Sexual Hormones in Human Skin

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Abstract
The skin locally synthesizes significant amounts of sexual hormones with intracrine or paracrine actions. The local level of each sexual steroid depends upon the expression of each of the androgen- and estrogen-synthesizing enzymes in each cell type, with sebaceous glands and sweat glands being the major contributors. Sebocytes express very little of the key enzyme, cytochrome P450c17, necessary for synthesis of the androgenic prohormones dehydroepiandrosterone and androstenedione, however, these prohormones can be converted by sebocytes and sweat glands, and probably also by dermal papilla cells, into more potent androgens like testosterone and dihydrotestosterone. Five major enzymes are involved in the activation and deactivation of androgens in skin. Androgens affect several functions of human skin, such as sebaceous gland growth and differentiation, hair growth, epidermal barrier homeostasis and wound healing. Their effects are mediated by binding to the nuclear androgen receptor. Changes of isoenzyme and/or androgen receptor levels may have important implications in the development of hyperandrogenism and the associated skin diseases such as acne, seborrhoea, hirsutism and androgenetic alopecia. On the other hand, estrogens have been implicated in skin aging, pigmentation, hair growth, sebum production and skin cancer. Estrogens exert their actions through intracellular receptors or via cell surface receptors, which activate specific second messenger signaling pathways. Recent studies suggest specific site-related distribution of ERα and ERβ in human skin. In contrast, progestins play no role in the pathogenesis of skin disorders. However, they play a major role in the treatment of hirsutism and acne vulgaris, where they are prescribed as components of estrogen-progestin combination pills and as anti-androgens. These combinations enhance gonadotropin suppression of ovarian androgen production. Estrogen-progestin treatment can reduce the need for shaving by half and arrest progression of hirsutism of various etiologies, but do not necessarily reverse it. However, they reliably reduce acne. Cyproterone acetate and spironolactone are similarly effective as anti-androgens in reducing hirsutism, although there is wide variability in individual responses.

Introduction
Several functions of the human skin appear strongly dependent on biologically active sexual hormones, namely androgens, estrogens, and progestins. Their effect is mediated by binding to nuclear receptors, and lack of functional receptors prevents the action of sexual hormones on the skin [1]. The effects of sexual hormones can differ from cell type to cell type and among cells of different locations [2]. Androgen effects on the pilosebaceous unit are best known and best characterized. Nevertheless there is considerable variability in the response of the pilosebaceous unit to androgens that is not yet completely understood [3].

Androgens and skin
Androgens relevant to the skin
Among the circulating androgens, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) are predominantly produced in the adrenal cortex. Androstenedione is produced approximately equally by the adrenal cortex and the ovaries, and somewhat less by the testes [4]. These are weak prohormones that act only after conversion
to the more potent androgens, testosterone and 5α-dihydrotestosterone (DHT). Testosterone is mainly secreted by the testes in males beginning at puberty; in reproductive-age females it arises almost equally from the ovary and adrenal cortex by a combination of secretion and conversion of androstanediene in peripheral organs. DHT is mainly synthesized in peripheral organs, including skin, in both genders.

Testosterone, particularly free testosterone, is the major circulating androgen because of its concentration and potency [3]. The less potent DHEA-S is the androgen with by far the highest serum concentration in both sexes and is related to sebum production prepubertally [5] and to cystic acne in adults [6]. Androstanediene and DHEA have also been shown to stimulate sebum secretion in humans [7]. Male levels of testosterone stimulate full axillary and pubic hair growth, but in the absence of DHT formation do not suffice to stimulate male beard growth and scalp hair recession [8].

The androgen receptor and skin

Testosterone and DHT act through a single nuclear receptor, the androgen receptor (AR), with DHT being the more active ligand [9]. AR is an X-chromosome-encoded, ligand-activated, intracellular transcription factor that belongs to the steroid/nuclear receptor superfamily [2, 10]. Like all nuclear receptors, AR is a soluble molecule with a proclivity for employing transcriptional regulation as a means of promoting its biological effects. In common with other steroid receptors, AR is compartmentalized in the cytoplasm, where it exists in polymeric complexes that include the heat shock proteins hsp 90, hsp 70, and hsp 56. Association of androgens with AR results in dissociation of the heat shock proteins. This in turn exposes a nuclear translocation signal previously buried in the receptor structure and initiates transport of the ligand-receptor complex to the nucleus. There, AR occupies androgen response elements in the promoter regions of androgen-regulated genes to initiate the signaling cascade. AR is present in epidermal and follicular keratinocytes, sebocytes, sweat gland cells, dermal papilla cells, dermal fibroblasts, endothelial cells, and genital melanocytes [11, 12]. It is stabilized by ligand binding and is up-regulated in genital skin fibroblasts and sebocytes [13, 14].

Polymorphisms that confer enhanced receptor activity have been variably associated with the androgen-dependent skin disorders. Studies of CAG trinucleotide repeats have shown them to have been inconsistently related to hirsutism [9, 15]. Failure to account for possible skewed X-inactivation of the affected alleles, for which there are likewise conflicting data, contributes to some of the disparities. A recent linkage study has reported that genetic variability in a shorter GCN repeat of the AR gene, which confers a higher gene effect, has an etiological faction of 0.46 for early-onset familial male-pattern baldness, confirming an earlier study [16]. Thus, this may prove to be a more clear-cut determinant of sensitivity to androgen than the CAG repeat.

