Age and skin site related differences in steroid metabolism in male skin point to a key role of sebocytes in cutaneous hormone metabolism

Markus Haag,1 Tina Hamann,1 Alexandra E. Kulle,2 Felix G. Riepe,2 Thomas Blatt,1 Horst Wenck,1 Paul-Martin Holterhus2 and Reto Ivo Peirano1,*

1Research and Development; Beiersdorf AG; Hamburg, Germany; 2Division of Pediatric Endocrinology; Department of Pediatrics; Christian-Albrechts-University of Kiel (CAU)/University Hospital of Schleswig—Holstein (UKSH); Kiel, Germany

Abbreviations: 3β-HSD, 3β-hydroxysteroiddehydrogenase; 17β-HSD, 17β-hydroxysteroiddehydrogenase; BMI, body mass index; DHEA, dehydroepiandrosterone; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDF, human dermal fibroblasts; HSD3B1, 3β-hydroxysteroiddehydrogenase Type 1; IQR, interquartile range or middle fifty; MRM, Multiple reactions monitoring; NHEK, normal human epidermal keratinocytes; nM, nanomol/L; SBF, suction blister fluid; SPE, solid phase extraction; SZ95, SZ95 sebocytes; UPLC-MS/MS, ultraperformance liquid chromatography quadrupole mass spectrometry

Hormone concentrations decline with aging. Up to now it was not clear, whether the decrease of hormone concentrations in blood samples are also present in cutaneous suction blister fluids, and whether skin from different anatomical sites shows different hormone concentrations.

Analysis of suction blister fluids and paired blood samples from young (mean 27.8 y) and old (mean 62.6 y) male subjects by UPLC-MS/MS showed that DHEA concentration in blood samples was age-dependently significantly reduced, but increased in suction blister fluids, while androstenedione behaved in an opposite manner to DHEA. Testosterone decreased age-dependently in blood samples and in suction blister fluids. Regarding skin sites, DHEA was lower in samples from upper back compared with samples from the forearm. In contrast, the concentrations of androstenedione and testosterone were higher in samples from upper back.

In vitro analyses showed that SZ95 sebocytes, but neither primary fibroblasts nor keratinocytes, were able to use DHEA as precursor for testosterone biosynthesis, which was confirmed by expression analysis of 3β-hydroxysteroiddehydrogenase in skin biopsies.

In conclusion, we show an inverse pattern of DHEA and androstenedione concentrations in blood vs. suction blister fluids, highlighting age-dependent changes of dermal testosterone biosynthesis, and a stronger metabolism in young skin. Furthermore, sebocytes play a central role in cutaneous androgen metabolism.

Introduction

Human skin is both, a hormone-sensitive organ and a peripheral tissue, able to synthesize hormones by itself.1,2 Testosterone is the most important androgen in males but it is also synthesized at significant levels in females at all ages.3 Testosterone can be converted to estradiol, which is an estrogen, synthesized by enzymatic aromatization in peripheral tissues.4,5 The concentration of estradiol in blood is much lower than that of testosterone.6,7 Both steroid hormones, testosterone and estradiol, are able to influence the appearance of human skin by inducing the synthesis of collagens, which preserve the structural integrity of the extracellular matrix and therefore help to maintain a youthful skin appearance characterized by a thick and elastic dermis without wrinkles.8,10

Testosterone is mainly synthesized by the gonads and to a lesser extent by the adrenals always using dehydroepiandrosterone (DHEA) as a precursor.11 As a consequence of age-dependent diminished metabolic productivity of steroidogenic organs, the levels of DHEA and testosterone in blood decline with advancing age.12,13 These age-dependent changes in hormone concentrations can aggravate the visible signs of skin aging such as wrinkles and sagging.5,10,14

For testosterone synthesis using DHEA as a precursor only two key enzymes are needed.15,16 The first step is the oxidative conversion of the hydroxyl group at the third carbon atom of DHEA by the 3β-hydroxysteroiddehydrogenase (3β-HSD), leading to the formation of androstenedione. Subsequently, the keto group at the 17th carbon atom of androstenedione is reduced by 17β-hydroxysteroiddehydrogenase (17β-HSD), resulting in the active testosterone. Aromatization of testosterone by aromatase (CYP19A1) leads to the generation of estradiol.17

It has been reported previously that significant amounts of testosterone can be derived from DHEA by local synthesis within
human skin itself. Sebocytes seem to be important mediators in this process because the existence of all needed enzymes for testosterone metabolism has been confirmed.

