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REVIEW

Menopause and the Skin: Old Favorites and New Innovations in Cosmeceuticals for Estrogen-Deficient Skin

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ABSTRACT

Estrogen is a pivotal signaling molecule; its production is regulated by the expression of the aromatase (CYP19A1) gene from ovarian and peripheral tissue sites, and it is transmitted via estrogen receptors to influence many important biological functions. However, the narrative for this overview focuses on the decline of 17β-estradiol levels from ovarian sites after menopause. This estrogen-deficient condition is associated with a dramatic reduction in skin health and wellness by negatively impacting dermal cellular and homeostatic mechanisms, as well as other important biological functions. The changes include loss of collagen, elastin, fibroblast function, vascularity, and increased matrix metalloproteinase(s) enzymatic activities, resulting in cellular and extracellular degradation that leads to dryness, wrinkles, atrophy, impaired wound healing/barrier function, decreased antioxidant capacity [i.e., defense against reactive oxygen species (ROS) and oxidative stress], decreased attractiveness and psychological health, and increased perception of aging. While topical estrogen may reverse these changes, the effects of today's low-dose systemic hormone treatments are not well established, raising the need for more concentrated local administration of hormones or newer cosmeceutical agents such as selective estrogen receptor modulators (SERMs), including phytoestrogens that have become major active ingredients for skin care products, especially when addressing estrogen-deficient skin. Two example compounds are presented, an analog of resveratrol (i.e., 4′-acetoxy resveratrol) and the isoflavonoid equol, both of which are involved in a variety of biochemical/molecular actions and mechanisms, as demonstrated via in vitro and clinical studies that enhance human dermal health, especially in estrogen-deficient skin.

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PLAIN LANGUAGE SUMMARY

Estradiol levels decline to near zero after menopause. Estrogen deficiency adversely affects many physiological functions, including skin changes such as atrophy, wrinkles, hydration, poor wound healing/barrier function, decline in perceived facial attractiveness, and

even psychological health. Women with menopausal skin changes seek cosmetic and medical treatments that enhance their self-perception and inhibit skin aging, particularly in exposed areas (face, neck, and hands). It is widely accepted that traditional treatments such as local hormone treatment are effective in reversing (estrogen-deficient) aging skin deterioration. But, the uncertainly of the effects of long-term systemic menopausal treatment and, more recently, aversion to systemic hormones has led to newer therapeutic agents that can send estrogen's important skin-health signals via selective estrogen receptor modulators (SERMs) other than estrogen itself. Many plant-derived compounds (phytoestrogens) that contain estrogen-agonist SERMs now play major roles in treatments for aging and estrogen-deficient skin. The targets are the estrogen receptor beta molecules that are abundant in skin (keratinocytes/fibroblasts). The variation in effect and the influence of coexisting influences such as environmental exposure, race, and aging are reviewed. While several botanicals are mentioned in this overview, two promising cosmeceuticals are examined, an analog of resveratrol [4′-acetoxy resveratrol (4AR)], which enjoys a high public profile in the health arena, and the isoflavonoid compound equol. Both 4AR and equol are SERMs that have peer-reviewed in vitro and clinical study results supporting improvement of estrogen-deficient menopausal skin.

Keywords: Aging; Cosmeceuticals; Estrogen; Estrogen deficient skin; Equol; 4′-Acetoxy resveratrol; Hormone therapy; Menopause; Polyphenols; Skin

Key Summary Points

Menopause represents an estrogen-deficient hormonal state with general and dermal health concerns, where the skin reflects a conspicuous decline in physical attributes due to the lack of estrogen's positive effects.

Women with estrogen-deficient skin associated with menopause seek cosmetic and medical treatments to improve dermal health and physical characteristics to enhance their self-perception and inhibit skin aging.

Traditional treatments, such as low-dose menopausal hormone treatment (MHT), are adequate to marginal in reversing estrogen-deficient skin. This has led to newer therapeutic agents that can send estrogen's important signal in a specific positive manner via selective estrogen receptor modulators (SERMs). Many plant-derived compounds (phytoestrogens) have this SERM characteristic and now play a major role as cosmeceuticals in the skin care industry.

Reviewed here are two phytoestrogen/botanicals, namely an analog of resveratrol [4′-acetoxy resveratrol (4AR)] and a newer isoflavonoid compound, equol. Both are cosmeceuticals that have peer-reviewed in vitro and novel clinical study results that support the improvement of estrogen-deficient skin.