Androgen metabolism and its transcriptional regulation in the skin

The skin can be regarded as a peripheral organ that locally synthesizes significant amounts of androgens with intracrine or paracrine actions [3, 11]. Having recognized the key effects of biologically active androgens on skin, their local synthesis and degradation have gained special interest. The local level of each sex steroid depends upon the expression of each of the androgen- and estrogen-synthesizing enzymes in each cell type, with sebaceous glands and sweat glands being the major contributors [9, 17].

The skin, especially sebocytes, is capable of synthesizing cholesterol, which is utilized in cell membranes, formation of the epidermal barrier and sebum; however, sebocytes express very little of the key enzyme, cytochrome P450c17, necessary for synthesis of the androgenic prohormones DHEA and androstanediene [18]. However, DHEA and androstanediene, and possibly DHEA-S, can be converted by sebocytes and sweat glands, and probably also by dermal papilla cells, into more potent androgens like testosterone and DHT [2]. Five major enzymes are involved in the activation and deactivation of androgens in skin [19]. In a first step, steroid sulfatase hydrolyzes DHEA-S to DHEA in skin [20]. The sebaceous gland has been suggested to carry out this reaction since strong steroid sulfatase immunoreactivity was found in acne skin, primarily associated with the monocytes infiltrating the lesions [21], but further evidence is required to corroborate this preliminary report. This enzyme activity has also been detected in the dermal papillae of human terminal hair follicles [22]. Subsequently, 3β-hydroxysteroid dehydrogenase/A5α-isomerase (3β-HSD) converts DHEA to androstanediene. Two isoforms of this enzyme have been described. Human skin seems to express exclusively the type 1 isoform. Several studies led to the conclusion that type 1 3β-HSD is mainly located in the sebaceous glands [17].

In a further step, androstanediene is activated by conversion to testosterone through androgenic 17β-hydroxysteroid dehydrogenase (17β-HSD). The cutaneous expression of 17β-HSD was mainly demonstrated in the pilosebaceous unit and epidermal keratinocytes. To date, twelve isoforms of this enzyme have been identified [19, 23]. 17β-HSD types 3 and 5 form the active androgen testosterone from androstanediene, whereas the oxidative reaction of 17β-HSD types 2 and 4 is in the reverse direction, inactivating the potent sexual steroids. The human sebaceous gland possesses the cellular machinery needed to transcribe the genes for 17β-HSD types 1–5; among them a strong 17β-HSD2 and 17β-HSD5 expression have been reported [17, 18, 24]. The predominance of the strongly pro-oxidative 17β-HSD2 suggests its protective role against the effects of locally excessive amounts of potent androgens [18]. Greater reductive activity of androgenic 17β-HSD types 3 and 5 was noted in sebaceous glands from the facial than from non-acne prone areas, suggesting an increased net production of potent androgens in facial areas. In addition, 17β-HSD3 expression was detected in human sebocytes, but not in keratinocytes, further indicating the importance of the sebaceous gland in cutaneous androgen activation [17]. However, scrotal skin 17β-HSD3 and 17β-HSD5 expression levels seem to be age-dependent, in that 17β-HSD3 mRNA is highly expressed before 2 years of age and again during the teenage years, whereas 17β-HSD5 mRNA is predominantly expressed between the age 2 and 13 years and again after 30 years of age [24]. In hair follicles, 17β-HSD is localized in outer root sheath cells, where it primarily inactivates androgens; early studies showed androstanediene as the major metabolite of cultured human hair follicle keratinocytes incubated with radiolabeled testosterone [19]. Anagen hairs mainly express high levels of type 2 and moderate levels of type 1 17β-HSD [25]. 17β-HSD enzyme activity has also been shown in cultured epidermal keratinocytes and in the microdissected apocrine sweat glands.

5α-Reductase irreversibly converts testosterone to DHT, the most potent naturally occurring androgen in tissue [26]. Two isoforms have been described; type 1 dominates in the skin [27].
Expression of this enzyme is predominant in sebaceous and sweat glands, with lesser activity in epidermal cells and hair follicles [9]. Type 2 activity predominates in beard hair follicles. Finally, two isoenzymes of 3α-hydroxysteroid dehydrogenase (3α-HSD) catalyze active androgens to compounds that do not bind the androgen receptor (three isoforms of 3α-HSD were initially identified, but the type 2 isozyme has been found to predominantly form testosterone and has been renamed 17β-HSD type 5) [17,28]. By glucuronidation or sulfation, water soluble steroid metabolites are eliminated through the kidney. Alternatively, aromatase may convert testosterone and androstenedione to estrogens in sebaceous glands, outer as well as inner root sheath cells of anagen terminal hair follicles, and dermal papilla cells [17,29].