Up to now, the potential age dependence of testosterone biosynthesis in human skin in relation to its precursors, to the skin site and with respect to the age-dependent endocrine changes in plasma has not been investigated. To address this question, the concentrations of DHEA, androstenedione and testosterone were measured in blood and suction blister fluids of male volunteers. Suction blister fluid is mainly derived from the interstitial fluid of the skin. Its composition represents most of the components of serum at lower concentrations, but also locally generated metabolites. Because of this property, analysis of suction blister fluid composition is a powerful tool to determine local hormone concentrations in human skin, and to compare them with systemic hormone concentrations in parallel blood samples. Our data provide valuable new insights into the cutaneous mechanisms of sex-hormone metabolism.

Results

Influence of age on steroid hormone concentrations in blood and suction blister fluid of male subjects.

To investigate age-dependent differences of steroid hormone concentrations in blood and suction blister fluid (SBF), an in vivo study was performed. The study was approved by the ethical committee of the University of Freiburg, Germany, and conducted in accordance to the Declaration of Helsinki. Concentrations of DHEA, androstenedione and testosterone in blood samples and SBF derived from the upper back of young (27.8 y) and old (62.6 y) male subjects were determined by UPLC-MS/MS after solid phase extraction.

As shown in Figure 1, the concentrations of DHEA, androstenedione and testosterone were differently influenced by age. The concentration of DHEA in blood samples of young subjects (Median = 46.8 nM, interquartile range (IQR) = 39.8 nM) was significantly higher (p = 0.016) than in those derived from old subjects (Median = 21.6 nM, IQR = 7.4 nM) (Fig. 1A). On the contrary, the concentrations of androstenedione (Fig. 1B) and testosterone (Fig. 1C) were higher in SBF of young male subjects (median 9.0 nM, IQR = 16.9 nM), which was two fold higher than in samples of old male subjects (median = 0.6, IQR = 0.2 nM) (Fig. 1D).

The concentration of testosterone in blood samples declined age-dependently from 12.9 nM (IQR = 4.5 nM) to 10.8 nM (IQR = 4.9 nM) (Fig. 1E). In SBF, testosterone concentration was age-dependently reduced by 35%, from 4.9 nM (IQR = 3.2 nM) to 3.2 nM (IQR = 3.0 nM) (Fig. 1F).

Influence of skin site on steroid hormone concentrations. In order to assess the influence of skin site on dermal hormone levels, suction blisters of young male volunteers were collected from the upper back and the forearm of the same subject in parallel. In Figure 2, the concentrations of DHEA, androstenedione and testosterone are shown.

Depending on the skin site the concentrations of steroid hormones were differently altered. The concentrations of DHEA (Fig. 2A) were lower in SBF collected from the upper back compared with the forearm (median = 9.0 nM, IQR = 16.9 nM vs. 18.1 nM, IQR = 11.7 nM). In contrast, the concentrations of androstenedione (Fig. 2B) and testosterone (Fig. 2C) were higher in SBF of the upper back (median = 12.5 nM, IQR = 3.7 nM) compared with the forearm (median = 9.0 nM, IQR = 7.4 nM) (Fig. 2D).
Steroid hormone concentrations in SBF from different skin sites. (A–C) SBF from upper back (n = 8) and forearm (n = 8) of young male subjects were generated in parallel. Concentrations of DHEA, androstenedione and testosterone were subsequently determined by UPLC-MS/MS after solid phase extraction. Statistical significance differences were marked with an asterisk (p < 0.05).

