Cutaneous hormone production is distinct between anatomical sites and between males and females.

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Title: Cutaneous hormone production is distinct between anatomical sites and between males and females.

Short Title: Characterization of skin hormone production

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This study was conducted in Dallas, Texas, USA and approved by the University of Texas Southwestern Institutional Review Board. (STU 2019-0145)

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Abbreviations: AF-antecubital fossa; LC-MS/MS-liquid tandem mass spectrometer; BMI-body mass index; HSD3B1-3-beta-hydroxysteroid dehydrogenase 1; DHEA – dehydroepiandrosterone; °C – Celsius; mL – milliters; mm – millimeters; rpm – revolutions per minute; SEM – standard error of the mean; uL – microliters; APCI – atmospheric pressure chemical ionization
ABSTRACT

The skin acts as an endocrine organ capable of hormone production and response. Moreover, many skin conditions clinically improve with anti-androgen therapies. Despite their importance, we have an incomplete understanding of the composition of hormones produced by the skin. In our current study, we have characterized the hormonal landscape of the skin across anatomical sites and between the sexes through analysis of skin secretions. In this observational pilot study, we collected skin secretions from the antecubital fossa, forehead, back, and axilla of twelve male and ten female subjects using the commercially available adhesive patches, Sebutape. We then developed a method to extract and quantify hormones from these secretions through liquid chromatography tandem mass spectrometry. We were able to detect seven hormones and observed anatomical site differences in glucocorticoids, cortisone and 11-deoxycorticosterone. Most notably, we observed marked elevations in dehydroepiandrosterone in the axilla and androstenedione on the forehead. We also detected differences in several sex steroid hormones between male and female subjects with majority consistent with known systemic hormone differences. Through this approach, future studies will determine how hormonal composition of skin secretions is altered in skin diseases.
INTRODUCTION

The skin acts as an endocrine organ, containing the enzymatic machinery needed for de novo hormone synthesis (Nikolakis et al., 2016). These hormones are observed to play a significant role in skin disease and the cyclical hormonal fluctuations women experience during the menstrual cycle have been linked to flares in inflammatory skin disorders (Raghunath et al., 2015). Moreover, diseases with hormonal derangements like hyperandrogenism and hypecortisolism have cutaneous manifestations such as acne. Androgens are known to increase sebum production and hyperproliferation of keratinocytes, both elements of acne’s pathogenesis (Fritsch et al., 2001; Zouboulis et al., 1999). Thus, anti-androgen therapies are effective for conditions clinically linked to hormonal irregularities (Searle et al., 2020). Due to the predilection of acne for specific areas of the body, the potential for anatomic variability in hormone production and control also seems likely.

Sebaceous, eccrine and apocrine glands each contain unique enzymes and receptors with the capacity for hormonal synthesis and response (Nikolakis et al., 2016; Wollina et al., 2007). The density and location of these glands varies at different body sites. Sebaceous glands exist in highest density on the scalp, forehead, and face (Zouboulis CC, n.d.). In contrast, eccrine glands exist in highest density on the palms and soles. Apocrine glands are abundant in the axilla and groin. The sebaceous gland produces hormones that regulate sebaceous gland lipid production, cell proliferation, and sebum secretion (Nikolakis et al., 2016; Thiboutot et al., 2003; Zhang et al., 2021; Zouboulis et al., 1999). Eccrine glands secrete water for thermoregulatory control. Apocrine glands expand at puberty producing hormones and pheromones (Zouboulis CC, n.d.). Given the anatomic variability, we hypothesized that there were likely differences in hormonal secretions at distinct body sites.
Due to differences in gonadal hormone production, serum levels of sex steroid hormones vary between males and females and have profound effects on the skin (Dao and Kazin, 2007). Males tend to have thicker skin due to androgens promotion of dermal thickness (Azzi et al., 2005). Additionally, dimorphism in hair growth is attributable to shifts in androgens, as evidenced by beard growth in men and androgenetic alopecia. Many different cell types of the skin contain hormone receptors (Zouboulis, 2004). However, how hormone production differs between genders and across skin sites is currently unknown.

