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ORIGINAL ARTICLE

Detection and distribution of endogenous steroids in human stratum corneum

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ABSTRACT

Objectives: The objective of the study was to investigate the presence and distribution of endogenous steroids in human stratum corneum (SC) with respect to sex, age, anatomical site, and depth into SC, using a noninvasive sampling technique and a sensitive analytic method for quantitation.
Materials and methods: Corneocytes in the SC samples removed by sequential tape stripping from the forearm, forehead, and back sites were processed and analyzed using a validated liquid chromatography–tandem mass spectrometry method for the quantitation of hydrocortisone, cortisone, and testosterone.
Results: In the 32 volunteers surveyed, testosterone was only detected at the forearm site in a single volunteer. Both hydrocortisone and cortisone were detected in 5–7 individuals out of 16 from both the age 20–35 years and age 50–65 years groups. Maximal amounts of hydrocortisone and cortisone found in SC of forehead, forearm, and back were 0.37 ng/cm^2, 0.96 ng/cm^2, and 0.49 ng/cm^2; and 0.20 ng/cm^2, 0.12 ng/cm^2, and 0.06 ng/cm^2, respectively, and were either higher than or comparable to those reported in human hair in terms of concentration by SC weight. In the population with either hydrocortisone or cortisone detected, no significant differences relating to sex, age groups, and anatomical sites were observed for the amount of hydrocortisone and cortisone in the SC. However, significantly higher amount of cortisone was found in the surface layers of SC than deeper layers in the age 50–65 years group.
Conclusion: The results demonstrate that, with the achievable sensitivity of current analytical technology, physiological concentrations of endogenous steroids, such as hydrocortisone and cortisone, can be found in the SC of some individuals.

Introduction

Skin is the largest human organ, accounting for approximately 12–15% of body weight and consists of a complex layered structure, which forms a barrier between the body and the outside environment. It is structured in three layers: epidermis, dermis, and subcutaneous layer. The stratum corneum (SC) is the outermost layer of the epidermis with a thickness of approximately 10–20 μm, which is the rate-limiting barrier to percutaneous absorption and protects the body against the outer environment.[1,2]

Hormones influence the development and function of human skin, which also produces and releases hormones. Other than the adrenal gland, ovary, and testis, skin has been well recognized as the site of steroid hormone formation and metabolism. The skin and sebaceous glands are capable of synthesizing cholesterol *de novo* from acetate, and express genes coding and activity for enzymes obligatory for steroidogenesis; for example, the cytochromes P450scc, P450c17, and P450c21 convert cholesterol to androgens and other steroids.[3] Especially in postmenopausal women, almost all active sex steroids are made in target tissues by an intracrine mechanism. Human skin also expresses elements of the central hypothalamic–pituitary–adrenal axis including pro-opiomelanocortin, corticotropin-releasing hormone,

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the corticotropin-releasing hormone receptor-1, key enzymes of corticosteroid synthesis and synthesizes glucocorticoids.[4,5] These hormones exhibit a wide range of biological activities on the skin. For example, glucocorticoids induce hair growth, stimulate sebocyte proliferation, and regulate keratinocyte differentiation.[6] Androgens stimulate proliferation of sebocytes and dermal papilla cells, while estrogens improve collagen content and quality, increase skin thickness and enhance vascularization. Several studies have reported the production of cortisol in cultured human epidermal melanocytes and dermal fibroblasts,[7,8] and in histocultured human hair follicles.[9] Physiological concentrations of different endogenous steroids have also been found in hair samples.[10–12] Given that SC is the terminal differentiational layer of the skin, steroids produced in the local tissue or diffused from systemic circulation should eventually reach SC.

To the best of our knowledge, there are no existing literature data concerning endogenous steroid content of the human SC. In this study we employed a sensitive method applying liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) to quantitate the endogenous steroids in human SC harvested by tape-stripping technique,[13,14] and to investigate their distribution with respect to sex, age, anatomical site, and depth into the SC.

Materials and methods

Chemicals and reagents

Hydrocortisone and cortisone were supplied by Sigma Chemical Co. (St. Louis, MO, USA), whereas testosterone was purchased from Fluka (Buchs, Switzerland). LC–MS grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (Lab-Scan, Rongmueng, Pathumwan Bangkok, Thailand), formic acid (Riedel-deHaën, Seelze, Germany) and water (Alps Chem Co., Taipei, Taiwan) were of LC grade. For extraction of steroids, dichloromethane was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Sodium hydroxide (Showa, Tokyo, Japan) and hydrochloric acid (Riedel-deHaën) were also used.