As shown in Table 1, only SZ95 sebocytes were able to use DHEA as precursor for synthesis of androstenedione and testosterone. In the supernatant of the SZ95 sebocytes, androstenedione accounts for 43.1% (+/- 4.0%) and testosterone for 31.5% (+/- 49.4%) of all detected steroid hormones, showing that the added precursor DHEA is used for generation of androstenedione and testosterone, with only 22.2% (+/- 13.0%) of DHEA being left after 24 h of hormone incubation.

Primary fibroblasts and keratinocytes did not use DHEA as precursor for generating the active androgens androstenedione and testosterone. Nearly the complete added amount (94.4% +/- 3.2% for fibroblasts and 98% +/- 0.7% for keratinocytes) of the initially added DHEA was still detectable after 24 h of incubation.

Expression of 3β-HSD type I enzyme in vitro. The initial step in generating testosterone using DHEA as precursor is the oxidative conversion of DHEA to androstenedione, which is catalyzed by the 3β-HSD enzyme. In contrast to the adrenals, where the 3β-HSD type II enzyme is expressed, the skin expresses the type I isozyme. Using qPCR-analysis, 3β-HSD type I transcription in SZ95 sebocytes could be confirmed. In contrast, neither primary fibroblasts nor keratinocytes showed any detectable signal for 3β-HSD type I mRNA transcription (data not shown).

To confirm the qPCR-results, protein expression of the 3β-HSD type I enzyme in vivo. To confirm the role of sebocytes in cutaneous androstenedione metabolism from DHEA, 3β-HSD type I expression was visualized by cryosections of male scalp skin. A representative stain of 3β-HSD type I immunoreactivity is shown in Figure 3. 3β-HSD enzyme expression was mainly limited to sebaceous glands. A weak staining was also noticeable in the lower parts of the hair follicle duct (Fig. 4B–C). However, the magnified views of a sebaceous gland (Fig. 4D) and the epidermis with upper parts of the hair follicle duct (Fig. 4E) confirmed that 3β-HSD type I expression is limited to sebaceous glands.

Discussion

Steroid hormone concentrations are influenced by age. Our in vivo study shows that the age-dependent concentrations of DHEA and androstenedione in SBF and blood samples behave in an opposite manner. We could confirm previous studies showing significantly decreasing DHEA and testosterone levels in blood samples during the aging process.26,27 The observed age-dependent decrease of DHEA levels in blood samples of old male subjects has been attributed to reduced systemic metabolism.11,28 In sharp contrast to the blood data, the dermal concentrations of DHEA are elevated in SBF of old male subjects compared with SBF of young male subjects.

Table 1. Relative steroid hormone concentrations in cell culture supernatants after DHEA treatment

|           | DHEA | Androstenedione | Testosterone |
|-----------|------|-----------------|-------------|
| SZ95-Sebocytes | 22.2% | 43.1% | 31.5% |
| Fibroblasts    | 94.4% | 0.2%  | 0%    |
| Keratinocytes  | 98.0% | 0.4%  | 0%    |

Relative concentrations of DHEA, androstenedione and testosterone in supernatants from SZ95 sebocytes (n = 3), primary fibroblasts (n = 7) and primary keratinocytes (n = 4) 24 h after addition of DHEA. Sum of all detected steroid hormones was set to 100% for normalization of results.
Androstendione levels behave in an opposite manner since there is an age-dependent increase of androstendione in blood and a decrease in SBF. The percental change of dermal testosterone concentration between samples of young and old male subjects is even greater in SBF than in blood (35% vs. 16%), showing a higher local testosterone decline in skin of aged male subjects compared with blood.

The inverse age-dependent androgen patterns in blood vs. skin support the concept of an independent androgen metabolism in the skin undergoing separate skin-specific age-dependent changes. There seems to be an increased metabolism of DHEA to androstenedione and subsequently to the active downstream product testosterone in young skin vs. old skin. Therefore, young skin is able to use DHEA more efficiently for the synthesis of androgens than aged skin.

**Figure 3.** 3β-HSD type I expression in vitro. Confluent cultured SZ95 sebocytes (SZ95), primary dermal fibroblasts (HDF) and primary epidermal keratinocytes (NHEK) were harvested. Total protein fractions were isolated and subjected to western blot analysis using an anti 3β-HSD type I antibody. An anti-GAPDH antibody served as loading control.