DIGITAL FEATURES

This article is published with digital features, including a summary slide and plain language summary, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13214081.

INTRODUCTION

The narrative for this overview presents: (a) an introduction to estrogens and their impact on human health, estrogen biosynthesis by the aromatase gene/enzyme, and estrogen hormonal action via estrogen receptors, (b) estrogen as an essential hormone in skin function, health, and wellness during premenopause, (c) specifically, how estrogen levels change with aging and especially at and after menopause, which influences skin estrogen biosynthesis and estrogen receptor expression, resulting in estrogen deficiency and alterations in skin components, and (d) traditional treatments, such as hormone replacement therapy (HRT), and new innovations in cosmeceuticals for estrogen-deficient skin in women.

We gathered data (from 2000 to August 2020) assessing current therapeutic options using the keywords: estrogen-deficient skin, menopause, skin, dermal, estrogen, and cosmeceuticals for estrogen-deficient skin using different keyword combination. The following databases were utilized: Web of Science (currently maintained by Clarivate Analytics covering over 12,000 journals and 160,000 conference proceedings) and PubMed maintained by the US National Library of Medicine at the National Institutes of Health (USA). Also, we included other background references (where appropriate) on the topics of estrogen, skin, aging, menopause, natural products, and cosmeceuticals (without a year-limit range for searching these topics). This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

ESTROGENS AND HEALTH

The impact of estrogens, specifically the potent sex steroid hormone, 17β-estradiol, controls many aspects of health [1–5]. There are two other major natural estrogens in the circulation, estrone and estriol (primarily during pregnancy) [1, 5].

While estrogens widely influence many important functions such as homeostatic actions, cell proliferation and death, liver protein expression, lipid metabolism, energy balance, glucose metabolism, immune and cardiovascular regulation, gonadotrophin feedback and gametogenesis, brain-neuronal development/memory processing and repair/neurodegeneration, bone growth, and others, this review is focused on estrogen and dermal health, especially in estrogen-deficient skin in women [1–6].

ESTROGEN BIOSYNTHESIS IN SKIN

The enzyme responsible for estrogen production is encoded by the aromatase cytochrome P450 (CYP19A1) gene, which converts C19 androgens to C18 estrogens [3, 4]. This enzyme has a dual effect: (1) the removal of the androgen molecule or the "detoxification of androgens by aromatase" by the removal of one carbon and (2) the production of the estrogen molecules that are, mole for mole, 100 to 1,000 times more biologically active or potent compared with their parent androgens [3, 4]. So testosterone, through the more abundant androgen metabolic pathway, is reduced by the 5α-reductase enzyme to 5α-dihydrotestosterone (5α-DHT), which is known to inhibit estrogen actions such as wound healing and skin repair [6–9].

Skin estrogen biosynthesis has been reported in keratinocytes, melanocytes, and fibroblasts [10–13]. Also, estrogen induces the structural protein ezrin that enables the intercellular bridges that furnish epidermal integrity [14], induces the hydrophilic glycosaminoglycan hyaluran that underlies skin thickness and opacity, induces elastin which gives the skin its resilience to deformation, and induces the expression of several types of collagens that are the basis of the mass of the dermis [14].

ESTROGEN PRODUCTION, LEVELS IN WOMEN: EFFECTS ON SKIN

At puberty, the developing ovarian follicles begin secreting estrogens. Of the two principle estrogens, 17β-estradiol is approximately seven times more potent than estrone. This is because of its interaction characteristics with estrogen

