Age-Dependent Transformation of Skin Biomechanical Properties and Micromorphology during Infancy and Childhood

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TO THE EDITOR

It is well known that skin continues to undergo structural and functional changes after birth. These include skin surface acidification (Fluhr et al., 2012); increased hydration over the first few weeks of life, followed by decreased hydration (Nikolovski et al., 2008); and changes in cell organization and structure (Bensaci et al., 2015; Fluhr et al., 2014). In terms of biomechanical properties, skin elasticity decreases over the first decade of life (Sugihara et al., 1991) and collagen and elastin fiber density increases for several decades (Vitellaro-Zuccarello et al., 1994).

Studies of the biomechanical maturation of the skin have focused primarily on changes that occur in adults during aging, and most studies of skin maturation in children have focused on the maturation of the epidermis, which increases in thickness with age and has high cell turnover in the first months of life (Stamatas et al., 2010). One recent study showed that skin biomechanical properties evolve throughout infancy (Visscher et al., 2017), but such studies remain rare.

Here, we investigated the correlation between the maturation of biomechanical properties of the skin and the evolution of skin topography and micromorphology from infancy to early adulthood (study approved by Provincial Ethical Committee of Modena, University Hospital of Modena). We recruited a cohort of 70 subjects in seven age groups: 1–15 days, 5 weeks, 5–7 months, 2 years, 4–5 years, 7–8 years, and 20–35 years (Supplementary Table S1 online); all patients or their parent or guardian gave their informed written consent. Skin properties were examined by cutometry and reflectance confocal microscopy in vivo, and by immunohistochemistry in a limited number of foreskin biopsy samples (see Supplementary Material online for detailed methods).

Cutometry showed that skin elasticity, as measured by the ratio of immediate retraction to maximum distention (Ur/Uf), increased from infancy to 2 years of age and then plateaued (Figure 1a). The viscoelastic component, calculated as the ratio of immediate to delayed distension (Uv/Ue), decreased from infancy to adulthood (Figure 1b). Total recovery (Ua) was slightly higher at older ages (Figure 1c) and total deformation (Uf) did not vary between age groups (Figure 1d). The parameters related to skin elasticity and recovery (Ur/Uf, Ua/Uf, Ur/Ue, and Ua) were positively correlated with age and body surface area, whereas Uv/Ue was negatively correlated with both of these and Uf did not have any significant correlations (Figure 1e). None of the parameters were correlated with stratum corneum hydration.

The viscoelastic properties of the skin are related to the presence of interstitial fluid in the dermal extracellular matrix, and thus changes in Uv/Ue may reflect the water content of the epidermis and the dermis (Dobrev, 2002). Although we cannot completely rule out this possibility, none of the parameters examined were significantly (ie, $P < 0.01$) correlated with stratum corneum hydration as measured by cutometer (Figure 1e), suggesting that the changes in these skin biomechanical properties are related to structural maturation rather than hydration. This is consistent with reports that skin biomechanical properties are related to the structure of the extracellular matrix in adults, specifically elastin fibers, fibrillin microfibrils, rete ridges, and, to a lesser extent, collagen fibrils (Langton et al., 2017).

Skin microstructure was examined by reflectance confocal microscopy, which allows fast, in vivo imaging of the cyto-architectural aspects of the skin. The thickness of the stratum corneum and the supra papillary epidermis increased with age (Figure 2a), consistent with previous findings (Stamatas et al., 2010). The homogeneity and furrow architecture of the stratum corneum changed dramatically between infants and older children (Supplementary Figure S1a–S1d). Reflecting spheroids were observed throughout childhood and were most prevalent during infancy (Supplementary Figure S1e–S1f), and the nature of these structures warrants further investigation. The observed evolution from poorly defined to well-defined keratinocyte outline (Supplementary Table S2 online) might reflect the fact that keratinocyte proliferation is high during the first months of life (Stamatas et al., 2010). Dermal papillae increased in number with age, consistent with a previous study by Miyauchi et al. (2016), but contrary to this previous study, rete ridge thickness was stable across age groups (Figure 2a). This contradiction could be explained by a difference in age group stratification in the studies.