**Figure 4.** 3β-HSD type I expression in vivo. Skin biopsies of human scalp skin were cryosectioned. DAPI staining (A) was used to visualize histological structures. Positive 3β-HSD type I immunoreactivity was confirmed using an anti-3β-HSD type I antibody (B). A merge image (C) of DAPI staining and 3β-HSD type I immunoreactivity showed that the immunoreactivity was restricted to sebaceous glands and lower parts of the hair follicle duct, which is confirmed by a magnification of a sebaceous gland (D) and epidermis (E).
Steroid hormone concentrations are influenced by skin site. In order to analyze whether the cutaneous steroid hormone metabolism is also altered by topological features, such as the difference of sebaceous gland density, SBF from upper back and forearm were taken in parallel. Upper back and forearm were chosen, because these skin sites contain a different sebaceous gland distribution, with a higher density of sebaceous glands in the upper back, and a sebaceous gland scarcity in forearm skin.29,30

The analysis of steroid hormone concentrations shows that the dermal concentration of DHEA is lower in SBF from upper back compared with the forearm, but concentrations of the metabolic products of DHEA, androstenedione and testosterone, were higher in SBF from upper back. These two characteristic steroid hormone patterns indicate a higher metabolism from DHEA to androstenedione and testosterone in the upper back compared with the forearm. Because of the fact that all samples were taken in parallel, our data show that cutaneous hormone metabolism is clearly influenced by skin site, and not only by the concentrations of steroids in the blood. The observed differences in hormone concentrations between the upper back, which is rich in sebaceous glands, and the forearm with only sparse sebaceous glands, supports the published hypothesis, that sebocytes play a key role in the cutaneous androgen metabolism, and local androstenedione and testosterone allocation.20,31

Sebocytes generate testosterone using DHEA as a precursor. To analyze whether sebaceous glands have a role for the observed skin site-specific differences in generation of androstenedione and testosterone, the metabolism of testosterone using DHEA as a precursor was analyzed in vitro. The experiments showed that only SZ95 sebocytes, but not primary fibroblasts or keratinocytes were able to use DHEA as a precursor for generating androstenedione and testosterone. In contrast, if androstenedione was used as testosterone precursor, generation of testosterone could be observed in the cell culture supernatants of SZ95 sebocytes, fibroblasts and keratinocytes (data not shown), confirming results indicating that 17β-HSD is expressed by all of these skin cell types.2,32

To investigate these findings further, the expression of the 3β-HSD type I enzyme was verified by qPCR-analysis and western blots. As anticipated, 3β-HSD type I enzyme was only detectable in samples of SZ95 sebocytes, but not in samples of primary fibroblasts and keratinocytes. To verify the in vivo function of sebaceous glands in cutaneous androstenedione generation, the localization of 3β-HSD type I enzyme was visualized by immunofluorescence stainings of facial skin biopsies. As predicted by qPCR-Analysis and western blots of cell cultures, 3β-HSD type I immunoreactivity was limited to sebaceous glands. The observed weak staining of lower parts of the hair follicle duct could be due to the fact that sebocytes release their cytoplasm, containing 3β-HSD type I, into the duct after holocrine lysis.33,34 Hence, our data confirm the previous published in vitro results, that sebaceous glands are important in the initial step of cutaneous testosterone metabolism by generating androstenedione from DHEA.16,20,31

Human skin is an endocrine tissue and also a target of the produced hormones itself.1–2 In this respect, cutaneously produced testosterone could be one factor that induces sebocyte proliferation.35,36 Moreover, testosterone and also estradiol, which are generated by aromatization of testosterone in the skin, are able to promote collagen synthesis.10,37 Therefore, high cutaneous testosterone biosyntheses leading to maintenance of high cutaneous testosterone concentrations in vivo could help to preserve the integrity of the extracellular matrix.9,14

Conclusion

The concentration of DHEA and androstenedione in cutaneous samples and blood samples, with respect to the age of the subjects, behave in an opposite manner, which shows that the dermal metabolism of androgens is age-dependently modified. Moreover our data show that young skin is able to produce more testosterone than old skin. The observed site-specific differences support the hypothesis that sebaceous glands are likely to be modifiers in these processes by controlling the initial conversion of DHEA to androstenedione.