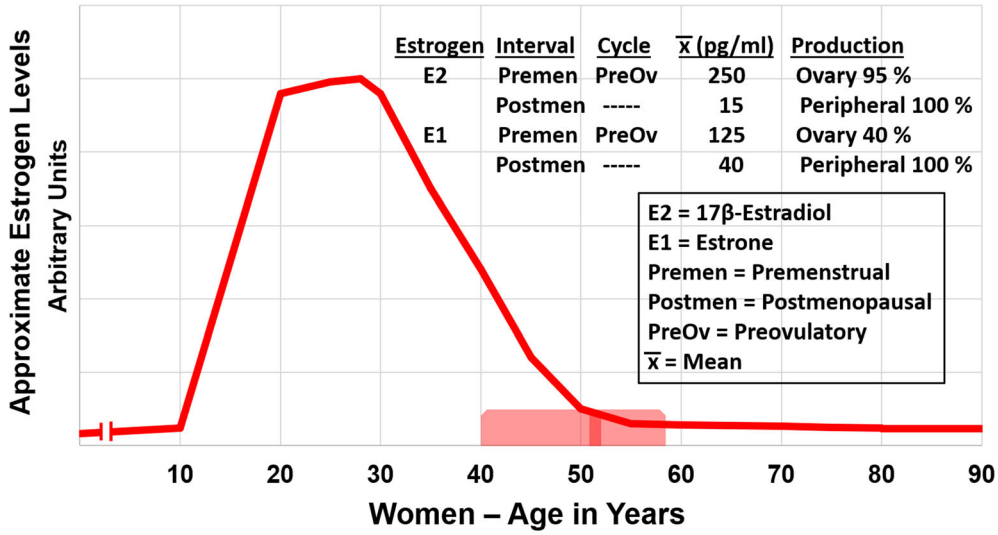

Fig. 1 Approximate production of estrogens (profile) in women with age. Estrogen levels peak in the late 20s. Estrogen levels during perimenopause fluctuate greatly around a normal range until menopause, when no more responsive follicles are available. In the USA, most women experience menopause from 40 to 58 years of age, with the average at 51 years of age (see red rectangular bar above the *x*-axis). In postmenopausal women, all the estrogen production is derived from peripheral tissues, primarily from adipose tissue [15]. Estrogen levels (17β-estradiol and estrone) in the reproductive interval (i.e., approximately 12–40 years of age) and changes in these levels during the perimenopause and postmenopausal intervals have been reported in detail elsewhere [17]

receptors. Estriol has little estrogen agonist activity for the same reason [4, 15]. During the adult reproductive period, overall estrogen levels originating from the ovaries peak in the late 20s [15] (Fig. 1). Skin collagen and elastin peak around 30 years of age, which corresponds with the peak in estrogen production [16]. In this regard, several reports suggest positive correlations between circulating estrogen levels and: (a) perceived age, (b) attractiveness, (c) enhanced skin health, and (d) facial coloration in women [17]. There are several reviews on the importance of estrogen and skin [13, 17–21], only the main points will be noted here.

As human beings age, the first signs of dermal aging begins around 30 years of age when estrogen levels begin to decline, the skin thins, dries, wrinkles, becomes pigmented unevenly [and with continued age liver spots (solar lentigines) form], and wound healing is delayed [13, 17–23]. Specifically, the appearance of wrinkles around the eyes and mouth, and frown lines along the forehead are seen with uneven skin color and a general loss of skin tone (pale appearance) [16, 21–23]. Sagging skin and thin skin are due to the loss of definition/abundance of the underlying collagen and especially the elastin fibers in the dermal layer that provide the full, robust, and elastic recoil properties of youthful skin associated with normal premenstrual estrogen levels, see below [13, 17–23]. Estrogen also enhances moisture/hydration (via hyaluronic acid, mucopolysaccharides, and sebum production) where skin turgor, dermal thickness, and keratinocyte and fibroblast proliferation is increased [13, 17–21]. Additional positive influences of estrogen include increased cellular viability and extracellular matrix components, such as fibrillin and tissue inhibitor of matrix metalloproteinases (TIMP) and inhibition of matrix metalloproteinases (MMPs) [13, 17–21], counteracting radical oxygen species (ROS) and oxidative stress (OS) via its antioxidant properties, and finally activating nuclear factor erythroid 2-related factor (NRf2) that leads to the increased expression of other antioxidants and detoxifying enzymes [24–31].