Participants

Sixteen Chinese males and 16 Chinese females were recruited in this study. All individuals had no known skin disorders that needed topical corticosteroid treatment at the time of the study, and were not taking any medication for at least 1 month prior to the study. After the volunteers signed the informed consent form approved by the Institutional Review Board for Human Research, National Cheng Kung University Hospital, they were categorized into two groups according to their ages: Group 1 (age 20–35 years), and Group 2 (age 50–65 years). Each group included 8 males and 8 females, coded as Nos. 1–16, respectively.

Collection of SC samples

Forehead, ventral forearm, and back were selected as the test sites. Participants were instructed not to apply any soap or cosmetics to those sites for at least 12 hours prior to the study. On the day of the study, the skin sites were cleaned with tissues and marked with a marker pen and the tapes were consistently applied to the same area. Booktape (Scotch 845; 3M Co., St Paul, MN, USA) was used in the study, provided on a roll and cut into pieces of 2 cm × 2 cm to give a surface area of 4 cm.[2] The tape was pressed onto the skin site at the forehead, forearm, and back with five constant-pressure strokes and was removed with a forceps in one swift movement. The procedure was repeated 20 times and the tape-strips were divided into five, seven, and eight strips per glass tube on each site. Two volunteers were selected to investigate the effect of sample collection time on the amounts of endogenous steroids detected in the tape strips. The second and third collection of SC samples was conducted at approximately 1-month intervals between each collection at the same three sites using the above-described procedure. Throughout the entire study, tape stripping of the SC was performed by the same operator, which typically removed approximately 70–80% of the SC.

Determination of SC protein content

The protein content of SC removed by 20 tape strippings from three individuals at different skin sites was evaluated to validate tape-stripping techniques. The assay was based upon the Bradford dye reaction (BioRad Laboratories, Hercules, CA, USA).[1,15] Each sample was incubated with 2 mL of 1N NaOH and shaken in an incubator shaker overnight. After neutralization with 2 mL of 1N HCl and centrifugation, 200 μL of the supernatant was pipetted into a plastic tube, to which 600 μL deionized water and 200 μL of the dye reagent were added. After 5 minutes, the optical density of the sample was measured at 595 nm on a spectrometer (Varian Cary 50; Mulgrave, Victoria, Australia). A standard curve was prepared with lyophilized bovine serum albumin in concentrations ranging from 5 μg/mL to 30 μg/mL.

Sample extraction

An aliquot of 0.3 mL/tape of reporter lysis buffer (Promega Corporation, Madison, WI, USA) was added to each glass tube containing five, seven, or eight strips, and gently rotated overnight. The solution was extracted twice with dichloromethane/water (1/1, v/v). The organic layer was collected and filtered through 0.22 μm syringe filter (Chrom Tech, Apple Valley, MN, USA). The sample solution was evaporated to dryness and stored at −20°C until assayed. On the day of analysis, the residues were re-suspended in 100 μL of methanol, and 40 μL of each sample was then injected into the LC–MS/MS. Endogenous steroid content in the SC expressed in ng/cm^2 was calculated as follows: ng/cm^2 = concentration obtained from calibration curve in μg/mL / 4 cm^2 × 0.1 mL × 1000.

LC–MS/MS

We have adapted the LC–MS/MS method from Hauser et al.[16] An Alliance 2695 separation system from Waters Co. (Milford, MA, USA) was used. Separation was achieved at 20°C on a Cosmosil 5C18-AR II column (4.6 mm × 150 mm, 5 μm, Nacalai Tesque Inc., Tokyo, Japan). Eluent A was composed of water/acetonitrile (95/5, v/v) and eluent B of water/acetonitrile (5/95, v/v), both containing 0.1% formic acid. Elution was performed at a flow rate of 0.5 mL/min starting from 30% B (0–2 minutes), linearly increased to 70% B (2–10 minutes), and returned to 30% B (10–15 minutes). The analysis time for a sample was 15 minutes. Sample analyses were carried out on a Quattro Premier XE tandem mass spectrometry equipped with an electrospray ionization interface (Waters Co.). Nitrogen was used as desolvation and cone gas, with flow rates of 700 L/hour and 50 L/hour, respectively. Source and desolvation temperatures were set at 120°C and 350°C, respectively. The electric potential applied on the capillary was 3.0 kV, and the sample cone voltage was set individually for each compound. Argon was used as collision gas at a pressure of 1.0×10^{-4} mbar and a collision energy setting adapted for each compound. Steroids were detected using multiple reaction monitoring (MRM) of the two most abundant product ions per compound. Dwell time for each transition was 25 ms. Data processing and quantitation were performed by the MassLynx 4.1 (Waters Co.).