Our study aimed to characterize hormonal composition of the skin by collecting skin secretions using commercially available, Sebutape®, and quantifying the amount of sex steroid hormones through liquid chromatography tandem mass spectrometry (LC-MS/MS). We hypothesized that sex steroid hormone amounts would differ between the sexes and at different anatomical sites.

RESULTS

Seven hormones were detected from the samples of the 22 participants by LC-MS/MS: dehydroepiandrosterone (DHEA), androstenedione, testosterone, progesterone, 11-deoxycortisol, cortisol, and cortisone. To accurately assess each site, quadruplicate samples were taken at the antecubital fossa, forehead, back and axilla (Figure 1a). The tape was left on the skin for 15 minutes and then placed in a tube with methanol, vortexed, and discarded. The solution was stored at -20º C before analysis by LC-MS/MS (Figure 1b-f). Outliers were detected and removed within the biological replicates using Grubbs test, which determines if the most extreme value in a data set is a significant outlier. As shown in Figure 1, variability within the quadruplicate samples was small, illustrating both method reproducibility and a local biological
hormonal landscape sufficient to establish hormonal profiles characteristic for the surveyed sites (Figure 1g).

Hormonal differences between anatomical sites were observed (Figure 2). DHEA was significantly higher in the axilla compared to the AF \((p < 0.0001)\), forehead \((p < 0.0001)\), and back \((p < 0.0001)\) (Figure 2a). Androstenedione was higher in the forehead compared to the AF \((p < 0.0001)\), back \((p < 0.0001)\) and axilla \((p = 0.0002)\) (Figure 2c). There was no site difference noted for testosterone (Figure 2b). Progesterone had the least scatter of all the hormones analyzed without any significant differences between sites (Figure 2d). Of the glucocorticoids, 11-deoxycortisol was increased in the forehead in comparison to the axilla \((p = 0.02)\) (Figure 2e). Cortisol had no significant differences between anatomical sites (Figure 2f). Cortisone was decreased in the AF in comparison to the forehead \((p = 0.003)\) and back \((p = 0.02)\) (Figure 2g).

Our study detected several differences between the sexes at specific anatomical sites (Figure 3). DHEA was higher in the AF \((115.5 \text{ vs. } 30.8; p = 0.005)\), forehead \((180.1 \text{ vs. } 71.27; p = 0.03)\), and the back \((114.5 \text{ vs. } 39.9; p = 0.001)\) in males as compared to females (Figure 3a). Androstenedione was significantly higher in both the forehead \((10.1 \text{ vs. } 7.1; p = 0.04)\) and back \((5.6 \text{ vs. } 3.8; p = 0.03)\) in males compared to females (Figure 3b). Testosterone was also higher in the forehead \((7.1 \text{ vs. } 2.15; p = 0.008)\) and back \((7.1 \text{ vs. } 2.5; p = 0.02)\) in males compared to females (Figure 3c). In contrast, progesterone trended lower in males than females on the forehead \((26.9 \text{ vs. } 33.8; p = 0.12)\) and back \((27.02 \text{ vs. } 34; p = 0.19)\); although neither were statistically significant (Figure 3d). 11-deoxycortisol was decreased in males as compared to females on the back \((2 \text{ vs. } 3.2; p = 0.04)\) (Figure 3e). No notable differences were detected in cortisol (Figure 3f). Cortisone trended lower in males compared to
females in the axilla (30.1 versus 45.2; \( p = 0.06 \)); although, not statistically significant (Figure 3g).

Participants with outlier values were assessed individually. One participant had eight outlier values, two participants had four outlier values, one participant had three outlier values, three participants had two outlier values, and six participants had one outlier values. No commonalities were obvious of the participants with outliers in reference to gender, medications, or medical history. However, two participants were outliers for BMI. One of these participants had three outlier values, cortisone on the forehead and testosterone on the back and axilla. The other had outlier values in androstenedione of the axilla.

