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INVESTIGATIVE REPORT

Epidermal Thickness at Different Body Sites: Relationship to Age, Gender, Pigmentation, Blood Content, Skin Type and Smoking Habits

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Epidermal thickness and its relationship to age, gender, skin type, pigmentation, blood content, smoking habits and body site is important in dermatologic research and was investigated in this study. Biopsies from three different body sites of 71 human volunteers were obtained, and thickness of the stratum corneum and cellular epidermis was measured microscopically using a preparation technique preventing tissue damage. Multiple regressions analysis was used to evaluate the effect of the various factors independently of each other. Mean (SD) thickness of the stratum corneum was 18.3 (4.9) µm at the dorsal aspect of the forearm, 11.0 (2.2) µm at the shoulder and 14.9 (3.4) µm at the buttock. Corresponding values for the cellular epidermis were 56.6 (11.5) µm, 70.3 (13.6) µm and 81.5 (15.7) µm, respectively. Body site largely explains the variation in epidermal thickness, but also a significant individual variation was observed. Thickness of the stratum corneum correlated positively to pigmentation ($p = 0.0008$) and negatively to the number of years of smoking ($p < 0.0001$). Thickness of the cellular epidermis correlated positively to blood content ($P = 0.028$) and was greater in males than in females ($P < 0.0001$). Epidermal thickness was not correlated to age or skin type. *Key words: epidermis; human; regional differences; skin biopsies; stratum corneum.*

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To understand epidermal reactions to biochemical or biophysical stimuli, knowledge of epidermal thickness and its dependence on factors such as body site, age, sex, pigmentation, blood content and smoking are important. Many authors have reported on measurements of epidermis (1 – 10) in relation to various factors without drawing firm conclusions (5 – 7, 9 – 11). These studies have had different purposes: collection of baseline data in normal skin (2), investigation of the effect of previous sun exposure comparing different body sites (3, 4), the relation between epidermal

thickness, pigmentation and human photosensitivity (5), ultraviolet radiation (UVR) transmission (8) or the effect of different constitutive factors (1, 6, 7, 9 – 11). Most studies have included only a few subjects (1, 2, 4), and studies presenting large materials have generally been based on biopsies obtained either from many different parts of the body (7, 10, 11) or from one region only (5, 6, 8).

Light microscopy may still be considered the "gold standard" for measurement of epidermal thickness, and by which other methods are compared. However, the morphology of the skin becomes distorted during the different steps of preparation. Swelling or shrinkage changes the dimension of the epidermal layers, leading to inaccurate estimations of epidermal thickness (12). The conventional formalin-paraffin processing used in the earlier studies (3, 7) is unsuitable for determination of stratum corneum thickness, because this preservation technique distorts the anatomy of the horny layer (12, 13).

In this study we applied cryopreparation, a special non-formalin processing technique, in order to prevent crystallization in the water content, and to minimize changes in the skin structure and epidermal thickness during the different preparation steps (13). We obtained biopsies from three different body sites of 71 human volunteers and evaluated inter-individual variance, differences between body sites and the influence of age, gender, skin type, pigmentation, blood content, smoking habits and body site on the thickness of both the stratum corneum and the cellular epidermis.

MATERIALS AND METHODS

Subjects

A total of 71 healthy volunteers of Nordic ancestry participated. None were suffering from diabetes mellitus. Detailed information about the subjects is summarized in Table I. The local ethics committee approved the study (Ref. KF01-017-00, Copenhagen County Ethics Committee) and informed consent was obtained prior to participation.