Materials and Methods

Determination of steroid hormone concentration. DHEA, androstenedione and testosterone were simultaneously measured by ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) according to previously published method.3 UPLC-MS/MS was performed by using a Quattro Premier/Xe mass spectrometer connected to an Acquity System (Waters Corporation). In brief: 0.1 ml serum, cell supernatant or suction blister fluid sample were mixed with 10 μl of internal standard deuterium labeled 17-hydroxyprogesterone-d8 (Cambridge Isotope Laboratories, DLM-6598–0) and cortisol-d4 (Cambridge Isotope Laboratories, DLM-2218–0) were used. Calibrators and controls were prepared in steroid free serum for serum samples, and in DMEM medium (Gibco, 31053–028) for cell supernatant and suction blister fluids, and also extracted by SPE. Oasis MAX u-elution plates (Waters, WAT058943) were used for extraction and washed with 5% NH₄OH solution (Merck, 105428) and 10% methanol (Fluka, 34966). All steroids were eluted with isopropanol (Fluka, 34965). The UPLC Quattro premier/Xe system (Waters Corporation) was operated in multiple reaction mode and all steroids were measured in the positive ion mode. For each steroid two different MRM transitions were determined (supplemental data). The total run time was 5 min. Quantification was linear from 0.1 to 200 nM (r² > 0.992), and reproducible with an inter- and intra-assay coefficient < 15%. In addition the method was validated for suction blister fluids. Steroid-free suction blister fluid was generated by stirring suction blister fluid from different volunteers two times with active charcoal and subsequent filtration. The limit of detection for the determined hormones was 0.1 nM for androstenedione and testosterone and 0.5 nM for DHEA.

Collection of SBF and blood samples. Participants of this study were grouped in collectives of young (27.8 y, SD 2.4 y) and old (62.6 y, SD 2.4 y) healthy subjects (eight subjects each).
Participants who received hormone replacement therapy or suffer from hormone related diseases were excluded. The use of topical hormones was also not allowed. The Body Mass Indexes (BMI) of all subjects ranged between 20 and 30 kg/m². Suction blisters were prepared on upper back of all subjects and on forearms of the young subjects.

Fasting blood samples were collected with a 2.7 ml Sarstedt EDTA-Monovette rapidly in a short time period in the morning, immediately before generating suction blisters as described before. Blood plasma was received according to manufactures instructions and stored at −80°C for later analysis. After formation of suction blisters, the liquids were collected with a 1 ml syringe (Terumo, BS-01T and NN-2719R) and stored at −80°C for later steroid hormone analysis. For interpretation of data median values and interquartile ranges (IQR) were determined using Statistica 8 (Statsoft). IQR is the difference between the third and first quartile (IQR = Q75% − Q25%) and also called middle 50.

This study was approved by the Freiburg ethics committee and performed following the Declaration of Helsinki. All subjects gave their written informed consent.

Cell culture experiments. For functional analysis of cell specific testosterone metabolism, SZ95 sebocytes (n = 3), primary fibroblasts (n = 7) and keratinocytes (n = 4) were seeded at 80.000 cells/well in 24-well dishes.\(^\text{58}\) For cultivation of SZ95 sebocytes, Sebomed (Biochrom, F8205), and for fibroblasts DMEM (PAA, E15) and stored by −80°C for later steroid hormone analysis. For interpretation of data median values and interquartile ranges (IQR) was also not allowed. The Body Mass Indexes (BMI) of all subjects ranged between 20 and 30 kg/m². Suction blisters were prepared on upper back of all subjects and on forearms of the young subjects.