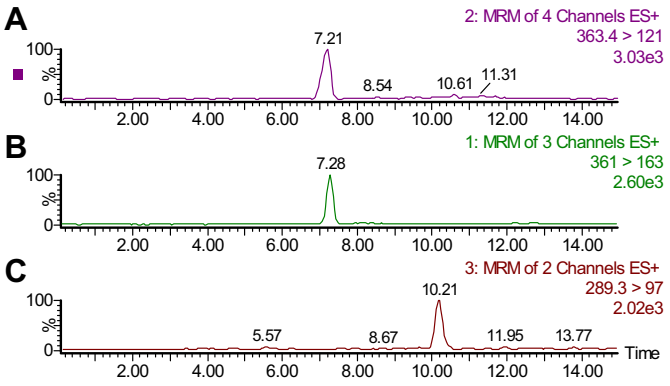

Figure 1 Multiple-reaction monitoring chromatograms of standard solutions containing (A) 5 ng/mL hydrocortisone, (B) 1 ng/mL cortisone, and (C) 0.8 ng/mL testosterone analyzed by liquid chromatography–tandem mass spectrometry.

Method evaluation

In a pilot study with samples from three volunteers, androstenedione was detected in none of the samples. Hence, the method was validated for simultaneous quantitation of hydrocortisone, cortisone, and testosterone. Standard stock solutions of the three steroids were prepared at 100 μg/mL in methanol and stored at −20°C. Linearity of the standard solutions was evaluated at concentrations of 0.3–50 ng/mL. The limit of detection (LOD) and limit of quantitation were defined as the concentrations that gave signal-to-noise ratios of 3 and 10, respectively. Intraday and interday precision were determined by spiking the standard solutions at three concentration levels (1.0 μg/mL, 0.1 μg/mL, and 0.025 μg/mL) onto five 2 cm × 2 cm tapes (20 μL per tape), followed by the extraction procedure described above. The responses were compared with a set of calibration standards based on peak area and recovery was determined by comparing the representative peak areas of the extracted samples to the standards at the same concentration.

Statistical analysis

Statistical analysis was performed using SPSS software version 12 (SPSS Inc., Chicago, IL, USA). Both analysis of Mann–Whitney and Kruskal–Wallis H test were used to evaluate differences in the concentrations of endogenous steroids between the sex, age, anatomical site, and depth into SC. The significance level was set at $p < 0.05$.

Results and discussion

LC–MS/MS

LC–MS/MS has become the method of choice for steroid quantitation. Methods have been described for the determination of steroids, such as hydrocortisone, cortisone, and testosterone.[10,17] The main advantage of LC–MS/MS over GC–MS/MS is the possibility to reduce sample preparation procedure. Recently, Carvalho et al[18] published an LC–MS/MS method for the analysis of cortisol and cortisone in serum with LOD of 2.75 nmol/L (1 ng/mL) and 0.85 nmol/L (0.3 ng/mL), respectively. Unfortunately, the sensitivity for testosterone achieved by LC–MS/MS was generally inferior to that achieved by GC–MS/MS method which requires prederivation for sample preparation (LOD of 0.1 ng/mL urine vs. 0.1 pg/mg hair, respectively).[11,16]

Table 1 Linearity, limit of detection, and limit of quantitation for analysis of hydrocortisone, cortisone, and testosterone standard solutions ($n = 3$).

Component	Concentration (ng/mL)	Interday CV%	Intraday CV%	LOD (ng/mL)	LOQ (ng/mL)
Hydrocortisone	1	2.29	1.31		
	5	2.17	3.26	0.08	0.1
	10	1.90	1.18		
Cortisone	0.5	3.77	4.64		
	1	4.05	6.12	0.02	0.05
	5	1.47	4.56		
Testosterone	0.3	2.98	1.89		
	0.8	1.68	6.60	0.01	0.03
	2	5.79	7.61		

CV = coefficient of variation.