Table 1 Changes: in estrogen-deficient skin

	References
General	
↑ Dryness/pruritis	[20, 22–24]
↑ Wrinkles	[17–20, 22, 23, 33, 64]
↑ Thinning/atrophy	[20, 22, 23]
↑ Impaired wound healing	[18–23, 33, 35]
↑ Perceived age	[17, 35]
↓ Attractiveness (facial coloration)	[16, 17, 35]
↓ Overall skin health (turgor, tone, etc.)	[16, 17, 22, 23, 35]
↓ Barrier function	[10, 18, 22, 23, 33]
↓ Psychological health	[16, 35]
↓ Antioxidant capacity	[20, 21, 32]
↓ ROS defense against oxidative stress	[20, 21, 35]
Epidermis	
↑ Flattening of the dermal–epidermal junction	[17, 18, 22, 23]
↓ Melanocyte activity	[22, 23]
↓ Langerhans cells	[22, 23]
↓ Re-epithelization	[20, 23, 33]
↑ Number of pores	[23]
Dermis	
↓ Hydration (glycoaminoglycan, mucopolysaccharide, and hyaluronic acid content via fibroblasts)	[18–20, 32–35]
↓ Collagen synthesis/content (type I and type III)	[17–21, 34, 35, 64]
↓ Elastic fibers (elasticity)	[17, 19–21, 33–35, 64]
↓ Fibroblast function (insulin-like growth factor and TGF-β)	[17, 21, 34]
↑ Matrix metalloproteinases (MMPs)	[17, 23, 35]
↓ Cellular and vascularity (blood flow)	[19, 23, 35]
Hypodermis	
↓ Overall volume/distribution of subcutaneous fat	[17, 22, 23]

ROS reactive oxygen species, *TGF-β* transforming growth factor beta

THE MENOPAUSE AND ESTROGEN-DEFICIENT SKIN

There are several reviews that cover the importance of estrogen-deficient skin during postmenopause [17–21, 23, 32–35], only the main basic endocrine, skin biology, and some clinical points will be noted here (see Table 1).

Menopause is a period of particular interest regarding skin biology and treatment. The term

"menopause" marks a milestone in aging women—1 year of no menstrual periods [36]. The cause is the failure of ovarian follicles to produce sufficient estrogen to stimulate the growth of the endometrium. While menopause can occur prematurely in women as a result of systemic or ovarian disease, or as the result of ovarian ablation, most attention is focused on skin changes in women who, from the age of about 45 years, begin to undergo high and low erratic swings of estrogen as their gonadotropin-responsive ovarian follicles become exhausted [15, 17].

By age 45–55 (the average of menopause in the USA is 51 [36]), all responsive follicles are gone and there ensues a decades-long period in which the main source of estrogens is local formation (peripheral conversion) of androgens secreted by the ovarian stroma and adrenal glands [15, 17]. The degree of skin atrophy present at a specific time depends on the previous exposure to estrogen, the amount of local estrogen produced by the skin and subcutaneous fat, and effects of aging [15, 37].

Regardless of the above, few menopausal women escape skin atrophy [33]. The most obvious places that this is noticeable is in areas that have not been shaded from actinic rays and where an upright posture facilitates gravity-fed sagging; the face, neck, and forearms-hands [22, 23, 33]. A simple test of the extent of these changes is performed by gently grasping the skin on the back of the hand, pulling it upward and observing when the skin is released how quickly the fold falls back to its original shape. In postmenopausal women the fold may take 3–4 times as long to reconstitute itself as is the case in premenopausal women [38, 39]. This is due to the lack of hydrophilic glycosaminoglycans (GAGs) and proteoglycans (PGs) [40, 41], low hydration of the dermis [18–20, 32–35], the lack of a tight association between epidermis, dermis, and sub dermis [17, 18, 22, 23] caused by decreased expression of collagen and elastin [17, 18, 21].

Also, collagen to collagen cross-linking is important where they provide strength and stability, while excessive or nonspecific cross-links create stiffness and lack of recoil, which is a component of wrinkle formation, along with a reduction in muscle mass, skin thickness, and dehydration of the stratum corneum [41–43]. Notably, since hyaluran is plentiful during the premenopausal period, it may be that the collagen in skin is always cycling with hyaluran-spread collagen fibrils, making available estrogen-induced collagenase and other proteases. This is the case in the uterine cervix [44].

In addition to the above, the skin of menopausal women, particularly that of women who are many years postmenopausal is fragile to abrasion. This is related to the decrease in estrogen-induced ezrin in the epidermis. Ezrin is responsible for the interlinking of epidermal cells via "intercellular bridges" that maintain the integrity against the elements of the epidermis layer of the skin [14, 45].

Throughout the dermis and sub dermis, there is vascular fragility that may result in leakage of blood from the microvascular system. While this has not been studied, it is possible that this is related to the lack of estrogen-dependent vascular maintenance [46, 47].

All-in-all, the above changes during the menopause are difficult to hide and become a serious cosmetic issue [48].

