The skin acts as an endocrine organ capable of hormone production and response. Moreover, many skin conditions clinically improve with antiandrogen therapies. Despite their importance, we have an incomplete understanding of the composition of hormones produced by the skin. In this 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 12 male and 10 female subjects using commercially available adhesive patches. 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 the 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 such as hyperandrogenism and hypercortisolism 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, antiandrogen therapies are effective for conditions clinically linked to hormonal irregularities (Searle et al., 2020). Owing to the predilection of acne for specific areas of the body, the potential for anatomical 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 vary at different body sites. Sebaceous glands exist in the highest density on the scalp, forehead, and face (Zouboulis, 2019). In contrast, eccrine glands exist in the 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, 2019). Given the anatomical variability, we hypothesized that there were likely differences in hormonal secretions at distinct body sites.

Owing to the 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 because of androgen's promotion of dermal thickness (Azzi et al., 2005). In addition, 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 sexes and across skin sites is currently unknown.

This study aimed to characterize the hormonal composition of the skin by collecting skin secretions using commercially available Sebutape (Clinical and Derm LLC, Dallas, TX) and quantifying the amount of sex steroid hormones through
Figure 1. Methodology of cutaneous hormone sampling. (a) The antecubital fossa, forehead, axilla, and back were cleaned with alcohol, and four Sebutapes from Clinical and Derm LLC (Dallas, TX) 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 1 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 with LC-MS/MS using a SCIEX QTRAP 6500+ equipped with a Shimadzu LC-30AD (Shimadzu, Kyoto, Japan) HPLC.
RESULTS

Seven hormones were detected from the samples of 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 (AF), 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 −20 °C before analysis by LC-MS/MS (Figure 1b–f). Outliers were detected and removed within the biological replicates using Grubbs test, which determines whether the most extreme value in a dataset 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 than in the AF (P < 0.0001), forehead (P < 0.0001), and back (P < 0.0001) (Figure 2a). Androstenedione was higher in the forehead than in 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 with that in 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 with that in the forehead (P = 0.003) and back (P = 0.02) (Figure 2g).

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

DISCUSSION

In this study, we developed a method for analysis of the skin steroid hormones through the collection of skin secretions and quantification by LC-MS/MS. Our results show anatomical site and sex differences in the androgens DHEA and androstenedione. DHEA was observed in the axilla at 100-fold greater amounts than in the AF, forehead, and back (Figure 2a). The high amounts of DHEA likely reflect 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. Whereas 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 this 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, which converts DHEA to androstenedione and is expressed predominantly in sebocytes (Supplementary Figure S1) (Fritsch et al., 2001). Therefore, it would be interesting for future studies to determine the hormone levels in the palms and soles where sebaceous glands are absent and other eccrine glands are present.

In this study, androstenedione was present in greater amounts on the forehead, a skin site known for its high prevalence of acne, than in 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 with that in healthy controls.

Of the glucocorticoids, we observed greater amounts of 11-deoxycortisol in the forehead than in the axilla. In addition, we observed greater amounts of cortisol on the forehead and back than in the AF (Figure 2e and g). Several hormonal differences between the sexes were also detected (Figure 3). The androgens DHEA, testosterone, and androstenedione were increased at several anatomical sites in...
Figure 2. Steroid hormone levels of skin secretions in the AF, forehead, back, and axilla. (a) DHEA, (b) testosterone, (c) androstenedione, (d) progesterone, (e) 11-deoxycortisol, (f) cortisol, and (g) cortisone are shown with mean percentages ± SEM plotted. DHEA, testosterone, cortisol, and cortisone are shown on a logarithmic scale. Androstenedione, progesterone, and 11-deoxycortisol are 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 with Tukey's multiple comparisons test with a single pooled variance; $\alpha = 0.05$. Statistically significant $P$-values and $P < 0.02$ are shown. (h) Infographic depicting anatomical sites where quadruplicate Sebutapes were placed. AF, antecubital fossa; DHEA, dehydroepiandrosterone.

Figure 3. Male versus female comparisons of steroid hormones of skin secretions in the AF, forehead, back, and axilla. (a) DHEA, (b) testosterone, (c) androstenedione, (d) progesterone, (e) 11-deoxycortisol, (f) cortisol, and (g) cortisone are shown with mean percentages ± SEM plotted. DHEA, testosterone, cortisol, and cortisone are shown on a logarithmic scale. Androstenedione, progesterone, and 11-deoxycortisol are 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 Grubbs' test and removed from statistical analysis. *$P < 0.05$, **$P < 0.01$. Statistical significance was calculated using multiple unpaired $t$-tests with the linear step-up procedure of Benjamini, Krieger, and Yekutieli; $\alpha = 0.05$. Statistically significant $P$-values and $P < 0.02$ are shown. (h) Infographic depicting male (gray) and female (orange) anatomical sites where Sebutapes (Clinical and Derm LLC, Dallas, TX) were placed; axillary and back samples are not shown. AF, antecubital fossa; DHEA, dehydroepiandrosterone.
males than in 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 than in males, but none were statistically significant. This 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 SD were observed in progesterone (Figure 2d). No specific hormone was ubiquitously different across all anatomical sites 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 the 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. Although the function of androgens in the skin is not entirely clear, we hope that our research serves as a launching point for future investigations into hormone production and regulation in the skin and the role of skin hormones in skin diseases.