In the current study, separation of the three steroids was achieved in 15 minutes using a C18 column eluted with a water–acetonitrile gradient. As shown in Figure 1, all compounds were baseline separated when using their optimized MRM transitions. Generally, the two most intense transitions of a compound were chosen for quantification in MRM mode. It appeared that higher signal to noise ratios were obtained when protonated forms $[M+H]^+$ were detected in positive mode electrospray ionization interface. Hydrocortisone dissociation spectrum presented product ions at *m/z* 121, 267, 97, and 91. Cortisone presented extensive fragmentation with *m/z* 163, 121, and 105 as the most abundant product ions. Best sensitivity was obtained for testosterone, forming intense ions at *m/z* 97 and 109. The correlation coefficient of the calibration curve was >0.999 for all the three steroids showing linearity between 0.3 ng/mL and 50 ng/mL. Limits of detection and limits of quantification were in the ranges of 0.1–0.8 ng/mL and 0.3–1.0 ng/mL, respectively. Both intraday ($n = 3$) and interday ($n = 3$) precision was ≤15% at all levels examined. Extraction recovery ranged between 20% and 40% for tape-strips spiked with 0.025–1 μg/mL of the three steroids, which corresponded to 0.625–25 ng/cm^2 of five tape-strips. We used five tape strips as a unit for the calculation because five to eight tape strips were combined for quantitation of endogenous steroids in the SC. The results are summarized in Tables 1 and 2. Although several extraction solvents were tested with and without prior treatment of lysis buffer to disrupt the corneocytes, extraction recovery of the steroids from the tape strips appeared to be relatively low because the evaluation was done with steroids spiked directly onto the tapes without SC. The spiked steroids might be absorbed into the adhesives and were more difficult to extract. It would be more relevant to the actual situation if recoveries could be evaluated with steroids spiked onto the tapes with a blank SC; however, the unknown and highly variable content of endogenous steroids in the blank SC, which is to be investigated in the present study, would compromise the accuracy of the determination. Nonetheless, the data presented here

Table 2 Precision, limit of detection, and limit of quantitation for analysis of hydrocortisone, cortisone and testosterone in the tape strips ($n = 3$).

Component	Spike (μg/mL)	Concentration in the tape strips		Error (%)	Recovery (%)
		ng/4 cm^2 (5 tapes)	ng/cm^2 (5 tapes)		
Hydrocortisone	0.025	2.5	0.625	9.5	39.7
	0.1	10	2.5	−20.6	26.3
	1.0	100	25	9.7	24.8
Cortisone	0.025	2.5	0.625	−15.0	33.8
	0.1	10	2.5	−3.1	40.6
	1.0	100	25	3.9	32.7
Testosterone	0.025	2.5	0.625	−0.8	23.5
	0.1	10	2.5	−10.2	20.1
	1.0	100	25	−4.8	22.3

Table 3 Intra- and interindividual variability of the protein content in the SC removed by 20 tape strippings.

Volunteer	Site														
	Protein content (μg/cm^2)														
	Forehead				Forearm				Back				Mean	SD	CV %
	I[a]	II[b]	III[c]	Total[d]	I	II	III	Total	I	II	III	Total			
No. 1	71.2	64.8	52.0	188.0	89.2	59.2	49.6	198.0	59.2	41.6	46.8	147.6	177.9	26.7	15.0
No. 4	20.0	46.8	32.8	99.6	54.4	44.0	43.6	142.0	31.2	37.2	23.2	91.6	111.1	27.1	24.4
No. 8	38.8	54.8	32.0	125.6	51.2	61.6	65.2	178.0	74.8	76.0	74.0	224.8	176.1	49.6	28.2
Mean	43.3	55.5	38.9	137.7	64.9	54.9	52.8	172.7	55.1	51.6	48.0	154.7			
SD	25.9	9.0	11.3	45.4	21.1	9.5	11.1	28.4	22.1	21.2	25.4	66.9			
CV %	59.8	16.3	29.1	33.0	32.5	17.4	21.1	16.4	40.1	41.2	53.0	43.2			

CV = coefficient of variation; SD = standard deviation.
[a] I: 1st–5th tapes.
[b] II: 6th–12th tapes.
[c] III: 13th–20th tapes.
[d] Total amount was calculated as the sum of fractions I, II, and III, and represented total protein content per cm^2 of SC harvested by 20 tape trippings.

demonstrate the feasibility of the described procedures to extract endogenous steroids from the SC.

Validation of tape-stripping technique

The protein content of SC removed by 20 tape strippings from three participants at different skin sites are summarized in Table 3. The average amount of protein removed from the three individuals ranged from 111.1 μg/cm^2 to 177.9 μg/cm^2, and intraindividual variation of averaged SC protein content removed among three sites ranged from 15.0% to 28.2%. The amount of protein from the forehead, forearm, and back were in ranges of 99.6 μg/cm^2 to 188.0 μg/cm^2 (coefficient of variation (CV) = 33.0%), 142.0 μg/cm^2 to 198.0 μg/cm^2 (CV = 16.4%), and 91.6 μg/cm^2 to 224.8 μg/cm^2 (CV = 43.2%), respectively. There was no significant difference in protein content of the tape-stripped SC among anatomic sites. The overall mean protein content of SC removed by tape stripping (155.0 ± 34.5 μg/cm^2) was comparable to but less varied than previously reported (288 ± 124 μg/cm^2).[19] As it is expected that protein amount usually decrease after repeated tape stripping, we deliberately divided the 20 tape strips into three samples, i.e., tape strips 1–5 as the surface layer of SC, tape strips 6–12 as the middle layer, and tape strips 13–20 as the bottom layer, to obtain comparable protein amount in each sample. Protein content of the surface layer, middle layer, and bottom layer from different sites ranged from 43.3 μg/cm^2 to 64.9 μg/cm^2, 51.6 μg/cm^2 to 55.5 μg/cm^2, and 38.9 μg/cm^2 to 52.8 μg/cm^2, respectively. Protein content in three layers was not statistically different from each other. The overall protein content in each sample averaged 52.0 ± 15.2 μg/cm^2.