More recently, transcription factors regulating steroidogenesis in the classical steriodogenic organs have been demonstrated in the skin. Thus, a review of current literature provides a hint that SOX-9 may potentiate steroidogenesis by way of steroidogenic factor-1 (SF-1) to activate the steroidogenic acute regulatory protein (STAR), while DAX-1 exhibits an antagonizing function, and WT-1 plays a bimodal tuning role by up-regulating DAX-1 [30].SF-1 and DAX-1 are detected in skin and its appendages with a distinctive expression pattern. Prominent expression of DAX-1 has been confined to the epidermis, sebaceous glands, sweat glands, and outer root sheath of the hair follicles with weaker expression in the inner root sheath, matrix cells, and dermal papilla cells. Similarly, SF-1 has been detected in the epidermis, but displayed a scattered nuclear pattern across all layers [31,32]. SOX-9 and WT-1 were also detected in the skin [30]. These data demonstrate that important regulators of steroidogenesis are present in human skin and its appendages. These transcription factors may have a role in cutaneous steroidogenesis and thus be involved in hair follicle pathologies associated with steroids.

Androgens and the sebaceous gland
Sebaceous gland enlargement and production of sebum are dependent upon androgens, and at puberty, male sebum production modestly increases over that of females [9]. Skin in acne produces higher rates of testosterone and DHT than in healthy individuals. In addition, isolated elevations of plasma DHT and 3α-androstenediol glucuronide, postulated by some to be biochemical markers of cutaneous androgen metabolism and action, have been found in female patients with acne [33,34], but there is considerable evidence that these results primarily reflect adrenal steroid metabolites [9]. Androgens stimulate sebocyte proliferation, an effect dependent on the area of skin from which the sebaceous glands are obtained; facial sebocytes are mostly affected [34]. In contrast, androgens as single compounds seem to be unable to modify sebocyte differentiation, which is stimulated by co-incubation with peroxisome proliferator activated receptor (PPAR) ligands [35,36].

Androgens and the hair follicle
Androgens have strong effects on hair growth and appear to act through type 2 5α-reductase and the AR on dermal papilla cells [9,37]. Single nucleotide polymorphisms of the AR have been associated with hirsutism in women [38] and androgenetic alopecia in men [16]. Dermal papilla cells appear to mediate the growth-stimulating signals of androgens by releasing growth factors that act in a paracrine fashion on the other cells of the follicle [39]. Androgens cause enlargement of hair follicles in androgen-dependent areas (beard in male adolescents, axillary and pubic hair), but in scalp follicles of susceptible men paradoxically androgens foster miniaturization and shortage of hair in the anagen stage leading to common baldness [3]. These contradictory effects may be explained by genetically determined differences in the response of papilla cells to androgens at different body areas [9]. Notably AR mRNA was reported to be expressed in beard and axillary hair dermal papilla cells for both sexes, but only at a low level in those from occipital scalp hair [40]. In addition, very high doses of testosterone and DHT (10 μM) were shown to induce apoptosis of dermal papilla cells in association with activation of the bcl-2 pathway [41]. The skin of hirsute women forms excessive DHT, but it is unclear whether this is formed by the hair follicles themselves or by the associated sebaceous gland hyperplasia [42]. Men with a deficiency of type 2 5α-reductase provided the initial clue that conversion of testosterone to the more potent DHT by this enzyme enhances androgenic effects on hair follicles. These individuals produce little or no beard growth and do not develop androgenetic alopecia [43]. Subsequently the inhibition of type 2 5α-reductase by finasteride has been proven to slow or even reverse the progression of androgenetic alopecia [44]. Currently, higher levels of STAR and type 1 3β-HSD were detected in the scalp of men with androgenetic alopecia [45].

Further effects of androgens on human skin
Androgens appear to promote perspiration since males sweat at a greater rate than females in similar situations [46]. This difference between the sexes arises during puberty. Sweat glands contain over half of skin 5α-reductase activity and express the enzymes necessary to form DHT from DHEA, as well as AR. However, the sweat gland secretion rate is not directly influenced by androgens: androgen treatment has not stimulated sweat production in adult women, and anti-androgen application to the skin of males has not decreased the sweat rate. Therefore, androgens have been postulated to initiate the factors required for the different sweat secretion rate between the sexes during puberty, but do not maintain the function of the glands. It is likely that the androgen effect is exerted on the differentiation of the apocrine sweat glands. This type of sweat glands, a hybrid of this eccrine and apocrine glands, develops during puberty from eccrine or eccrine-like precursor glands, but the secretory rate is seven-fold higher in response to similar innervation. Given that these glands comprise up to 45% of the axillary glands in patients with hyperhidrosis, they probably play a major role in the pathophysiology of this condition. Hyperhidrosis has been reported to be the sole skin manifestation of androgen excess. A few studies have indicated that the apocrine gland of patients with excessive or abnormal odor (osmidrosis), irrespective of sex, is a typical androgen target organ [47]. Type 1 5α-reductase predominates in the apocrine sweat glands of such patients [48].