**DISCUSSION**

In this study, we developed a method for analysis of skin steroid hormones through the collection of skin secretions and quantification by LC/MS-MS. Our results demonstrate anatomical site and sex differences in the androgens DHEA and androstenedione. DHEA was observed in the axilla at 100-fold greater amounts compared to the antecubital fossa, forehead and back (Figure 2a). The high amounts of DHEA, likely reflects the production of DHEA by apocrine glands, which are present within the axilla (Labors et al., 1979). We predict that other apocrine sites, such as the groin, will show similar elevations in DHEA. While the role of DHEA in the skin is not well understood, it is interesting that this precursor steroid to more potent androgens is secreted in excess at all of the skin sites sampled in our study (Figure 2). Thus, the production of more potent androgens is not limited by DHEA availability in the skin and is more likely regulated by the activity of regulatory enzymes such as 3-beta hydroxysteroid dehydrogenase 1 (HSD3B1), which converts DHEA to androstenedione and is expressed
predominantly in sebocytes (Supplementary Figure S1) (Fritsch et al., 2001). Therefore, it would be interesting in future studies to determine the hormone levels in the palms and soles where sebaceous glands are absent and other eccrine glands are present.

In our current report, androstenedione was present in greater amounts on the forehead, a skin site known for its high prevalence of acne, compared to the other skin sites sampled (Figure 2c). Androstenedione in the serum has been correlated with acne, suggesting that the higher amounts of androstenedione on the forehead may indicate why acne often presents at this location (Labors et al., 1979). Notably, we did not sample the beard region, a location on the face that has been linked to acne in female patients with known hormonal derangements (Chanyachailert et al., 2021). In future studies we aim to quantify hormone production in the skin of male and female patients with acne, compared to healthy controls.

Of the glucocorticoids, we observed greater amounts of 11-deoxycortisol in the forehead compared to the axilla. Additionally, we observed greater amounts of cortisone on the forehead and back compared to the AF (Figures 2e and 2g). Several hormonal differences between the sexes were also detected (Figure 3). The androgens, DHEA, testosterone, and androstenedione were increased at several anatomical sites in males compared to females. Interestingly, progesterone, which is elevated during the luteal phase of the female menstrual cycle, showed trends of increased levels at several anatomical sites in females compared to males, but none were statistically significant. Our study included subjects on hormonal contraception and did not control for menstrual cycle timing, which may explain why we did not detect expected differences between male and female subjects. Interestingly, no outliers and a small standard deviation were observed in progesterone (Figure 2d). No one hormone was ubiquitously different at each anatomical site tested between genders, suggesting that the skin production and secretion
of hormones could be regulated locally despite systemic differences. Fittingly, fewer differences between the sexes were detected in the glucocorticoids. Future studies could investigate anatomical areas where sexual dimorphism is more obvious such as areas with facial hair.

In conclusion, through the use of Sebutape® and LC-MS/MS we were able to quantify steroid hormones of skin secretions taken from the AF, forehead, back and axilla. DHEA and androstenedione were found to be increased in the axilla and forehead, respectively. Differences were detected between the sexes; however, no hormone was altered across all sites. We completed all sampling in quadruplicate with high consistency among technical replicates and between biological samples, indicating the reliability of our method. We also used a commercially available tape and a short exposure time of 15 minutes, making this a feasible approach for translational studies of hormones throughout the field of Dermatology. While the function of androgens in the skin is not entirely clear, we hope our research serves as a launching point for future investigations into hormone production and regulation in the skin and the role on skin hormones in skin diseases.

MATERIALS & METHODS

Participant Recruitment

This study was approved by the University of Texas Southwestern institutional review board and written informed consent was obtained from all study participants. Recruitment occurred from January to March 2021 and the study was completed between March and April 2021.

Inclusion criteria included participants age of 18 to 40, BMI between 15-35, and willingness and ability to comply with the requirements of the protocol. Exclusion criteria
included participants outside the ages of 18 to 40, use of antibiotics in the last 6 months, use of topical medications, chronic skin disorders or other chronic medical conditions, use of lipid or hormonal altering medications (except for oral contraceptives), use of immunomodulator medications, women with irregular menstrual periods, or participants with history of surgery to endocrine organs. Basic demographic and medical information were collected from each patient. Twelve males and ten females met eligibility criteria and were included in the study (Table 1).