Detection of endogenous steroids

Endogenous steroids content was expressed as ng/cm^2 of SC in the surface, middle, and bottom layers. Among the 32 volunteers surveyed in the study, testosterone was only detected at the forearm site in a single volunteer (Group 2, No. 16, 0.07 ng/cm^2). However, resampling of SC in the same individual after 2 months did not show any detectable amount of testosterone in the SC. The inconsistent detection of testosterone might suggest the possibility of contamination from the working environment or contact with relatives and a nonphysiologic role of the hormone. Hydrocortisone was detected in five individuals from each group, whereas cortisone was detected in seven individuals from Group 1 (age 20–35 years) and six from Group 2 (age 50–65 years), respectively. The amounts of hydrocortisone and cortisone detected in different layers of the SC from different sites in thesevolunteers were summarized in Tables 4 and 5. Maximal amounts of hydrocortisone and cortisone found in SC of forehead, forearm, and back were 0.37 ng/cm^2, 0.96 ng/cm^2, 0.49 ng/cm^2; and 0.20 ng/cm^2, 0.12 ng/cm^2, and 0.06 ng/cm^2, respectively. There was no apparent pattern of detection for both hydrocortisone and cortisone with respect to sex, age group, or anatomical site. For those individuals with

Table 4 Amounts of hydrocortisone in human SC at different anatomical sites. (Only the individuals with detectable amounts are shown. Hydrocortisone was not detected in any samples collected from the rest of the volunteers.)

ID	Sex	Age	Site												Average (ng/cm^2)
			Forehead				Forearm				Back				
			I[a]	II[b]	III[c]	Total[d]	I	II	III	Total	I	II	III	Total	
			ng/cm^2				ng/cm^2				ng/cm^2				
1	F	23	—	—	—		—	0.18	—	0.18	—	—	—		0.06
11	F	29	—	—	—		—	0.02	—	0.02	0.06	—	—	0.06	0.03
12	F	29	—	—	—		0.12	0.12	—	0.24	—	—	—		0.08
15	M	29	0.12	0.15	0.10	0.37	—	0.96	—	0.96	—	0.10	—	0.10	0.48
16	M	31	—	—	—		—	0.15	—	0.15	0.06	0.08	0.35	0.49	0.21
1	F	54	—	—	—		0.09	—	—	0.09	—	—	—		0.03
3	F	58	—	—	0.14	0.14	—	—	—		—	—	—		0.05
6	F	57	—	—	0.13	0.13	—	—	—		—	—	—		0.04
4	M	58	—	—	—		—	—	—		—	0.12	—	0.12	0.04
9	M	57	—	—	—		—	0.04	0.07	0.11	—	—	—		0.04

— = below limit of quantitation.
[a] I : 1st–5th tapes.
[b] II: 6th–12th tapes.
[c] III: 13th–20th tapes.
[d] Total amount was calculated as the sum of Fractions I, II, and III, and represented total hydrocortisone content per cm^2 of SC harvested by 20 tape strippings.

Table 5 Amounts of cortisone in human SC at different anatomical sites. (Only the participants with detectable amounts are shown. Cortisone was not detected in any samples collected from the rest of the participants.)

ID	Sex	Age	Site												Average (ng/cm^2)
			Forehead				Forearm				Back				
			I[a]	II[b]	III[c]	Total[d]	I	II	III	Total	I	II	III	Total	
			ng/cm^2				ng/cm^2				ng/cm^2				
1	F	23	0.11	0.05	0.04	0.20	0.10	—	—	0.10	—	—	—		0.10
3	F	23	—	—	—		0.07	—	0.05	0.12	0.04	—	—	0.04	0.05
4	F	22	—	—	—		—	—	—		—	0.03	—	0.03	0.01
11	F	29	—	—	—		—	—	—		0.02	—	—	0.02	0.01
2	M	23	—	—	—		0.07	—	—	0.07	–	—	—		0.02
9	M	26	—	—	—		0.05	—	—	0.05	0.06	—	—	0.06	0.04
10	M	35	—	—	—		—	—	—		0.05	—	—	0.05	0.02
3	F	58	0.02	—	—	0.02	—	—	—		—	—	—		0.01
6	F	57	0.02	—	—	0.02	—	—	—		—	—	—		0.01
7	F	51	0.02	—	—	0.02	0.03	—	—	0.03	—	—	—		0.02
10	F	58	—	—	—		0.03	—	—	0.03	—	—	—		0.01
4	M	58	0.02	—	—	0.02	0.03	—	—	0.03	—	—	—		0.02
11	M	63	—	—	—		0.04	—	—	0.04	—	—	—		0.01