Interestingly, collagen fibers were fibrillar and showed a parallel orientation in newborns, whereas they were thicker, coarse, and multidirectional in older infants through adults (Figure 2a–2c). Mechanical forces are known to increase the diameter of collagen fibrils (Sanders and Goldstein, 2001). Our data support a contribution of mechanical forces to dermal maturation postnatally, including the reorganization of the collagen matrix and increased collagen fiber thickness

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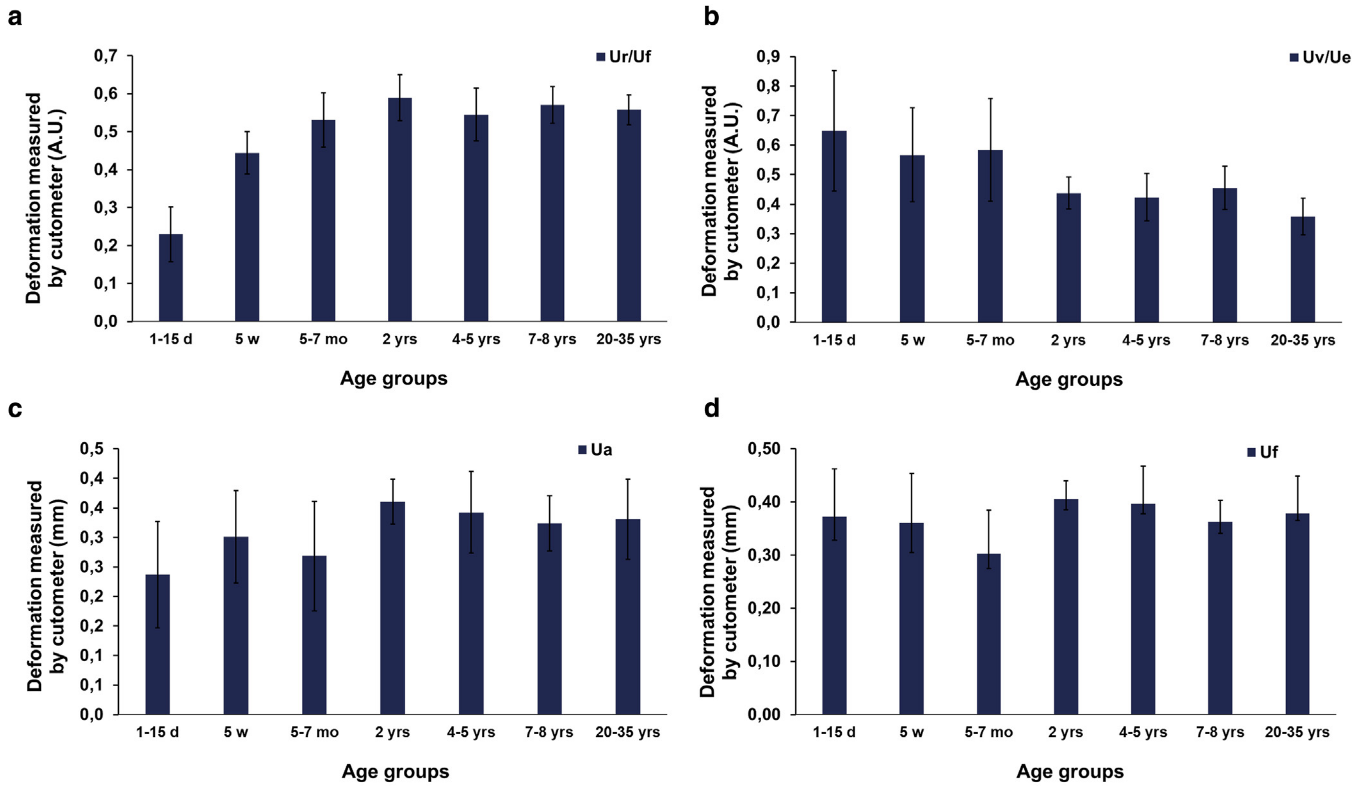