MATERIALS AND 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 2021 to March 2021, and the study was completed between March 2021 and April 2021.

Inclusion criteria included participants aged 18–40 years, with a body mass index between 15 and 35 kg/m², and willingness and ability to comply with the requirements of the protocol. Exclusion criteria included participants outside the ages of 18–40 years, the use of antibiotics in the last 6 months, the use of topical medications, chronic skin disorders or other chronic medical conditions, the use of lipid or hormonal-altering medications (except for oral contraceptives), the use of immunomodulator medications, women with irregular menstrual periods, or participants with a history of surgery to endocrine organs. Basic demographic and medical information was collected from each patient. A total of 12 males and 10 females met the eligibility criteria and were included in the study (Table 1).

Skin sampling
Participants were asked to complete a skin preparation before the sampling 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 the avoidance of topical medications for 7 days before the 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. Four anatomical areas were sampled: the AF, the axilla, the forehead, and the 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 (Thermo Fisher Scientific, Pittsburg, PA) in an 8 ml polystyrene methylene-rubber-lined vial (Thermo Fisher Scientific). The vials were vortexed every 15 minutes for an hour, and the tape was discarded after (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 × 100 mm test tubes with polystyrene-rubber-lined screw-cap test tubes. A cocktail of stable isotope standards was added, and samples were vortexed for ~5 seconds and then centrifuged at 3,500 r.p.m. for 10 minutes operated at 25 °C. The supernatant was decanted to a fresh tube and dried under a gentle stream of nitrogen at 40 °C. Samples were reconstituted in 200 μl warm (37 °C) 50% methanol and transferred to autosampler vials containing flat-bottom 350 μl inserts.

Steroid analysis was carried out using a Shimadzu LC20 ADXR (Shimadzu, Kyoto, Japan) HPLC system coupled to a SCIEX API 5000 triple quadrupole liquid chromatography–mass spectrometer operated in atmospheric pressure chemical ionization mode (Figure 1d and Supplementary Table S1). The full details of the method are included in the supplementary materials (Supplementary Text S1). A brief description of the method is as follows: a 20 μl aliquot of sample was injected onto a Phenomenex 150 × 3 mm 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 minute, 50% B at 3 minutes, and 100% B at 5.5 minutes where it was held at 100% B until 6 minutes and returned to 100% A at 7

| Table 1. Population Demographics |
|-----------------------------|--------------------------|
| Characteristics             | Population (n = 22) |
| Sex, n (%)                  |                          |
| Male                        | 12 (54.55)               |
| Female                      | 10 (45.55)               |
| Age, y, mean (range)        | 29.45 (22–40)            |
| BMI, kg/m², mean (range)    | 24.65 (20.8–34.99)       |
| Race and ethnicity, n (%)   |                          |
| White, non-Hispanic         | 13 (59.1)                |
| White, Hispanic             | 6 (27.27)                |
| Nonwhite                    | 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) |

Abbreviation: BMI, body mass index.
1Included Asian (n = 2) and multiracial of white and Indian (n = 1).
2Data only include female participants (n = 10).
3Data only include females who experience menstrual periods (n = 6).
minutes until 9.5 minutes 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 were removed (Figure 1g). The average of each participant’s remaining biological replicates at each anatomical site was averaged. Mean values ± SEM are plotted using GraphPad Prism, version 9.1.1 (GraphPad Software, San Diego, CA), to generate Figures 1 and 2. Individual outlier values were identified using the ROUT method, then denoted in gray on the figures, and removed from statistical analysis. DHEA, testosterone, cortisol, and cortisone are shown on a logarithmic scale to assess the skew of the larger data points. The 11-deoxycortisol, androstenedione, and progesterone are represented on a linear scale. Outliers for body mass index were identified using the ROUT method. Ordinary one-way ANOVA and Tukey’s multiple comparisons with a single pooled variance were 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 $P < 0.02$ were denoted on the graphs.

**Data availability statement**

All data are available in the text and supplementary materials.

**ORCIDs**

Juliana Pinede: http://orcid.org/0000-0002-8420-9412
Kaitlyn M. Eckert: http://orcid.org/0000-0001-5663-6380
Jeff G. McDonald: http://orcid.org/0000-0003-1570-4142
Tamia Harris-Tryon: http://orcid.org/0000-0002-4170-7083

**CONFLICT OF INTEREST**

The authors state no conflict of interest.