**Skin Sampling**

Participants were asked to complete a skin preparation prior to sampling in order to decrease external variables. The preparation included avoidance of bathing or face washing, avoidance of emollients, creams, or antimicrobial soap for 24 hours before sampling and avoidance of topical medications for 7 days prior to sampling. All sampling was completed between the hours of 12 PM and 3 PM over a 3-week period.

The sebum samples were collected using Sebutape® from Clinical and Derm LLC (Dallas, TX, USA). Four anatomical areas were sampled: the unilateral antecubital fossa (AF) and axilla, and the forehead and back, between the scapulae. The areas were cleaned with alcohol wipes. Four tape strips were placed side-by-side at each anatomical site and left to sit for 15 minutes (Figure 1a). The tapes were removed and placed in 3 mL of liquid-chromatography mass-spectrometry grade methanol (Fisher Scientific, Pittsburg, PA, USA) in a 8 mL PTFE/rubber-lined vial (Fisher Scientific, Pittsburg, PA, USA). The vials were vortexed every 15 minutes for an hour after which the tape was discarded (Figure 1b). The samples were stored at -20 °C (Figure 1c).
**Steroid Hormone Extraction and Analysis**

Sebutape samples were received in 8 mL screw-cap vials and were transferred to 16 × 100mm test tubes with PTFE-lines screw cap test tubes. A cocktail of stable isotope standards were added and samples were vortexed for ~5 sec, then centrifuged at 3500 rpm for 10 min operated at 25 °C. Supernatant was decanted to a fresh tube and dried under a gentle stream of nitrogen at 40 °C. Samples were reconstituted in 200 uL warm (37 °C) 50% methanol and transferred to autosampler vials containing flat-bottom 350 uL inserts.

Steroid analysis was carried out using Shimadzu LC20 ADxr HPLC system coupled to a SCIEX API 5000 triple quadrupole mass spectrometer (LC-MS) operated in atmospheric pressure chemical ionization (APCI) mode (Figure 1d; Supplementary Table S1). The full details of the method are included in the supplementary material (Supplementary Text S1). A brief description of the method is as follows. A 20uL aliquot of sample was injected onto a Phenomomenex 150 × 3mm 2.6 µm particle Kinetex column and resolved with a binary gradient where mobile phase A was 50% methanol and mobile phase B was methanol. The gradient began at 100% A, ramped to 25% B at 1 min, 50% B at 3 min, 100% B at 5.5 min where it was held at 100% B until 6 min and returned to 100% A at 7 min until 9.5 min for column re equilibration. The flow rate was 0.5 mL/min and the column temperature was 35 °C.

**Statistical Analysis**

Outliers within the biological replicate samples were identified using Grubbs' test, also called the extreme studentized deviate method, and removed (Figure 1e). The average of each participant’s remaining biological replicates at each anatomical site were averaged. Mean values ± standard error of the mean (SEM) are plotted using GraphPad Prism version 9.1.1 to generate
Figures 1 and 2. Individual outlier values were identified using the ROUT method, denoted in gray on the figures, and removed from statistical analysis. DHEA, testosterone, cortisol and cortisone are shown on a logarithmic scale to assess skew of the larger data points. 11-deoxycortisol, androstenedione, and progesterone are represented on a linear scale. Outliers for BMI were identified using the ROUT method. Ordinary one-way ANOVA and Tukey’s multiple comparisons with a single pooled variance was used to determine anatomical site differences of hormones. Unpaired t test with individual variances computed for each comparison with two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli was used to determine site differences between genders for each hormone. Comparisons with p-values less than 0.02 were denoted on the graphs.

DATA AVAILABILITY STATEMENT
All data are available in the text and supplementary materials.