Adult male skin is thicker and drier than female skin. This is in part because androgen stimulates epidermal hyperplasia and suppresses epidermal barrier function in fetal and adult human skin [49]. Testosterone replacement treatment has been reported to have a similar effect on barrier function in castrated adult mice and an adult man with hypopituitarism. Ashcroft and Mills [50] observed in a hairless mouse model that endogenous testosterone inhibition of cutaneous wound healing was AR-mediated. However, further work, especially in humans, is required to better understand the enhanced inflammatory response suggested by these and other authors [51,52].
Androgens, seborrhea and acne

The obligatory role of androgens in the pathophysiology of acne has long been recognized and corroborated by clinical and experimental observations and therapeutic experience. Clinical observations supporting the pathogenic role of androgens in acne, include close association between the normal onset of microcomedonal acne in prepubertal children with the adrenarcheal rise in circulating DHEA-S levels [53], acne formation in small children with virilizing tumors or congenital adrenal hyperplasia (CAH) [54], hyperandrogenism identified in women with sudden exacerbation of acne, persistent acne beyond 30 years of age and therapy-refractory acne, absence or rarity of acne in men with androgen insensitivity syndrome or early castration before puberty [55], induction of acne by systemic or topical administration of androgens or anabolic steroids [56], and positive associations between serum androgen levels and acne lesion counts in men and women [57]. In vitro studies using sebaceous gland organ culture, rat prepubial glands, and primary culture or immortalized human sebocytes have all demonstrated the expression of the enzymes necessary for the synthesis and metabolism of androgens [17,27,58,59]. However, the in vitro effect of supplemented androgens on the cell division and lipo-bogenesis varies, depending partly on the culture conditions [34,35,58,60,61]. Hormonal treatment of acne in female patients using various methods of suppressing androgen secretion or action suppresses sebum production by 12.5–65% and is beneficial as monotherapy for female patients with mild to moderate acne [62,63].

In addition to stimulation of sebum production, indirect evidence also suggests the importance of androgens on comedogenesis and inflammation: higher activity of type 1 5α-reductase in the follicular infrainfundibulum, indicating increased androgen genesis and inflammation: higher activity of type 1 5α-reductase or 17β-HSD mRNA levels, implying that stress and inflammation may also augment androgenesis in sebocytes [66].

Functional studies are needed to prove the significance of the expression of the steroidogenic enzymes identified and localized in human skin, especially in sebaceous glands. Moreover, quantitative differences of the enzyme activities between normal healthy and acne-prone skin should be precisely determined before the design of potential drugs and the advancement of their clinical use.

Expression of steroidogenic enzymes in acne patients

The distribution and strong activities of various hydroxysteroid dehydrogenases in human sebaceous glands in acne-prone as compared to non-acne skin have long been observed [67]. While there was no difference in the rates of enzymatic hydrolysis of steroid sulfatase between the freshly obtained epidermal tissue of acne-prone and normal skin [20], a novel non-estrogenic inhibitor of steroid sulfatase, 6-{[adamantylidene]hydroxybenzoxazole}-O-sulfamate, was shown to effectively block the enzyme in the skin leading to a reduction of sebum secretion in animal studies [68]. However, although an exclusive predominance of type 1 5α-reductase has been demonstrated in sebaceous glands, with higher enzyme activity in facial skin than in nonacne-prone skin [27,69], there seems to be no relationship between the activity of 5α-reductase or 17β-HSD in sebaceous glands and the presence or absence of acne in both sexes [70]. Moreover, in a 3-month, multicenter, randomized, placebo-controlled clinical trial, the use of a potent selective inhibitor of type 1 5α-reductase alone or in combination with systemic minocy-cline was not associated with clinical improvement of acne [71]. On the other hand, enhanced expression of type 2 5α-reductase was revealed in the hair follicle but not in the sebaceous glands in inflammatory acne lesions [72]. Greater activity of type 2 17β-HSD, the isozyme working in the opposite oxidizing direction to inactivate androgens [73], was found in sebaceous glands from nonacne-prone areas as compared to sebaceous glands obtained from facial skin [18].

Androgen excess

Cutaneous manifestations – seborrhea, acne, hirsutism, male-pattern alopecia, i.e., SAHA syndrome when full-blown [74] – are prominent symptoms of peripheral androgen excess. Androgen excess occurs by increased circulating androgens (hyperandrogenemia) or by increased intracellular levels of androgens in the skin (hyperandrogenism). Hyperandrogenemia arises from ovarian or adrenal dysfunction or tumors, disturbed peripheral metabolism of androgen precursors, or exogenous androgenic medications [3]. Polycystic ovary syndrome, a polymorphic disorder, accounts for over 90 percent of cases. This hyperandrogenic disorder is often associated with insulin resistance and acanthosis nigricans in obese women, a combination termed HAIR-AN syndrome [75]. Other important causes, in order of decreasing frequency, are CAH, hyperprolactinemia, Cushings’s syndrome, gonadal or adrenal neoplasms, disorders of sexual differentiation, and corpus luteum dysfunctions of pregnancy. Exogenous (iatrogenic) causes of androgen excess include testosterone, anabolic steroids, the androgenic progestin dana-zol, and valproic acid [3].