ESTROGEN RECEPTORS AND THEIR ACTION IN SKIN

The classical estrogen receptors (ER), ERα, and ERβ, are members of the superfamily of nuclear hormone receptors [5, 12, 17]. Specifically, human ERβ is homologous to ERα, particularly in the DNA-binding domain (97% amino acid identity) but share little homology in the other domains [5, 12, 17]. Based upon the dissimilar amino acid homology in the ligand-binding region, one may predict 17β-estradiol would display different affinities for the ERs, but surprisingly, 17β-estradiol has almost equal high affinity for ERα with a K_d = 0.13 nmol/L and for ERβ with a K_d = 0.15 nmol/L [5, 12, 17, 34]. There is tissue-specific expression in humans of the ERs, where ERβ is more widely expressed in skin compared with ERα, and this is especially the case in the human scalp [10–12, 17, 21]. ERα activation is a major factor in reproductive cancers (e.g. breast and prostate), whereas ERβ

activation appears to be chemoprotective [34, 49]. Finally, ERβ activation has been shown to promote wound healing, independent of estrogen's anti-inflammatory properties [50]. Thus, selective estrogen receptor modulators (SERM's) at ERs have proven to provide skin benefits.[21, 34, 35, 49, 51, 52].

It is now apparent that many cells express a nongenomic, G-protein-coupled seven-trans-membrane ER termed GPER, also known as GPER1 or GPR30, that directly triggers cellular signaling cascades [5, 12, 17]. Recently, it has been shown that GPER activation protects against epithelial barrier disruption by *Staphylococcus aureus* α-toxin [53].

Finally, multiple studies have established the presence of mitochondrial ERs, which suggests that estrogen plays a role in regulating cellular bioenergetics [48, 54, 55]. While not yet studied in skin, this estrogen regulatory mechanism may be important for good dermal health.

TRADITIONAL ESTROGEN-BASED SYSTEMIC AND TOPICAL TREATMENTS

Starting in the 1940's, treatment of menopausal women with estrogenic preparations became popular for symptoms such as hot flushes [56]. This menopausal hormone treatment (MHT) was later augmented with the addition of a progestin to avoid the development of endometrial hyperplasia or cancer. While the hormones were primarily administered orally, they also were given by sub-cutaneous implants and by topically applied gels [56] The chief estrogen preparation used in the US remains an equine urinary extract of mixed human and equine hormonal compounds termed conjugated equine estrogen (CEE) or Premarin© [17, 56].

The most common progestin compounded with CEE is medroxyprogesterone acetate (MPA), although recent studies have incriminated MPA in many adverse effects such as nausea, bloating, headache, changes in appetite, weight gain, tiredness, swelling, acne, hot flashes, breast tenderness [57], and should be avoided in favor of nonsynthetic progestins such as progesterone or SERMs that do not activate the endometrium [21, 51].

In the intervening years since the inauguration of MHT, many pharmaceutical compoundings have appeared, including 17β-estradiol and progesterone. The latter required the development of micronized forms to avoid intestinal metabolism [58]. Both CEE and estradiol have been available in gel/cream forms.

In addition to relief of menopausal symptoms, maintenance of bone and psychological health, there has been an interest in the effect of these "classical" forms of MHT on the skin. While there are many anecdotal and open label studies of the effects of these various forms of MHT on skin health that showed positive effects on skin thickness [18, 19], wrinkles [59], and other measures [17, 23, 33], randomized, blinded prospective trials are few, indeed. Most of the studies on the effects of estrogen on skin date from the time when the dosage of the estrogenic component of MHT was as much as ten times the amount in present day treatment. Furthermore, through all the hormones and skin literature, there is continued qualification of studies and results because of the large and usually unmeasured or uncontrolled effects of exposure to the elements, smoking, race, and aging [17, 23, 60–62].

Formal evaluations of the effect of these variables are found in the literature on MHT and skin cancer. These studies show that race—likely expressed as skin pigmentation—, exposure, smoking, and aging are confounding variables with more influence on the skin than MHT [63].