— = below limit of quantitation.
[a] I: 1st–5th tapes.
[b] II: 6th–12th tapes.
[c] III: 13th–20th tapes.
[d] Total amount was calculated as the sum of fractions I, II, and III, and represented total cortisone content per cm^2 of SC harvested by 20 tape strippings.

hydrocortisone or cortisone detected, the amounts of the two steroids were not statistically different between sex, age group, or anatomical sites. However, significantly higher amounts of cortisone were found in the surface layer of SC than deeper layers in Group 2 individuals (see Figures 2 and 3).

Two volunteers were selected to investigate the effect of sample collection time on the amounts of endogenous steroids detected in the tape strips. The results demonstrate that, in a 23-year-old female volunteer, hydrocortisone was detected in all three collection times and observed in the middle layers of the forehead, forearm, and back. By contrast, cortisone was detected in the first and third sampling times and observed in the same position of all layers of the forehead. In another 29-year-old male

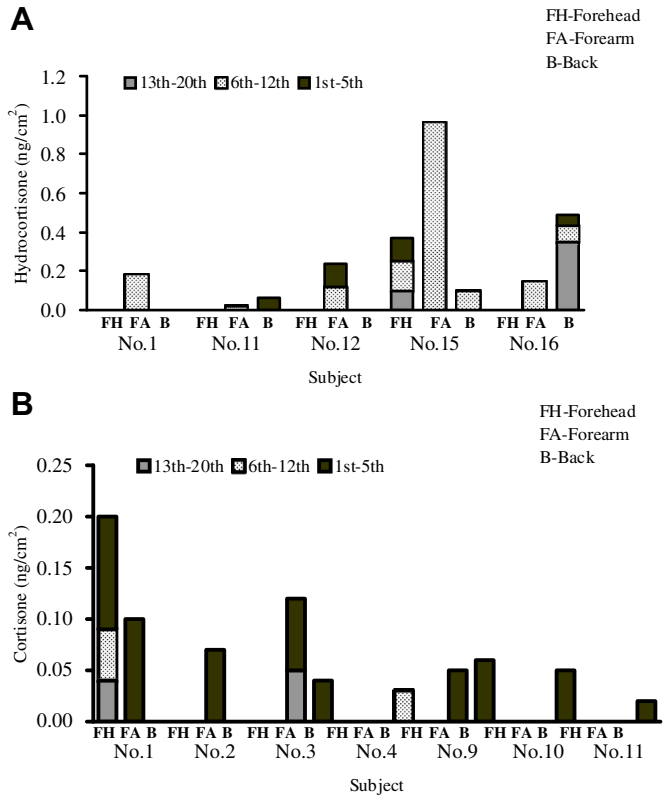

Figure 2 Amounts of (A) hydrocortisone and (B) cortisone in different layers of SC in individuals aged 20–35 years. In those participants with hydrocortisone or cortisone detected, the amounts of the two steroids were not statistically different between sex and anatomical sites ($p > 0.05$, Kruskal–Wallis H test). SC = stratum corneum.

