and winter (dashed line).

was higher. A similar, though less marked, correlation was observed between younger and older children.

Differences between the sexes

Six of the 16 girls studied in summer and 9 girls in winter experienced menarche. However, there was no clear difference in the findings between the boys and girls (data not shown). There was some difference in SC function between the girls who were not yet menstruating and those with normal menstrual cycles, but it was not clear whether they depended on the presence or not of menarche, or simply reflected age differences.

Testosterone level in saliva

The testosterone levels evaluated in the saliva of all children were remarkably lower than those of their mothers. There were no statistically significant differences in its level between boys and girls or between the different age groups.

DISCUSSION

This study examined the skin of Japanese children over 10 years of age, i.e. at the beginning of puberty, when physiological changes including active sebum secretion begin to occur. Tokudome & Tagami (6) reported extremely low skin surface hydration on the face and trunk in Japanese children aged 4–11 years, even compared with the skin of elderly individuals, who are well-known to develop senile xerosis. Our present biophysical data, obtained in children older than this, showed similar results, i.e. the children's skin showed a significantly low surface hydration state and low levels of skin surface lipids.

Even in normal adults, the dry and cold winter season has a great influence on the skin condition, and affects the face more than the forearm (7). Clinical observation revealed that approximately one-third of the children in our study exhibited visible dry skin changes in winter, in contrast to their mothers, the majority of whom did not show any dry skin in winter.

We found that the SC barrier function of the children, as assessed by TEWL measurements, exhibited distinct age- and location-dependent functional characteristics. On the flexor forearm, the SC barrier function tended to improve with increasing age, whereas it became poorer on the cheek of the older children. Sebum excretion, which starts to occur on the cheek at around the age of 14 years, worsens the poor facial SC barrier function by inducing the proliferation of skin surface microflora, which may exert an irritating effect on the skin.

CE is a thin insoluble structure enveloping corneocytes, and is formed via transglutaminase-catalysed cross-linking of various precursor proteins, including involucrin and loricrin (11). Thus, it provides a foundation for the organization of the intercellular lipids that are essential for the barrier function of the SC. The presence of immature CEs has been detected in the outermost layer of the SC of barrier-impaired skin, including even normal facial skin (10, 12). The ratio of immature CEs on the face does not depend on the age of the subjects (within the range 10–50 years), but it increases during the winter season; the ratio of immature CEs in the children was reported to be similar to that of adults (13). Our present results agree well with these findings. Although it is not known why the ratio of immature CEs in the 14-year-old age group tended to be higher than in other age groups, both children and adults, we speculate that the start of high sebum excretion in the children in this age group may lead to mild inflammation, possibly due to the proliferation of surface microflora as mentioned above.

The skin surface hydration state of the children was remarkably lower than that of the adults, as is clear from the development of dry skin in winter, even on the face. It is well known that dry skin is observed in elderly persons in winter. Such senile xerosis is due to reduced levels of skin surface lipids, intercellular lipids and natural moisturizing factors (NMF), together with the retention of effete corneocytes on the skin surface due to slower turnover of the SC, reflecting the less active epidermal proliferation in elderly individuals that is noted histologically as atrophic epidermis (14). In the present study, we also found similarly low skin surface lipids in the children's skin, reflecting lower production of all the components of skin surface lipids (data not shown).

Moreover, we observed much smaller corneocytes in the dry skin of children, as distinct from the larger corneocytes that are found in senile xerosis (14). It is well

known that corneocyte size reflects the turnover time of the SC in adults (15) and that corneocyte size increases with age (16, 17). Thus, our present finding of smaller corneocytes in the skin of children may reflect much more active epidermal proliferation, being distinct from the epidermis of senile xerosis. It is noteworthy that smaller corneocytes are seen in pityriasis alba, which is found even on the face of normal healthy children, but never in healthy adults, in winter (18). Pityriasis alba is also seen in atopic xerosis, which develops on the background of underlying mild inflammation accompanying enhanced epidermal proliferation that is noted histopathologically as mild acanthosis (19). Therefore, another possible pathomechanism underlying the dry skin in children in winter is the presence of mild subclinical underlying inflammation, as noted in pityriasis alba. It is likely that the decrease in the mass levels of intercellular lipids and altered ratios of fatty acids esterified to ceramide 1 in winter, as demonstrated by Rogers et al. (20), contribute to the decreased SC barrier function and xerosis.

It is of note that remarkably high excretion of skin surface lipids becomes detectable abruptly at the age of 14 years, being comparable with the adult levels on both the cheek and forehead. Takahashi et al. (17) also reported similar findings on the forehead, i.e. the amount of skin surface lipids drastically increases from approximately age 10 years, reaching a maximum in the 20s. In the present study, however, we could not detect any clear change in the saliva level of testosterone, the hormone that stimulates skin surface lipids production, with increasing age of children. The sudden up-regulation of skin surface lipids synthesis at 14 years of age may not be explained simply by an increase in circulating hormonal levels, but may also reflect the enhanced reactivity of the sebaceous gland receptors to the hormone at this age.

In addition to testosterone, female hormones are suspected to play a role in inducing pubertal change in the ceramide components of the SC (21). However, it is noteworthy that no differences in skin surface properties were seen between boys and girls in the present study. In a similar fashion, no gender difference has been observed for skin surface hydration or TEWL, although a significant difference in the secretion of skin surface lipids was reported between adult males and females (22, 23).

The results of this study may be summarized as follows: (i) there is a difference in skin surface physiology even between children of different age groups, reflecting their growth stage; (ii) some skin functions, such as the secretion of skin surface lipids, change remarkably in a short period during puberty even without any detectable increase in their saliva testosterone levels; and (iii) there are no significant differences between the sexes in the physiological parameters of the skin surface, in the age range examined.

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