Procedure

Skin reflectance measurements were obtained and biopsies were taken at defined areas of the dorsal aspect of the forearm, the shoulder over the scapula and the inner upper quadrant of the buttock. All measurements were performed in

Table I. *Data characteristics of the test subjects*

Number of subjects (male:female)	71 (37:34)
Median age, years (range)	47 (20–68)
Skin type (I:II:III:IV),[a] (%)	9:20:54:18
Smoking status ("never", "previous", "current"), (n)	32:27:12
Median number of years smoking[b] (75% quartiles)	15 (7; 30)

[a](20). [b]For previous and current smokers together.

clinically normal skin. Hair was gently removed with scissors. The skin measurements were performed during late autumn, when environmental exposure to solar UVR is negligible in Denmark. Altogether 71 measurements were obtained from the dorsal aspect of the forearm, 68 from the shoulder and 61 from the buttock. Skin type and smoking habits were clarified using a questionnaire.

Skin reflectance

Skin pigmentation (melanin) and blood content (haemoglobin) were measured with a skin reflectance meter (UV-Optimize, Model Matic 555, Matic, Naerum, Denmark). Quantification of light absorption in specific chromophores is calculated as reflected light relative to incident light. The peak wavelengths at 555 nm and 660 nm are used because of the optimal discrimination between absorption in haemoglobin and melanin. Pigmentation and blood content comprise an arbitrary scale with 100 steps (14, 15). Two-way analysis of variance showed no significant difference in blood content or pigmentation between repeated measurements at the same spot. Mean of all observations (residual standard deviation) was 25.1% (3.6) for blood content and 20.6% (1.5) for pigmentation.

Residual standard deviation is an expression of measure error and is equal to the squared root residual variance.

Skin biopsies

Two hundred 3-mm punch biopsies were taken under local anaesthesia (carbocain with adrenalin) injected subcutaneously. The biopsies were placed on filter paper with epidermis facing outwards, immediately snap-frozen in isopentane (2-methylbutan) and stored at −80°C until further processing to prevent drying and shrinking artefacts. From each biopsy 6–8 sections were cryostat-cut (5 μm thick) and stained with haematoxylin and erythrosine. The technically best section from each specimen was used to measure thickness. Three independent measurements were performed. Stratum corneum was measured using a calibrated ocular micrometer, the thickness of the cellular part of epidermis was determined using a calibrated square grid, and all measurements were adjusted for magnification optics (13). Total

epidermal thickness was calculated by adding corresponding values of the stratum corneum and the cellular part of epidermis. One trained pathologist performed all measurements.

Twenty randomly selected biopsies were evaluated twice. Two-way analysis of variance showed no significant difference between the two measurements. Mean of all observations (residual standard deviation) for the stratum corneum was 16 μm (1.2) and 63 μm (3.5) for the cellular epidermis.

Statistics

Epidermal thicknesses were Log_e (x) transformed for normality. The data for blood content and pigmentation for the separate body locations were normally distributed using the Shapiro-Wilks test, and for all body locations together the data were approximately normally distributed. Both analysis of variance and paired *t*-test were used to evaluate any differences between the body sites concerning epidermal thickness, pigmentation and blood content. Analysis of variance was used to compare differences between individuals and between body sites. Stepwise backward multiple linear regression analysis was used to estimate the influence of the factors age, sex, skin type, pigmentation, blood content, number of years smoking, smoking status and body site on epidermal thickness. $P < 0.05$ is considered to be significant.

RESULTS

Body-site-specific measurements of thickness, pigmentation and blood content are given in Table II. Significant differences between body sites on an intra-individual level ($p < 0.0001$), and between individuals, inter-individual ($p < 0.0001$), were found for thickness of both the stratum corneum and the cellular epidermis. The mean square for body site was about 34 times greater for body sites than for individuals, indicating that the difference between individuals is much smaller than the difference between body sites (Fig. 1).

Different factors influenced thickness of the stratum corneum and cellular epidermis. The results of the backward multiple regression analysis are given in Table III. Body site, the single variable with the strongest association with both stratum corneum and epidermal thickness, was capable of explaining 45% of the variation in the measured thickness of stratum corneum and 39% of the variation of cellular epidermis when evaluated independently of the other variables.