In CAH, defects of enzymes involved in adrenal cortisol synthesis result in inefficient cortisol biosynthesis [76]. This is compensated by an increased pituitary secretion of adrenocorticotropic hormone. Thus, normal cortisol blood levels may be achieved, but at the cost of an excess production of adrenal androgens, which is responsible for clinical signs. The enzyme most often defective in adrenal hyperplasia (over 95%) is 21-hydroxylase. In a few instances, other intermediary enzymes are responsible (11β-hydroxylase, 3β-HSD or P450 oxidoreductase) [76,77]. 21-Hydroxylase deficiency is an autosomal recessive disorder. Various mutations affecting both alleles of the gene lead to variable degrees of impairment of 21-hydroxylase activity [76]. Severe defects produce classic forms, which manifest themselves in infancy (virilization, genital ambiguity in girls, and, if mineralocorticosteroids are also deficient, salt wasting) or in childhood (pseudoprecocious puberty). Mild defects, one of the most common genetic disorders in man, cause nonclassic, late onset presentations, in which less severe signs occur later in childhood or during or after puberty including premature puberty, acne, hirsutism, or irregular menses; this form may even remain asymptomatic (cryptic form).

There may be a tendency to underestimate the role of androgen excess in men with acne. Indeed, a few published studies indicate the relevance of this phenomenon. Men with persistent acne had significantly higher serum levels of androgens than age-matched controls [78], and excess androgens of adrenal origin were frequently detected in men with severe (cystic) acne [6].
Hypogonadism

Skin signs of hypogonadism are thin, weak hair with a reduced number of terminal hair follicles on the face and the axilla and a feminine pattern of pubic hair, as well as lack of seborrhoea, acne and androgenetic alopecia, along with penis and testicular atrophy [79].

Treatment of androgen excess-associated disorders

The major thrust of drug design for treatment of androgen-associated disorders, so far, has been directed against several levels of androgen function and metabolism [19]. However, only partial effectiveness has been achieved either by androgen depletion, inhibition of androgen metabolism or blockade of the AR. In addition, major adverse events can occur, since effectiveness is only associated with systemic application of such compounds. Acne, hirsutism, and androgenetic alopecia of female pattern as manifestations of systemic or local androgen excess are best treated by eliminating the cause (e.g., tumors, drugs) or by interfering with androgen secretion or action. Oral contraceptives are used in women with polycystic ovary syndrome or idiopathic hirsutism [41]; both estrogens and progestins contribute to the androgen-suppressive effect [62,80]. Anti-androgens such as high-dose cyproterone acetate or spironolactone exhibit the strongest anti-androgenic activity among progestins [41]; they must be used with contraception because anti-androgens interfere with the differentiation of the genitalia of the fetal male. The combination of dospironone with a low dose estrogen seems to be as effective as the combination low-dose cyproterone acetate and estrogen [80,81]. In male acne patients with CAH, low-dose glucocorticoids (e.g., methylprednisolone 4 mg every other day at bedtime) have been used to suppress ACTH-mediated adrenal androgen production [82]. These hormonal treatments are best combined with other anti-acne regimens for a quicker relief [83]. Finasteride is the first selective androgen-metabolizing enzyme inhibitor introduced, targeting androgenetic alopecia in men [19].

Estrogens and skin

Estrogens and human skin

A number of studies have shown that estrogens have many important beneficial and protective roles in skin physiology [84,85]. Estrogens can delay or prevent skin aging manifestations by reducing epidermal thinning and maintaining skin thickness and hydration [86–88]. In postmenopausal women skin collagen content decreases at the rate of 2 % per year [89], while estradiol treatment can significantly increase hydroxyproline content [90]. Skin elasticity also correlates negatively with years since menopause, while hormone replacement therapy increases elasticity by 5 % over a year [91]. Estrogens also accelerate cutaneous wound healing [92], and many women notice an improvement in inflammatory skin disorders, such as psoriasis during pregnancy [93–95]. Epidemiological studies indicate that the mortality rates from non-melanoma skin cancer [96] and melanoma [97] are significantly lower in women. Binding studies on whole human skin homogenates have demonstrated the existence of estrogen-binding sites, although receptor levels vary with body site, with higher numbers in facial skin compared to thigh or breast [98,99]. Recent studies have begun to localize the molecular and cellular basis of these findings.

The estrogen receptors

Two distinct intracellular estrogen receptors (ER), ERα and ERβ, have been identified that belong to the superfamily of nuclear hormone receptors [100]. ERα and ERβ share approximately 60 % homology in the ligand binding domain, but bind estradiol with a similar affinity [101]. More recently it has been demonstrated that estradiol can also act independently of their classical genomic pathway [102]. Rapid cellular responses to estradiol, much faster than that can be attributed to genomic signaling, provide evidence that cell membrane estrogen receptors exist. These receptors can activate signaling cascades via conventional second messengers, including adenylate cyclase, cAMP, phospholipase C, protein kinase C, mitogen-activated protein kinase and ligand or voltage-gated ion channels [103–108]. In some cells ERβ counteracts ERα, in some cases acting as an ERα heterodimer to inhibit the transactivating function of ERα, and in other cases acting as a homodimer to regulate specific genes, many of which are anti-proliferative [109].