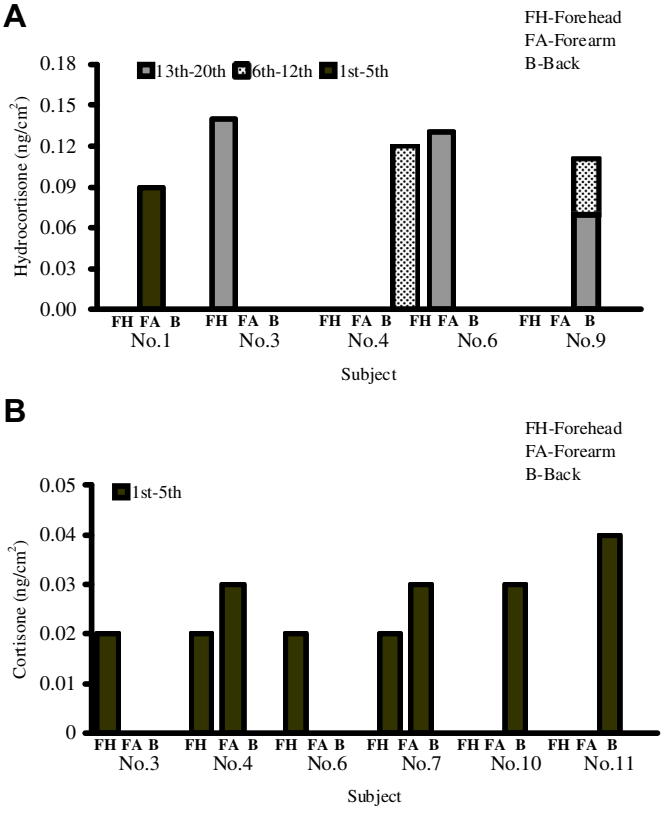

Figure 3 Amounts of (A) hydrocortisone and (B) cortisone* in different layers of SC in individuals aged 50–65 years. Amounts of cortisone were significantly higher in the surface layer of SC than deeper layers in these individuals (*$p < 0.05$, Kruskal–Wallis H test). SC = stratum corneum.

volunteer, hydrocortisone was detected in the first and third sampling and observed in the same position of middle layers of the forearm and back (data not shown). These results indicate that detectable amounts of endogenous steroids can be repeatedly found in the SC of the same individuals at different sampling times.

Because the protein content of SC removed in each site was approximately 155 μg/cm^2, maximal concentrations of hydrocortisone and cortisone detected in the SC would be calculated to be 6.2 ng/mg and 1.3 ng/mg protein, respectively. Unpublished data from our laboratory showed SC at the forearm removed by each tape-stripping (3M No. 845 book tape) averaged 368 ± 69 μg/cm^2. On SC weight basis, maximal concentrations of hydrocortisone and cortisone found would be 2.6 ng/mg and 0.5 ng/mg, which were either higher than or comparable to those reported in human hair (5.2–91 pg/mg and 12–163 pg/mg), respectively.[12]

The results also show that significantly higher amounts of cortisone were found in the surface layer of SC than deeper layers in Group 2 individuals. By contrast, in most cases hydrocortisone was not found in the surface layers. There may be two routes that endogenous steroids from the blood or synthesized in the skin could be incorporated into the SC: by active transport or passive diffusion into the epidermis and reaching the SC; and by secretion from sweat,[12] or possibly from sebum.[3,6] The former process would be likely to result in an increase in steroid concentration with depth into SC. By contrast, the latter would directly incorporate the steroids into the surface layers of the SC. 11β-Hydroxysteroid-dehydrogenase type 2 enzyme, which has a well-recognized function as a potent dehydrogenase that rapidly inactivates glucocorticoids converting hydrocortisone to cortisone, was found to be localized in superficial cells of duct of eccrine sweat glands of the skin and sebaceous glands.[20–22] The findings that more of cortisone and less hydrocortisone existed in the surface layers of the SC imply that they are mainly from body secretion. Some hydrocortisone may be converted to cortisone during the passage of sweat or sebum secretion. However, it is still intriguing that the phenomenon was only observed in the volunteers aged 50–65 years, but not in the younger individuals. Recently it was reported that 11β-hydroxysteroid-dehydrogenase type 1 enzyme activity was increased with age in human tissue explants and primary dermal fibroblast.[23] We postulate that type 2 enzyme activity might also vary with age, which would explain the observed differences in the distribution of endogenous steroids in SC between the two age groups. Nevertheless, the argument remains to be resolved.

Conclusion

The current study demonstrates for the first time that physiological concentrations of endogenous steroids, such as hydrocortisone and cortisone, can be found in the SC of some individuals, with the achievable sensitivity of current analytical technology. Maximal amounts of hydrocortisone and cortisone found in the SC of the forehead, forearm, and back were either higher than or comparable to those reported in human hair in terms of concentration by SC weight. These steroids may originate from systemic circulation or local synthesis in the skin. In the population with either steroid detected, no significant differences relating to sex, age groups, and anatomical site were observed for the amount of hydrocortisone and cortisone in the SC. However, a statistically higher amount of cortisone was found in the surface layers of SC than deeper layers in the age 50–65 years group. Issues remained to be clarified including whether the observed presence of steroid is by passive transfer or by active recruitment or local synthesis, and whether amounts of these endogenous hormones in the SC reflect the hormone levels in the physiological fluids, or the meaning of SC hormone levels under physiological and/or pathological conditions. The variation of SC turnover rate between ages, sites, and seasons that may influence the residence time of exogenous steroids in the epidermis can be one confounding factor in the study, and should also be taken into consideration.[24]