Both pigmentation and number of years of smoking correlated significantly with thickness of the stratum

Table II. *Epidermal thickness, pigmentation and blood content at different body sites; mean (SD)*

	Stratum corneum[a] (μm)	Cellular epidermis[a] (μm)	Total epidermis[a] (μm)	Pigmentation[a] (%)	Blood content[a] (%)
Forearm dorsal	18.3 (4.9)	56.6 (11.5)	74.9 (12.7)	35.2 (6.0)	22.4 (6.9)
Shoulder	11.0 (2.2)	70.3 (13.6)	81.3 (13.5)	25.5 (5.7)	31.1 (9.4)
Buttock	14.9 (3.4)	81.5 (15.7)	96.5 (16.1)	16.4 (4.2)	29.6 (5.7)
All body sites	14.8 (4.8)	68.9 (17.0)	83.7 (16.6)	26.2 (9.3)	27.6 (8.4)

[a]Significant differences between the examined body sites, *p*-values <0.0001, except between the shoulder and the buttock concerning blood content $p > 0.1$.
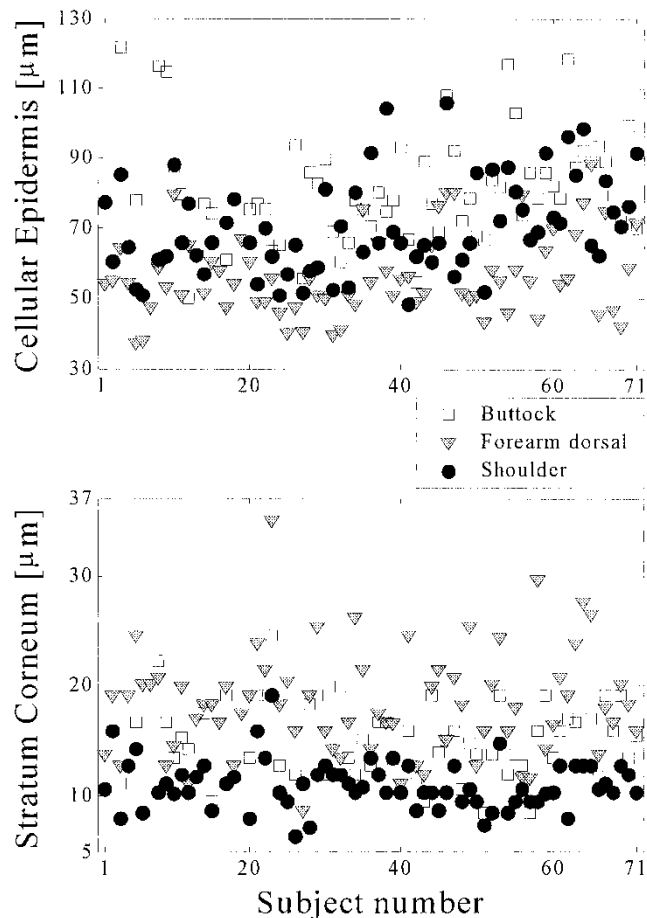
Fig. 1. Body site and inter-individual variations in thickness of the stratum corneum and cellular epidermis. The inter-individual variation in skin thickness is shown for each body site separately. The total variation in the material, including both body site and the inter-individual variations, corresponds to the total vertical spread of all the dots.

Table III. *Epidermal thickness and influencing factors using backward multiple regression analyses, p-values and squared correlation coefficient (r^2)*

	Stratum corneum	Cellular epidermis
Body site	<0.0001	<0.0001
Age	NS[a]	NS[a]
Gender	0.048	<0.0001
Skin type	0.025	NS[a]
Pigmentation	0.0008	NS[a]
Blood content	NS[a]	0.028
Number of years smoking	<0.0001	NS[a]
Smoking status	NS[a]	0.028
r^2 [b]	0.54	0.50

Thickness of the stratum corneum or the cellular epidermis as outcome variable and body site, age, gender, skin type, pigmentation, blood content, number of years smoking and actual smoking habits as explaining covariates.