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Parameter	Age r	Age P-value	Calculated BSA r	Calculated BSA P-value	Stratum corneum hydration r	Stratum corneum hydration P-value	N
Biological elasticity (Ur/Uf)	**0.585**	< 0.001	**0.621**	< 0.001	0.244	0.042	70
Gross elasticity (Ua/Uf)	**0.439**	< 0.001	**0.462**	< 0.001	0.282	0.018	70
Net elasticity (Ur/Ue)	**0.437**	< 0.001	**0.487**	< 0.001	0.297	0.013	70
Ratio viscoelastic to elastic distension (Uv/Ue)	**-0.555**	< 0.001	**-0.541**	< 0.001	-0.121	0.318	70
Total recovery (Ua)	**0.321**	< 0.001	**0.314**	< 0.001	0.131	0.28	70
Total deformation (Uf)	0.098	0.419	0.091	0.455	-0.028	0.819	70

Figure 1. Evolution of skin properties and correlations with age, BSA, and hydration. Skin biomechanical properties were assessed by cutometer in the seven indicated age groups (n = 9 to 11 per group). (**a**) Ur/Uf: tonicity parameter representing biological elasticity. (**b**) Uv/Ue: ratio of viscoelasticity to elastic distension. (**c**) Ua: viscoelasticity parameter representing the complete relaxation amplitude after pressure is released. (**d**) Uf: total deformation parameter representing the maximum amplitude and passive behavior of the skin to force. (**e**) Correlations between skin biomechanical parameters and age, calculated BSA, and stratum corneum hydration were calculated using Spearman's test. BSA, body surface area; r, Spearman's correlation constant. Significant correlations ($P < 0.01$) are indicated in bold.

(Sanders and Goldstein, 2001). We also noticed a structural pattern that has not been reported previously. Circular "cuffing" of the follicle by collagen fibers was observed in newborns only, and was very rare or completely absent in all other age groups (Figure 2a, 2d, 2e). This interesting feature may reflect the unique properties of postnatal hair follicle growth (Zhou et al., 2016).

The elastin component of the skin cannot be observed by reflectance confocal microscopy. Therefore, we analyzed its structural maturation by immunohistochemistry on foreskin samples from patients of various ages. Both fibrillin and elastin fibers increased in length and intensity with age, especially at the dermal–epidermal junction (Supplementary Figure S2 online).

The strength of the present study is the analysis of both structural and biomechanical properties of the skin in the same subjects over a wide range of age groups. In addition to evidence supporting the relationship between structural and biomechanical properties during skin maturation, we introduce the observation of collagen fiber cuffing around hair follicles in newborns. Collectively, these data demonstrate the biochemical and structural evolution of the dermis during postnatal development, as well as the translation of these changes into maturation of the biomechanical properties of the skin.

CONFLICT OF INTERESTS

GB, CDB, GB, NL, PM, and CB are current or former employees of Expanscience Laboratories; JF and RD provided consulting services for Expanscience; CPMG, NF, and BC provided services on a commercial basis for this study; GP and CM received a research grant; GP was investigator of the clinical study. This study was funded by Expanscience Laboratories.