Estrogens and epidermal keratinocytes

Recently, it has been demonstrated by immunohistochemistry that ERβ is the predominant estrogen receptor in adult human scalp skin, where it is strongly expressed in the stratum basale and stratum spinosum of the epidermis [110–112]. A recent study using semi-quantitative RT-PCR has confirmed ERβ mRNA expression in the skin of the midgestational human fetus [113]. Further work has demonstrated that human epidermal keratinocytes express only ERβ mRNA and protein by RT-PCR [114] and western blotting [115]. In contrast, an investigation of the high affinity estrogen-binding sites in human neonatal foreskin epidermal keratinocytes showed expression of both ERα and ERβ by immunocytochemistry and northern blotting [116]. The difference between these studies of ER expression may be due to culture conditions: since keratinocytes expressing both receptors were cultured in media containing phenolred, which has estrogenic activity [117], and estradiol can up-regulate ERα expression in cultured keratinocytes [116], skin ERα may be up-regulated by estrogenic compounds. Recent studies have also demonstrated that estradiol conjugated with bovine serum albumin can stimulate both epidermal keratinocyte proliferation and DNA synthesis [118], and estradiol increases phosphorylation levels of ERK1 and ERK2 kinases within 15 minutes in such cells [116]; both are indications that estradiol can activate non-genomic signaling pathways in the epidermis.

Estrogens and dermal fibroblasts

Primary cultures of human dermal fibroblasts from female skin have been shown to express both mRNA and protein for ERα and ERβ [119]. Although they co-express both receptors, immunocytochemistry showed some variation in their expression. ERβ was predominately nuclear, while ERα was expressed in both the cytoplasm and the nucleus. Furthermore, mRNA levels for ERβ were higher than levels of ERα. The same group also demonstrated that estradiol up-regulates ERβ expression in dermal fibroblasts cultured from postmenopausal women [120].

Estrogens and the pilosebaceous unit

The hair follicle

Estrogens appear to stimulate hair growth in man [9]. They are thought to prolong the anagen phase of scalp hair growth by increasing cell proliferation rates and postponing their transition to the telogen phase. In an organ culture system, estradiol
stabilizes hair shaft elongation in fronto-temporal male hair follicles [121]. Estrogens in low dosage modestly stimulate pubic and axillary hair growth of hypogonadal girls, independently of changes in androgen levels. It is possible that this effect of estrogens on hair growth is mediated in part by induction of androgen receptors, as is the case in brain, or by increase in insulin-like growth factor-I levels. In late pregnancy, when estrogen levels are high, a high proportion of scalp hair follicles remain in anagen. Postpartum, a large number of hair follicles simultaneously advance into telogen phase, causing loss of a large number of hairs. This postpartum telogen effluvium has been postulated to be caused by the rapid decrease of estrogens at the time of delivery causing a large number of hair follicles to simultaneously advance into telogen. On the other hand, estrogens significantly inhibit hair growth in a number of other mammalian species [84,85]. The estradiol-induced delay in the transition from the telogen to anagen phase in the mouse hair growth cycle appears to be mediated by the ERα of dermal papilla cells [122]. ER-null and aromatase-null mice are virilized and so have not proven to be a useful model for understanding the role of estrogens in hair and skin. In situ, immunohistochemical studies have shown that ERβ is strongly expressed in human scalp anagen hair follicles in contrast to ERα [29,112]. While there are no reports of ER expression in human telogen follicles, in the murine hair cycle ERα expression is maximal in the telogen follicle [123]. Human hair follicles in culture express ERβ, although the distribution pattern by immunohistochemistry appears to be gender-specific [124]; ERβ immunoreactivity was predominately in the female follicular dermal papilla, which appears to determine the type of hair produced [125], and although basal levels were much lower in male follicles, they could be up-regulated by estradiol. Dermal papilla cells cultured from female follicles expressed mRNA for both ERα and ERβ. Immunocytochemistry has demonstrated co-expression of ERα and ERβ; however there was some variation; ERβ was predominately nuclear, while ERα was expressed in the cytoplasm and the nucleus [29]. This is similar to the observation of ER expression in dermal fibroblasts [119]; however, in contrast to mRNA levels in dermal fibroblasts [120], in cultured follicular dermal papilla cells mRNA levels for ERα were approximately 2-fold higher than ERβ [29]. In addition, the expression of ERα mRNA in cultured follicular dermal papilla cells was down-regulated by dexamethasone, while ERβ expression remained unaffected [29].

The sebaceous gland
Estrogens suppress sebaceous gland size and function, both indirectly and directly, by pituitary-gonadal suppression of androgen production [9]. The estrogen effect is clear at the 35 μg dose of ethynylestradiol in oral contraceptive pills. The dose of estrogen required to suppress sebum production appears to be greater than the dose required to suppress ovulation [32]. In vitro studies with the addition of estradiol failed to show a regulatory effect on cell proliferation [35,58], while the inhibition of lipogenesis varied in different animal studies [35,125,126]. When administered systemically, estrogens produce a reduction in size and secretion of sebaceous glands in both human sexes [127]. Immunostaining of human skin has shown that ERα and ERβ are co-localized in different sebocytes [110–112], while basal sebocytes only display ERα [128]: the significance of this finding remains to be determined. The human epidermis, unlike most steroidogenic organs abundant in ERα, mainly expresses ERβ.