[a] NS = non-significant.

[b] r^2 express the part of the total variability that is explained by the significant variables only.

DISCUSSION

In correspondence with other studies (1, 4, 10), we found a difference between body sites in thickness of both the stratum corneum and the cellular epidermis. A considerable individual variation within a region has been observed (1, 2). However, the results of this study and that of Ya-Xian et al. (11) demonstrate that the influence of body site far exceeds individual differences.

The effect of body site on epidermal thickness might partly be due to pigmentation differences caused by body site dependent UVR exposure. Sunlight exposure has been shown to induce a thickening of the stratum corneum (16). Huzaira et al. (4) found a thinner stratum corneum in sun-protected body sites compared to sun-exposed body sites; the buttock was not included in these studies. In our study, measurements were obtained out of season for solar exposure in order to avoid this effect; still, a positive correlation between pigmentation and thickness of the stratum corneum was found even when body site was taken into account, but also for the buttock separately. This indicates an association between thickness of the stratum corneum and constitutive pigmentation not solar induced. However, it does not exclude an effect of acute UVR exposure on the stratum corneum – not examined in this study.

We found significant differences between body sites in relation to thickness of the cellular epidermis, with thickest cellular epidermis at the sun-protected buttock, but no correlation to pigmentation and thereby UVR exposure. Thickness of the cellular epidermis was correlated to blood content, which seems reasonable, i.e. a higher blood supply nourishing a thicker epidermis. This finding contradicts that of Monteiro-Riviere et al. (17), who found no correlation between

corneum independently of the presence of body site in the model. Pigmentation and thickness of the stratum corneum correlated positively, whereas number of years of smoking and thickness of the stratum corneum correlated negatively.

Both gender and blood content correlated significantly with thickness of the cellular epidermis independently of the presence of body site in the model. Males had a thicker cellular epidermis than females. Blood content and thickness of cellular epidermis correlated positively.

The other significant correlations between thickness of the stratum corneum and gender and skin type, and between thickness of the cellular epidermis and smoking status, could not be reproduced without the presence of body site in the model or when evaluating each body site separately. The impact of these variables on epidermal thickness is therefore doubtful.

No significant correlation was found between epidermal thickness measurements and age, not even when evaluated independently of the other variables ($p > 0.1$).

thickness of either stratum corneum or epidermis and Laser Doppler velocimetry determined blood flow in different animal species, although there is excellent consistency between blood content in the skin measured by the skin reflectance technique and blood flow evaluated by laser Doppler velocimetry (18).

We found no convincing correlation between epidermal thickness and skin type in accordance with Lock-Andersen et al. (5). This is supported by the lack of difference between black and white people concerning epidermal thickness (3, 8).

The importance of age and sex as variables in determining epidermal thickness is controversial, with the data pointing in both directions (1, 3, 5–7, 9–11, 18). In this study, thickness of both the stratum corneum and the cellular epidermis was independent of age; however, the narrow age range in our material (20–68 years) should be kept in mind. Ya-Xian et al. (11) found no gender difference in the number of cell layers in the stratum corneum; likewise, we found no correlation between thickness of the stratum corneum and gender. However, we did find that males had a significantly thicker cellular epidermis than females.

Cigarette smoking is another environmental factor besides UVR exposure that has been related to the development of changes in the skin associated with aging. The mechanisms by which tobacco smoke affects the skin are largely unknown and so far concerns about skin changes related to smoking have been confined to the dermis (19). In this study, thickness of the stratum corneum was correlated to number of years smoking, whereas no relation to smoking status was found. Unfortunately, our material included only 12 "current smokers". "Previous smokers" also contributed to the variable number of years of smoking and no data concerning the number of cigarettes consumed were obtained in this study. An effect of smoking on epidermal thickness therefore remains to be clarified.

ACKNOWLEDGEMENTS

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