While several studies, including a study by Wolff, Narayan and Taylor in 2005 [64], have suggested that MHT improves aging skin [17–21, 34, 35], at present, there is only one prospective randomized controlled trial of the effects of oral or transdermal MHT compared with placebo. This is the Kronos Early Estrogen Prevention Study (KEEPS) published in 2016, a 5-year, multicenter, double-blind, randomized placebo-controlled trial (NCT00144180). In this study, Owens and colleagues studied the effect of CEE + progesterone, or 17B estradiol + progesterone versus control on carefully measured

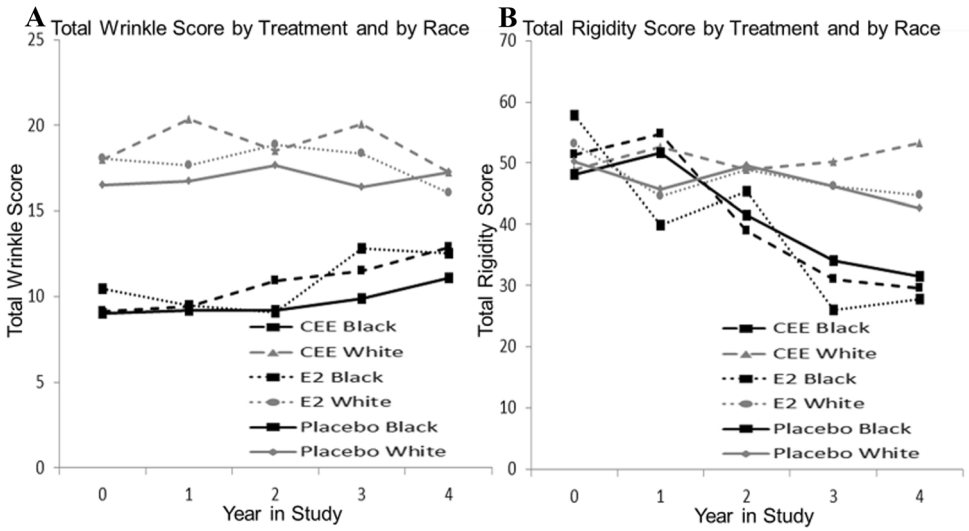

Fig. 2 Hormone effects on skin vary by race. A total of 116 subjects were assessed (CEE $n = 38$; E2 $n = 34$; Placebo $n = 44$; White $n = 77$; Black $n = 21$; Other $n = 16$) the average age at menopause was 53.2 ± 2.8 years, the average time since menopause was 1.6 ± 1.1 years and the average BMI (kg/m^2) was 26.0 ± 4.1. Reproduced with permission from [65]. **a** Mean total wrinkle score by treatment and by race over 4 years of follow-up. Racial groups (white and black) stratified by treatment group are indicated by gray and black lines, respectively. Total wrinkle score was not significantly different among treatment groups at any time point ($P = 0.24$). Black women, compared with White women, had the lowest total wrinkle scores across all 4 years ($P = 0.002$). **b** Men total rigidity score by treatment and by race over 4 years of follow-up. Racial groups (White and Black) stratified by treatment group indicated by gray and black lines, respectively. Total rigidity did not vary significantly among treatment groups at any time point ($P = 0.87$). Black women, compared with White women, had significantly decreased total facial rigidity after 4 years of follow-up ($P = 0.002$)

skin wrinkles and rigidity in women within 3 years of menopause who received treatment or placebo for 4 years. We show the most important illustration (Fig. 2) from this clinical study as reported by Owens, et al. [65].

The conclusion of this study was that race was the strongest predictor of the advancement of skin aging in the 4 years following menopause where Black women had the lowest wrinkle scores and significantly reduced facial rigidity compared with White women [65]. Also, MHT does not appear to affect skin wrinkles or rigidity at most facial locations [65].

Notably, in contrast, earlier studies showed that long-term hormonal therapy can indeed prevent skin aging in women [17–21, 64]. This may not be surprising, as estrogen plays many important roles in skin cells and glands such as keratinocytes, Langerhans cells, melanocytes, sebaceous glands, and fibroblasts, and decreased estrogen levels results in decreased capillary blood flow velocity to the skin [66]. However, as noted by Owen et al. [65] "previous findings may have been confounded by indication and selection bias that may account for the differences seen in earlier studies that were non-prospective, non-randomized, non-double-blind." Also, it is possible that the KEEPS data from the Owen study in 2016 [65] may have been "underpowered to detect a difference with MHT, or the relative dose was not potent enough, or that a different period of treatment would have led to decreased wrinkles and an objective difference in skin wrinkle scores."

The recent clinical trials appropriately use lower doses than was common in the last century, meaning these results may be more relevant than earlier studies. However, with the introduction of different compoundings and doses of MHT, more prospective clinical trials are greatly needed.