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CONFLICT OF INTEREST STATEMENT
All authors have no conflict of interest.
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AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization: JP, TH; Investigation: JP, KE; Resources: JP, KE; Formal analysis: JP, KE, TH; Methodology: KE, JM, TH; Data Curation: JP; Validation: KE; Writing – original draft: JP, KE; Writing – review and editing: JP, JM, TH; Visualization: JP, JM, TH; Project administration: JP; Supervision: JM, TH; Funding acquisition: TH.
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### Table 1. Population demographics

<table>
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<th>Characteristic</th>
<th>Population (n=22)</th>
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<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
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<td>Male</td>
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<tr>
<td>Female</td>
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<td><strong>Age, mean (range), years</strong></td>
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<tr>
<td><strong>BMI, mean (range), kg/m²</strong></td>
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<td><strong>Race and ethnicity, n (%)</strong></td>
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<td>White, non-Hispanic</td>
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<td>Non-white†</td>
<td>3 (13.64)</td>
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<td><strong>Female contraception‡, n (%)</strong></td>
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<tr>
<td>None</td>
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<tr>
<td><strong>Days since last menstrual period¶, mean (range)</strong></td>
<td>9.5 (4-22)</td>
</tr>
</tbody>
</table>

† Included Asian (n=2) and multi-racial of white and Indian (n=1)

‡ Data only includes female participants (n=10)

¶ Data only includes females who experience menstrual periods (n=6)
FIGURE LEGEND

Figure 1. Methodology of cutaneous hormone sampling. (a) The antecubital fossa, axilla, and back were cleaned with alcohol and four Sebutape® from Clinical and Derm LLC (Dallas, TX, USA) were placed at each site for 15 minutes. (b) The Sebutapes were placed in a tube with 3 mL of methanol and (c) vortexed every 15 minutes for an hour to allow the steroid hormones to dissolve into the solvent (d) before removing the tape. (e) The solution was stored at -20° C. (f) Steroid hormones were analyzed by LC-MS/MS using a SCIEX QTRAP 6500+ equipped with a Shimadzu LC-30AD (Kyoto, Japan) HPLC system and a 150 × 2.1 mm, 5µm Supelco Ascentis silica column (Bellefonte, PA, USA). (g) Outliers within biological replicates were detected and removed using Grubbs’ test. Quadruplicate Sebutapes from one participant are plotted to demonstrate the low scatter within replicates.

Figure 2. Steroid hormone levels of skin secretions in the antecubital fossa (AF), forehead, back and axilla. (a) Dehydroepiandrosterone (DHEA), (b) testosterone, (c) androstenedione, (d) progesterone, (e) 11-deoxycortisol, (f) cortisol, and (g) cortisone shown with mean percentages ± SEM plotted. DHEA, testosterone, cortisol, and cortisone shown on a logarithmic scale. Androstenedione, progesterone, and 11-deoxycortisol shown on a linear scale. Outliers within biological replicates were detected and removed using Grubbs’ test. Participants averages were plotted using GraphPad Prism version 9.1.1. Participant outliers (denoted in gray) were identified using the ROUT method and removed from statistical analysis. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Statistical significance was calculated using ordinary one-way ANOVA Tukey’s multiple comparisons test with a single pooled variance, α = 0.05. Statistically
significant \( p \)-values and \( p \)-values less than 0.02 are shown. (h) Infographic depicting anatomical sites where quadruplicate Sebutapes were placed.

**Figure 3. Male versus female comparisons of steroid hormones of skin secretions in the antecubital fossa (AF), forehead, back and axilla.** (a) Dehydroepiandrosterone (DHEA), (b) testosterone, (c) androstenedione, (d) progesterone, (e) 11-deoxycortisol, (f) cortisol, and (g) cortisone shown with mean percentages ± SEM plotted. DHEA, testosterone, cortisol, and cortisone shown on a logarithmic scale. Androstenedione, progesterone, and 11-deoxycortisol shown on a linear scale. Outliers within biological replicates were detected and removed using Grubbs’ test. Participants averages were plotted using GraphPad Prism version 9.1.1. Participant outliers (denoted in gray) were identified using Grubb’s test and removed from statistical analysis. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \), and ****\( p < 0.0001 \). Statistical significance was calculated using multiple unpaired \( t \)-tests with linear step-up procedure of Benjamini, Krieger and Yekutieli, \( \alpha = 0.05 \). Statistically significant \( p \)-values and \( p \)-values less than 0.02 are shown. (h) Infographic depicting male (gray) and female (orange) anatomical sites where Sebutapes were placed; axillary and back samples not shown.