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		Group 1 (1-15 d)	Group 2 (5 w)	Group 3 (5-7 mo)	Group 4 (2 yrs)	Group 5 (4-5 yrs)	Group 6 (7-8 yrs)	Group 7 (20-35 yrs)
Stratum corneum thickness (µm)		6.25 ± 2.20	6.77 ± 2.52	7.29 ± 2.69	8.43 ± 2.69	7.29 ± 2.69	8.86 ± 2.52	9.90 ± 1.65
Supra papillary epidermis thickness (µm)		9.9. ± 2.96	11.46 ± 3.30	11.98 ± 2.52	12.50 ± 2.69	13.03 ± 3.68	13.55 ± 5.03	12.50 ± 2.69
Rete ridge thickness (µm)		19.80 ± 4.11	16.67 ± 3.30	16.15 ± 1.65	16.15 ± 2.96	16.15 ± 2.96	18.76 ± 3.64	22.40 ± 3.52
Number of dermal papilla		4.60 ± 1.43	5.30 ± 1.83	6.00 ± 2.05	9.30 ± 2.54	9.30 ± 2.00	9.30 ± 1.70	10.60 ± 4.25
Collagen orientation (relative abundance)	Parallel	90%	30%	0%	10%	0%	20%	20%
	Multidirection	10%	70%	100%	90%	100%	80%	80%
Fibrillar collagen – 4-level abundance scale (mean±SD for each group)		3.20 ± 1.03	2.90 ± 1.59	2.70 ± 1.76	1.70 ± 1.49	3.40 ± 1.35	3.30 ± 1.33	1.30 ± 1.16
Coarse collagen – 4-level abundance scale (mean±SD for each group)		0.8 ± 1.03	1.10 ± 1.59	1.30 ± 1.76	2.30 ± 1.49	0.60 ± 1.35	0.70 ± 1.33	2.70 ± 1.16
Number of follicular structures (per image)		4.7 ± 2.58	2.50 ± 1.90	2.90 ± 1.97	1.00 ± 1.05	1.00 ± 1.70	0.90 ± 1.10	0.50 ± 0.52
Follicular collagen circular cuffing (relative abundance)	Present	60%	0%	10%	0%	0%	10%	0%
	Absent	40%	100%	90%	100%	100%	90%	100%

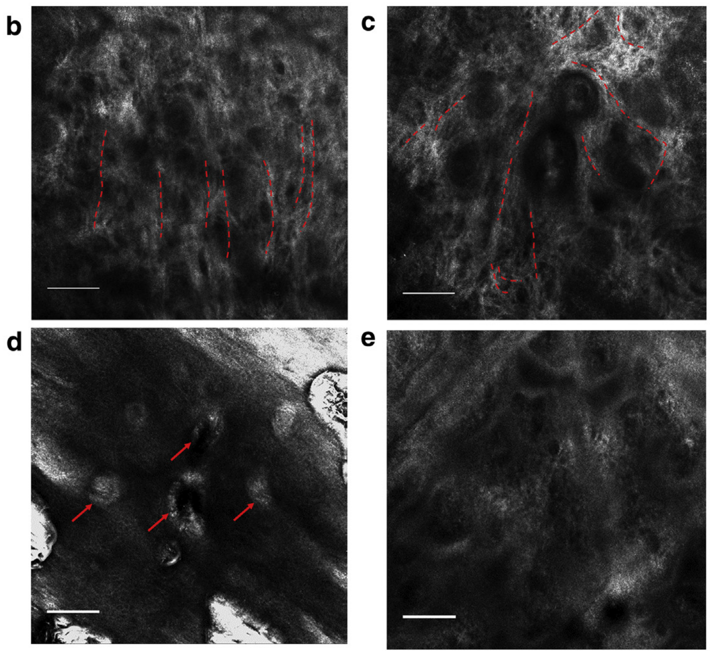

Figure 2. Evolution of skin microstructure with age observed by in vivo reflectance confocal microscopy. (**a**) Stratum corneum thickness, epidermal thickness, number of follicular structures, and number of dermal papilla were obtained directly from image analysis. The abundance of fibrillar or coarse collagen fibers was scored in each subject using a 0−4 scale by the investigator. The relative abundance of collagen fibers and presence of collagen cuffing was semi-quantitatively assessed by the investigator (gross value given per age group). (**b**) Parallel collagen fibers as observed in newborns. (**c**) Multidirectional collagen fibers as observed in adults. (**d**) Circular collagen follicle cuffing as observed in newborns (red arrows) (**e**). The absence of circular collagen follicle cuffing in adults. Sight area = 830 × 830 µm. Scale bar = 200 µm.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.07.034.

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