The apocrine gland
Similar to the sebaceous gland, the apocrine gland develops from the hair follicle and remains attached to it, increasing in size and activity with sexual maturity. A recent study has confirmed by both immunohistochemistry and RT-PCR that ERβ is expressed in the human axillary apocrine gland [129]. Although the apocrine secretory epithelium exhibited strong nuclear and cytoplasmic staining for ERβ, there was no expression of ERα, as also confirmed by RT-PCR [129].

The ecrine glands
Although the role of estrogens in the ecrine glands is unclear, recent immunohistochemical studies by two separate laboratories have demonstrated the presence of ERβ, but not ERα in human ecrine glands [110,112].

Estrogens and aromatase
Aromatase, the product of the CYP19 gene, catalyzes three consecutive hydroxylation reactions converting C19 androgens to C18 estrogens [130]. Aromatase is also present in various extra-gonadal tissues and its expression is regulated in part by means of tissue-specific promoters through the alternative splicing mechanism on multiple exon 1 variants[131]. At least six variants of exon 1 have been described; exons 1a, 1b, 1c, 1d, 1e and 1f that are specific for expression in the placenta, skin fibroblasts/fetal liver/adipose tissue/vascular tissue, ovary, ovary/prostate, placenta, and fetal brain, respectively [132,133]. By immunohistochemical examination, aromatase was found in the outer root sheath of anagen, terminal hair follicles and in sebaceous glands, but rarely in telogen hair follicles [134]. The higher expression of aromatase in the scalp hair follicles of women than men, particularly on the occiput, has suggested that local estrogen formation from testosterone may play a role in protecting them from alopecia [134]. Aromatase activity has been reported in the pilosebaceous unit [135], keratinocytes cultured in serum-free medium [136], fibroblasts from both genital and non-genital skin [137] and fibroblasts from adipose tissue [138]. However, in a recent study by Chen et al. (unpublished data), the mRNA expression of aromatase was under the detection limit in the total scalp extract from either bald or occipital area of men with androgenetic alopecia.

Estrogens and acne
Although the efficacy of ethynylestradiol-containing oral contraceptives has been confirmed and approved in acne treatment, very little is known about the role of estrogens in pathogenesis of acne formation. The quantitative difference of ERα and ERβ between normal and acne skin remains to be determined, although significant differences in the number of estrogen receptors between normal and acne-bearing skin was found to exist in both sexes [127].

Estrogens and melanocytes
Normal melanocytes
Hyperpigmentation of the face is commonly seen in pregnant women and may be accompanied by increased pigmentation in areas such as the areolae, linea alba and perineal skin, all of which usually fade following parturition [139]. Estrogen containing oral contraceptives can also cause facial hyperpigmentation [140] and estrogen-containing ointments can cause intense pigmentation of the genitals, mammary areola and linea alba in male and female infants [141]. Recently, using a double immu-
of fluorescence method, human scalp epidermal melanocytes have been shown to express both ERα and ERβ in situ [142], and others have confirmed by ligand binding studies that cultured human epidermal melanocytes contain ER [143]. A more recent immunocytochemical study has reported that human melanocytes cultured from adult foreskin express ER, although it is unknown whether this is ERα or ERβ [144]. Human female scalp melanocytes express ERα and ERβ in culture [145], and the induction of pigmentation in these cells up-regulates ERα but not ERβ [146].

Melanoma
The effects of estrogens on melanoma are poorly understood, apparently because estrogen receptors are variably expressed in melanoma cell lines [147–149]. Thornton et al. have recently shown that pigment induction leads to an increase in the expression of both ERα and ERβ in a poorly pigmented melanoma cell line [145, 146] suggesting that pigment cell phenotype may be regulated via ER.

Future therapeutic concepts of estrogen-related disorders
Since estrogens, presumably acting via ER, have important effects on skin ageing, pigmentation, hair growth, sebum production, wound healing and skin cancer, there is a pressing need to understand the complex interactions between estrogens, their receptors and other signaling events. The recent advances in the development of selective ligands, selective ER modulators and activators of non-genotrophic estrogen-like signaling, may help to answer the outstanding questions regarding estrogen-associated disorders in human skin.

Progestins and skin

Human skin and progestins
All progestins have the unique effect of increasing body core temperature. Natural progesterone has no known influence on human skin other than exerting this effect at normal luteal phase levels. This progestin action results from raising the thermoregulatory set-point at which sweating occurs [150]; evidence for a direct effect on cutaneous vasomotor tone is inconclusive [151]. However, synthetic progestins have varying degrees of clinically significant androgenic, anti-androgenic, anti-mineralocorticoid and glucocorticoid side-effects (Table 1). Nevertheless, androgenic progestins such as norethindrone and levonorgestrel have been important in cutaneous medicine in combination with estrogen in the treatment of hirsutism and acne. Consequently, pharmaceutical development efforts have centered on developing new generations of synthetic progestins such as drospirenone with selectively improved anti-androgenic and anti-mineralocorticoid profiles.

