Title: Characterization of cutaneous sex steroid hormone production through analysis of skin secretions

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KEY POINTS

- Sex steroid hormones of skin secretions varies between males and females
- Dehydroepiandrosterone is produced in elevated amounts in the axilla
- Androstenedione is produced in elevated amounts in the forehead

ABSTRACT

Importance: Systemic sex steroid hormone aberrations often manifest in skin disease. The sebaceous, apocrine, and eccrine glands all play an important role in the response and production of these hormones in the skin. However, our ability to quantify hormonal secretions at the skin surface is limited.

Objective: Our study aims to characterize the hormonal landscape of the skin at different anatomical sites and between the sexes through analysis of skin secretions.

Design: In this observational pilot study, we collected skin secretions from twelve male and ten female control subjects using commercially available, Sebutape®, from the antecubital fossa, forehead, back, and axilla. We then developed a method to extract and quantify the amount of sex steroid hormones from these secretions through liquid chromatography tandem mass spectrometry (LC-MS/MS).

Setting: Outpatient clinic

Participants: 34 participants were enrolled in the study, with 22 participants meeting criteria. Eligibility criteria included age of 18 to 40 and BMI between 15-35. Exclusion criteria included participants outside the ages of 18 to 40, use of antibiotics in the last 6 months, history of hormonal aberrations or chronic skin disorders, and use of hormone altering medications (except oral contraception).

Results: Our study detected anatomical site differences most notably in elevated dehydroepiandrosterone in the axilla and androstenedione in the forehead. Several hormonal differences were also detected between male and females consistent with known systemic hormone differences between the sexes.

Conclusions: We developed a method to quantify the hormonal levels in skin secretions using Sebutape®. Our approach found that hormonal composition varies based on sex and anatomical site. Additional studies will need to be completed to determine relevant hormonal shifts in clinical skin conditions.

INTRODUCTION

The skin acts as an endocrine organ, containing the enzymatic machinery needed for *de novo* hormone synthesis (1). These hormones are observed to play a significant role in skin disease. The cyclical hormonal fluctuations women experience during the menstrual cycle have been linked to flares in inflammatory skin disorders (2). Moreover, diseases with hormonal derangements like hyperandrogenism have cutaneous manifestations such as acne. Thus, anti-androgen therapies are effective for conditions clinically linked to hormonal irregularities (3). 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 have the capacity for hormonal synthesis and response (1,4). The sebaceous gland produces androgens that regulate sebaceous gland lipid production, cell proliferation, and sebum secretion (1,5,6). Eccrine glands secrete water for thermoregulatory control. Apocrine glands expand at puberty producing androgens and pheromones (7). Density and location of these glands varies at different body sites. Sebaceous glands exist in highest density on the scalp, forehead, and face (7). In contrast, eccrine glands exist in highest density on the palms and soles. Apocrine glands exist exclusively in the axilla and groin. 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 (8). 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.

METHODS

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 use of antibiotics within 6 months, history of hormonal aberrations or chronic skin disorders or use of hormonal altering medications (except for oral contraceptives). Basic demographic and medical information were collected from each participant (Table 1). Participants were asked to avoid bathing and topicals 24 hours before sampling and stop the use of topical medications 7 days prior to sampling. Sampling was completed between 12 PM and 3 PM.

Four anatomical areas were sampled: the unilateral antecubital fossa (AF), the axilla, the forehead and the back, between the scapulae. The areas were cleaned with alcohol wipes. Four Sebutape® strips (Clinical and Derm LLC) were placed side-by-side on the skin for 15 minutes. Tapes were removed and placed in 3 mL of LC-MS grade methanol (Fisher Scientific, Pittsburg, PA, USA) in a PTFE/rubber-lined tube (Fisher Scientific, Pittsburg, PA, USA). The tubes were vortexed every 15 minutes for an hour after which the tape was discarded. The samples were stored at -20 degrees Celsius until processing by LC-MS/MS. Samples were dried and resuspended in warm MeOH. The samples were transferred to GC vials using a Pasteur pipette and a volumetric internal standard was added. The samples were quantified using the linear range of a calibration curve created by serial dilution.

RESULTS

Four sex steroid hormones were detected from the sebum samples of the 22 included participants: dehydroepiandrosterone (DHEA), testosterone, androstenedione, and progesterone (Figure 1). DHEA was significantly higher in the axilla compared to the AF ($p<0.0001$), forehead ($p<0.0001$), and back ($p<0.0001$) (Figure 1a). There was no site difference noted for testosterone (Figure 1b). Androstenedione was shown to be higher on the forehead compared to the AF ($p<0.0001$), back ($p<0.0001$), and axilla ($p=0.0002$) (Figure 1c). Progesterone had minimal scatter but no significant differences between sites (Figure 1d).