Blood plasma was received according to manufactures instructions and stored at −80°C for later analysis. After formation of suction blisters, the liquids were collected with a 1 ml syringe (Terumo, BS-01T and NN-2719R) and stored by −80°C for later steroid hormone analysis. For interpretation of data median values and interquartile ranges (IQR) were determined using Statistica 8 (Statsoft). IQR is the difference between the third and first quartile (IQR = Q75% − Q25%) and also called middle 50.

This study was approved by the Freiburg ethics committee and performed following the Declaration of Helsinki. All subjects gave their written informed consent.

Reference
16. Luu-The V, Bélanger A, Labrie F. Androgen biosynthetic pathways in the human prostate. Best Pract Res Clin Endocrinol Metab 2008; 22:207-21; PMID:18471780; http://dx.doi.org/10.1016/j.beem.2008.02.004

17. McTernan PG, Anwar A, Eggo MC, Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Labrie F, Bélanger A, Cusan L, Gomez JL, Candas B. Anatomical variation in the amount and composition of human sebaceous gland tissue. Neuro Endocrinol Lett 2008; 29:201-4; PMID:18404141

18. Beilke E, Gorenov V, Wickers C, Von Zur Mühlen A, Von Büren E, Brabant G. Age-related changes of serum sex hormones, insulin-like growth factor-1 and sex-hormone binding globulin levels in men: cross-sectional data from a healthy male cohort. Clin Endocrinol (Oxf) 2000; 53:689-95; PMID:11155090; http://dx.doi.org/10.1046/j.1365-2265.2000.01159.x

19. Bhatia S, Parker CR, Jr. Adrenal androgens and aging. Semin Reprod Med 2004; 22:361-8; PMID:15635503; http://dx.doi.org/10.1055/s-2004-861552

20. Fritsch M, Orfanoz CE, Zouboulis CC. Sebocytes are the key regulators of androgen homeostasis in human skin. J Invest Dermatol 2001; 116:793-800; PMID:11348472; http://dx.doi.org/10.1046/j.1523-1747.2001.01312.x

21. Itami S, Takaya S. Activity of 3 beta-hydroxysteroid dehydrogenase delta 4-5 isomerase in the human skin. Arch Dermatol Res 1982; 274:289-94; PMID:6219636; http://dx.doi.org/10.1007/BF00403732

22. Dumont M, Luu-The V, Dupont E, Pelletier G, Labrie F. Characterization, expression, and immunohistochemical localization of 3 beta-hydroxysteroid dehydrogenase/delta 5 delta 4 isomerase in human skin. J Invest Dermatol 1992; 99:415-21; PMID:1401999; http://dx.doi.org/10.101111/1523-1747.ep1216131

23. Volden G, Thorstus AK, Bjornson I, Jellum E. Biochemical composition of suction blister fluid determined by high resolution multicomponent analysis (capillary gas chromatography-mass spectrometry and two-dimensional electrophoresis). J Invest Dermatol 1980; 75:421-4; PMID:7430710; http://dx.doi.org/10.1111/1523-1747.ep1224077

24. Kool J, Reubsaet L, Wesseldijk F, Maravilha RT, Dumont M, Luu-The V, Belanger A, Pelletier G, El-Alfy M. Sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 1997; 82:2396-402; PMID:9253307; http://dx.doi.org/10.1210/jc.82.2396

25. Rhéaume E, Lachance Y, Zhao HF, Breton N, Dumont M, Luu-The V, Dupont E, Pelletier G, Labrie F. Characterization, expression, and immuno-histochemical localization of 3 beta-hydroxysteroid dehydrogenase/delta 5 delta 4 isomerase in human skin. J Invest Dermatol 1992; 99:415-21; PMID:1401999; http://dx.doi.org/10.111111/1523-1747.ep1216131

26. Stárka L, Dusková M, Hill M. Dihydrotestosterone and testosterone synthesized in cultured human SZ95 sebocytes derives mainly from dehydroepiandrosterone. Exp Dermatol 2010; 19:470-2; PMID:20337700; http://dx.doi.org/10.1111/j.1600-0625.2009.00996.x

27. Hikoma T, Maibach HI. Gender differences of enzymatic activity and distribution of 17 beta-hydroxysteroid dehydrogenase in human skin in vitro. Skin Pharmacol Physiol 2007; 20:168-74; PMID:17396051; http://dx.doi.org/10.1159/000101386