Mode of action and effects of progestins
All progestins have corticoid, anti-corticoid, androgenic, and anti-androgenic effects. Discovery of these properties dates to discovery of the anti-mineralocorticoid effect of progesterone itself [152], which led to the development of the progesterone analogue spironolactone as an anti-mineralocorticoid [153] with little progestational activity [154]. Subsequently, the progesterone analogue cyproterone acetate was found to be a potent anti-androgen [80, 82, 155]. The anti-androgenicity of spironolactone was later recognized [156] and served as the basis for the recent development of the unique progestin drospirenone [80, 81]. Meanwhile, the search for orally active progestins led to the discovery of the progestational effects of the 19-nortestosterone analogues that are the gestogenic components of most oral contraceptives [157]. They are structurally androgenic and generate estrogenic or anti-estrogenic metabolites to varying degrees. The molecular basis of the relatively promiscuous pattern of signaling by progestins is due to high homology among the DNA binding domains (75%) and modest homology among the ligand binding domains (≥50%) of progesterone, mineralocorticoid glucocorticoid and androgen receptors [158, 159]. A new weakly gestogenic 19-nortestosterone derivative, tibolone, which has estrogenic effects on climacteric symptoms and bone and significant androgenic effects, is now marketed in Europe [160, 161]. A new generation of “pure” progestins based on 19-norprogestone is under development. The clinically important endocrine properties of commonly used gestogens are summarized in Table 1 [80, 81, 152–154, 156, 162–166].

The effects of progestins are not necessarily direct or genomic. Combination with estrogen in oral contraceptives enhances gonadotropin suppression of ovarian androgen production. Progestosterone itself also complements estrogen effects on fluid retention by lowering the osmotic threshold for vasopressin release [150]. Unique structural features of individual agents are responsible for other actions, such as inhibition of 5α-reductase and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Endocrine profiles of commonly used progestins. Potency estimates are semiquantitative composites of clinical and animal studies</th>
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<tbody>
<tr>
<td>Type of progestin</td>
<td>Anti-androgenic activity</td>
</tr>
<tr>
<td>Progesterone-derived</td>
<td></td>
</tr>
<tr>
<td>Chlormadinone acetate</td>
<td>±</td>
</tr>
<tr>
<td>Cyproterone acetate</td>
<td>+</td>
</tr>
<tr>
<td>Drospirenone</td>
<td>+</td>
</tr>
<tr>
<td>Medroxyprogesterone acetate</td>
<td>±</td>
</tr>
<tr>
<td>Progesterone</td>
<td>±</td>
</tr>
<tr>
<td>Nortestosterone-derived</td>
<td></td>
</tr>
<tr>
<td>Desogestrel</td>
<td>0</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>0</td>
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<tr>
<td>Norethindrone</td>
<td>0</td>
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<td>Norgestimate</td>
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</tbody>
</table>

* * Indicates the most potent side-effect of its class, + indicates a small but clinically significant effect of doses used therapeutically, and ± indicates an equivocal effect.
inactivation of cytochrome P-450 by spironolactone [151, 167] or 3β-HSD inhibition by cyproterone acetate [17,80,155]. Medroxyprogesterone acetate, but not progesterone itself, inhibits estrogen-induced vasodilation, which involves both genomic and non-genomic activation of nitric oxide synthase, while norethisterone has a non-genomic vasodilator effect [165, 168, 169]. Although 3β-HSD, the enzyme capable of converting pregnenolone to progesterone, has been characterized and localized in human skin, the production of progesterone has not yet been directly demonstrated in sebaceous glands [170]. Progesterone receptors are detected in nuclei of human sebaceous gland cells, and the effect of progesterone on sebum production in animal models varies in different studies, depending on the species and sex of the animals [127,171–174].

**Progestins and skin disorders**

The major use of progestins in skin disorders is in the treatment of hirsutism and acne vulgaris, where they are prescribed as components of estrogen-progestin combination pills and as anti-androgens [175]. Estrogen-progestin treatment can reduce the need for shaving by half and arrest progression of hirsutism of various etiologies, but does not necessarily reverse it. In contrast, they are effective in reducing acne. It is unclear whether those combinations with non-androgenic progestins are more effective in reducing lesions. However, they are to be favored because they do not inhibit the beneficial effect of estrogen on the serum lipid profile; those with drospirenone have the additional advantage of counteracting estrogen-induced sodium retention [63,176,177]. Cyproterone acetate and spironolactone are similarly effective as anti-androgens in reducing hirsutism, although there is wide variability in individual responses. Whether progestins play a direct role in hair cycling is unknown. Although telogen effluvium has been attributed to high estrogen or prolactin levels, the settings in which these hormones are incriminated (pregnancy and oral contraceptive use) [178,179] are related to high progestin states as well. Progestins are effective in reducing post-menopausal hot flashes [161]. This is likely to mainly result from action on the central nervous system [180].

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