Androgens are known to be higher in men and progesterone higher in females (9). While our data demonstrate this trend, it was not strictly the result (Figure 2). DHEA was higher in the AF (115.5 versus 30.8; $p=0.005$), forehead (180.1 versus 71.27; $p=0.03$), and the back (114.5 versus 39.9; $p=0.002$) in males as compared to females (Figure 2a). Testosterone was also higher in the forehead (7.1 versus 2.1; $p=0.008$) and back (7.1 versus 2.5; $p=0.02$) in males compared to females (Figure 2b). Androstenedione was significantly higher in both the forehead (10.7 versus 7.1; $p=0.05$) and back (5.6 versus 3.8; $p=0.04$) in males compared to females (Figure 2c). In contrast, no significant differences in progesterone were identified between the sexes; however, the forehead (26.9 versus 33.8; $p=0.12$) and back (27 versus 33.9; $p=0.19$) displayed a trend toward decreased levels of progesterone in males compared to females but none reached statistical significance (Figure 2d).

DISCUSSION

In this study we quantify anatomical site differences in the androgen production in the skin. Differences between the sexes were also observed in several anatomical sites in almost all detected hormones.

Increased DHEA was observed in the axilla in comparison to the antecubital fossa, forehead and back (Figure 1a). The axilla is the only site tested containing apocrine glands which is consistent with a study showing DHEA as a notable hormone in apocrine gland secretions (10). 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 at increased quantities in the axilla (Supplemental Figure 1). The more potent androgens, testosterone and DHT, are linked to acne pathogenesis (10). Lack of conversion of DHEA to these hormones could explain why the axilla is not an acne prone site. We also found increases in androstenedione, also a precursor to more potent androgens, on the forehead as compared to other sites (Figure 1c) (Supplemental Figure 1). Increased conversion of these precursors to more potent androgens could potentially contribute to skin disease. Future studies could look at skin secretions of patients with skin pathology affecting the forehead and axilla compared to our control population.

Several hormonal differences between the sexes were detected (Figure 2). In accordance with systemic levels, 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 female's menstrual cycle, showed trends of increased levels at several anatomical sites in females compared to males, but none were statistically significant. Minimal scatter was also observed in progesterone (Figure 1d) which could indicate tight regulation of this hormone. Additionally, no hormone was ubiquitously different between the sexes at all anatomic sites suggesting hormones secreted by glandular structures can regulate

secretion despite serum levels. Future studies could investigate anatomical areas where sexual dimorphism is more obvious such as areas with facial hair.

CONCLUSIONS

Through the use of Sebutape® and LC-MS/MS, we were able to quantify sex 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 which were consistent with known systemic hormone differences; however, no hormone was ubiquitously altered across all sites. While the function of these hormones in the skin is not well understood, we hope our research serves as a launching point for future investigations into hormone production and regulation in the skin.

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TABLE

Table 1. Population demographics

Characteristic	Population (n=22)
Sex, n (%)	
Male	12 (54.55)
Female	10 (45.55)
Age, mean (range), years	29.45 (22-40)
BMI, mean (range), kg/m²	24.65 (20.8-34.99)
Race and ethnicity, n (%)	
White, non-Hispanic	13 (59.1)
White, Hispanic	6 (27.27)
Non-white†	3 (13.64)
Female contraception‡, n (%)	
Intrauterine device	2 (20)
Oral contraceptive pills	5 (50)
None	3 (30)
Days since last menstrual period¶, mean (range)	9.5 (4-22)

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

‡ Data only includes participants with female sex (n=10)

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

FIGURE LEGEND

Figure 1. Sex steroid hormone levels as determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of skin secretions in the antecubital fossa, forehead, back and axilla. (A) Dehydroepiandrosterone (DHEA) and (B) testosterone shown on a logarithmic scale. (C) Androstenedione and (D) progesterone shown on a linear scale. Mean percentages $\pm$ SEM plotted. 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. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, # $p < 0.1$. Statistical significance was calculated using ordinary one-way ANOVA Tukey's multiple comparisons test with a single pooled variance, $\alpha = 0.05$. Statistically significant p -values and p -values less than 0.02 are shown.

Figure 2. Male versus female comparisons of sex steroid hormone levels as determined by LC-MS/MS analysis of skin secretions in the antecubital fossa, forehead, back and axilla.

(A) Dehydroepiandrosterone (DHEA) and (B) testosterone shown on a logarithmic scale. (C) Androstenedione and (D) progesterone shown on a linear scale Mean percentages $\pm$ SEM plotted. 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. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, # $p < 0.1$. 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.

A **B** **C**

Androstenedione

D

Progesterone

A

DHEA

B

Testosterone

C

Androstenedione

D

Progesterone