28. Dharia S, Parker CR, Jr. Adrenal androgens and aging. J Clin Endocrinol Metab 2000; 97:4279-84; PMID:10760294; http://dx.doi.org/10.1159/000080509

29. Plewig G, Kligman AM, Jansen T. Acne and rosacea. J Am Acad Dermatol 2001; 45(Suppl):S116-24; PMID:11511861; http://dx.doi.org/10.1067/mjd.2001.017

30. Greene RS, Downing DT, Pachi PE, Strauss JS. Anatomical variation in the amount and composition of human skin surface lipid. J Invest Dermatol 1970; 54:240-7; PMID:5436951; http://dx.doi.org/10.1111/j.1523-1747.1970.02280318

31. Chen W, Tsai SJ, Shyu HM, Tsai JC, Zouboulis CC. Testosterone synthesized in cultured human SZ95 sebocytes derives mainly from dehydroepiandrosterone. Exp Dermatol 2010; 19:470-2; PMID:20337700; http://dx.doi.org/10.1111/j.1600-0625.2009.00996.x

32. Hikoma T, Maibach HI. Gender differences of enzymatic activity and distribution of 17 beta-hydroxysteroid dehydrogenase in human skin in vitro. Skin Pharmacol Physiol 2007; 20:168-74; PMID:17396051; http://dx.doi.org/10.1159/000101386

33. Nelson LR, Bulun SE. Estrogen production and action. J Invest Dermatol 1999; 103:721-5; PMID:9796366; http://dx.doi.org/10.1111/j.1523-1747.ep1239601

34. Schneider MR, Paus R. Sebocytes, multifaceted epithelial cells: lipid production and holocrine secretion. Int J Biochem Cell Biol 2010; 42:181-5; PMID:19944183; http://dx.doi.org/10.1016/j.biocel.2009.11.017

35. Zouboulis CC, Xia L, Akamasu H, Sehlmann H, Fritsch M, Hornemann S, et al. The human sebocyte culture model provides new insights into development and management of seborrhea and acne. Dermatology 2001; 196:21-31; PMID:9557220; http://dx.doi.org/10.1159/0000007861

36. Abdel-Naser MB. Selective cultivation of normal human sebocytes in vitro; a simple modified technique for a better cell yield. Exp Dermatol 2004; 13:562-6; PMID:15335357; http://dx.doi.org/10.1097/00010675.2004.00187.x

37. Nelson LR, Buhun SE. Estrogen production and action. J Am Acad Dermatol 2001; 45(Suppl):S116-24; PMID:11511861; http://dx.doi.org/10.1067/md.2001.117432

38. Zouboulis CC, Sehlmann H, Neitzel H, Orfanoz CE. Establishment and characterisation of an immortalized human sebaceous gland cell line (SZ95). J Invest Dermatol 1999; 113:1011-20; PMID:10594745; http://dx.doi.org/10.1046/j.1523-1747.1999.00771.x

39. Plewig G, Klignman AM, Jansen T. Acne and rosacea. Springer, 2000.

40. Greane RS, Downing DT, Pachi PE, Strauss JS. Anatomical variation in the amount and composition of human skin surface lipid. J Invest Dermatol 1970; 54:240-7; PMID:5436951; http://dx.doi.org/10.1111/j.1523-1747.1970.02280318

41. Chen W, Tsai SJ, Shyu HM, Tsai JC, Zouboulis CC. Testosterone synthesized in cultured human SZ95 sebocytes derives mainly from dehydroepiandrosterone. Exp Dermatol 2010; 19:470-2; PMID:20337700; http://dx.doi.org/10.1111/j.1600-0625.2009.00996.x

42. Hikoma T, Maibach HI. Gender differences of enzymatic activity and distribution of 17 beta-hydroxysteroid dehydrogenase in human skin in vitro. Skin Pharmacol Physiol 2007; 20:168-74; PMID:17396051; http://dx.doi.org/10.1159/000101386