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References

1. Breternitz M, Flach M, Präßler J, Elsner P, Fluhr JW. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping; a randomized, controlled study. *Br J Dermatol* 2007;**156**:231–40.
2. Netzlaff F, Lehr CM, Wertz PW, Schaefer UF. The human epidermis models EpiSkinLM, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. *Eur J Pharm Biopharm* 2005;**60**:167–78.
3. Thiboutot D, Jabara S, McAllister JM, et al. Human skin is a steroidogenic tissue: steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes, and an immortalized sebocyte cell line (SEB-1). *J Invest Dermatol* 2003;**120**:905–14.
4. Slominski A, Wortsman J. Neuroendocrinology of the skin. *Endocr Rev* 2000;**21**:457–87.
5. Slominski A. Neuroendocrine system of the skin. *Dermatology* 2005;**211**:199–208.
6. Zouboulis CC. The human skin as a hormone target and an endocrine gland. *Hormone* 2004;**3**:9–26.
7. Slominski A, Zbytek B, Szczesniewski A, et al. CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH. *Am J Physiol Endocrinol Metab* 2005;**288**:E701–6.
8. Slominski A, Zbytek B, Szczesniewski A, Wortsman J. Cultured human dermal fibroblasts do produce cortisol. *J Invest Dermatol* 2006;**126**:1177–8.
9. Ito N, Ito T, Kromminga A, et al. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal (HPA) axis and synthesize cortisol. *FASEB J* 2005;**19**:1332–4.
10. Wheeler MJ, Zhong YB, Kicman AT, Coutts SB. The measurement of testosterone in hair. *J Endocrinol* 1998;**159**:R5–8.
11. Shen M, Xiang P, Shen B, Bu J, Wang M. Physiological concentrations of anabolic steroids in human hair. *Forensic Sci Int* 2009;**184**:32–6.
12. Raul JS, Cirimele V, Ludes B, Kintz P. Detection of physiological concentrations of cortisol and cortisone in human hair. *Clin Biochem* 2004;**37**:1105–11.
13. Tsai JC, Lin CY, Sheu HM, Lo YL, Huang YH. Noninvasive characterization of regional variation in drug transport into human stratum corneum *in vivo*. *Pharm Res* 2003;**20**:632–8.
14. Tsai JC, Lu CC, Lin MK, Guo JW, Sheu HM. Effects of sebum on drug transport across human stratum corneum *in vivo*. *Skin Pharmacol Physiol* 2012;**25**:124–32.
15. Jacobi U, Gautier J, Sterry W, Lademann J. Gender-related differences in the physiology of the stratum corneum. *Dermatology* 2005;**211**:312–7.
16. Hauser B, Deschner T, Boesch C. Development of a liquid chromatography–tandem mass spectrometry method for the determination of 23 endogenous steroids in small quantities of primate urine. *J Chromatogr B* 2008;**862**:100–12.
17. Kushnir MM, Rockwood AL, Roberts WL, et al. Performance characteristics of novel tandem mass spectrometry assay for serum testosterone. *Clin Chem* 2006;**52**:120–8.
18. Carvalho VM, Nakamura OH, Vieira JG. Simultaneous quantitation of seven endogenous C-21 adrenal steroids by liquid chromatography tandem mass spectrometry in human serum. *J Chromatogr B* 2008;**872**:154–61.
19. Voegeli R, Heiland J, Doppler S, Rawlings AV, Schreier T. Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry. *Skin Res Technol* 2007;**13**:242–51.
20. Krozowski Z. The 11 beta-hydroxysteroid dehydrogenases: functions and physiological effects. *Mol Cell Endocrinol* 1999;**151**:121–7.
21. Smith RE, Maguire JA, Stein-Oakley AN, et al. Localization of 11β-hydroxysteroid dehydrogenase type II in human epithelial tissues. *J Clin Endocrinol Metab* 1996;**81**:3244–8.
22. Thiboutot D, Martin P, Volikos L, Gilliland K. Oxidative activity of the type 2 isozyme of 17β-hydroxysteroid dehydrogenase (17β-HSD) predominates in human sebaceous glands. *J Invest Dermatol* 1998;**111**:390–5.
23. Tiganescu A, Walker EA, Hardy RS, Mayes AE, Stewart PM. Localization, age- and site-dependent expression, and regulation of 11β-hydroxysteroid dehydrogenase type 1 in skin. *J Invest Dermatol* 2011;**131**:30–6.
24. Haake AR, Holbrook K. The structure and development of skin. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz S, Fitzpatrick TB, editors. *Fitzpatrick's dermatology in general medicine*. 5th ed. New York: McGraw-Hill; 1999. p. 70–114.