IS ESTROGEN TREATMENT SAFE?

Over the last two decades, there has been a change in the attitudes of both professionals and the public regarding the medical uses of estrogen. This is due to a misapprehension of the effects of MHT on menopausal women. In 2002, the NIH stopped the estrogen-containing arms of a large randomized trial of menopausal treatments [67, 68]. The misapprehension was due to the inclusion in the Women's Health Initiative (WHI) of > 10-year postmenopausal women with age-related risk factors for cardiovascular complications. This resulted in an excess of venous thromboembolism among subjects older than 59 years at the time of commencement of the trial with MHT. By the time that this error was noticed, along with the lack of adverse effects on perimenopausal women less than 60, the administration of estrogen-containing MHT had fallen below 25% compared with previous years, and many doctors were aggressively opposing MHT. Furthermore, with the loss of marketable product, pharmaceutical manufacturers discontinued development and testing of estrogen-containing products. However, it is clear that contemporary MHT started in healthy women before they have reached 6–10 years past the menopause is free of excess cardiovascular complications [69]. MHT use is an individual issue for the woman and her caregiver. This applies to women with histories of successfully treated estrogen-sensitive lesions [70].

ESTROGEN-ONLY TREATMENT FOR SKIN HEALTH

Since menopause is linked to the failure of ovarian function, the presence of the uterus is not relevant to issues regarding menopause. That being noted, surgical removal of the uterus for gynecological disease or cancer often is accompanied by removal of the ovaries. In those cases, women of premenopausal age undergo premature menopause and may be treated with MHT for hot flushes. In these cases, there is no need for the addition of progestin to protect against endometrial growth. The types of estrogen are the same as described regarding MHT for menopausal hot flushes, etc. There are no studies on the skin of premature menopausal women taking estrogen. At the time of writing, it is safe to consider estrogen alone treatment (ET) to be comparable to MHT as regards dermal health [19, 71].

TOPICALLY APPLIED HORMONE TREATMENT OF SKIN

Although the skin has a protective epidermal layer, fat soluble molecules, such as steroid hormones, are well absorbed and bound by hormone receptors in the epidermis, dermis, and subdermis. Accordingly, preparations such as CEE creams have been available for decades. More recently, gels and hormone-eluting silastic patches are available and may be utilized to maintain or repair aging skin. In general, there is plentiful evidence of the positive effects of locally applied estrogen and other SERMs [19, 21, 23, 51, 59, 72]. However, in practice, the results of the use of topical estrogen remains subject to the effects of the overarching non-dermatologic factors; race, actinic exposure, smoking, and aging.

Finally, the use of topical gels, creams, and patches raises the possibilities of adverse effects of high dose exposure. Since the role of estrogen in the development of melanoma and non-melanoma skin cancers seems to be minimal [67, 69–71], the main issue is possible systemic overdose via topical administration. This is an unstudied issue. Perhaps the most troubling possible generator of adverse consequences is the effect of aging on the cardiovascular status of women. The WHI has amply shown the incidence of intravascular thrombosis and its accompanying effects—cardiovascular episodes and stroke must be kept in mind—for women 10 years or more past the menopause should not be exposed to estrogen without the supervision of a physician [73, 74].

BIOIDENTICAL HORMONES

The term "bioidentical hormone" technically refers to a compound with the same molecular structure as a hormone that is endogenously produced (e.g., 17β-estradiol). However, in popular culture, the term refers to the use of custom-compounded multihormone regimens (pills, gels, sublingual tablets, or suppositories) with dose adjustments based upon serial hormone monitoring. The hormones most-commonly compounded are estradiol, estrone, estriol, progesterone, testosterone, and dehydroepiandrosterone (DHEA) [75, 76].

The use of carefully documented bioidentical hormone therapy has been recently reviewed and presented to be effective and safe in postmenopausal women for dermal care, especially for anti-aging of the skin [77], however, there is a lack of uniformity in the various formulations that can be compounded, and attention should be exercised under careful guidance by a licensed health professional.

COSMECEUTICALS FOR ESTROGEN-DEFICIENT SKIN

Cosmeceuticals represent the blending of cosmetics and pharmaceuticals [78]. The term cosmeceutical was first coined by Dr. Albert Kligman in 1984 to describe topical products that afford both cosmetic and therapeutic benefits [79]. The pharmaceutical claims, in general, are subject to safety and efficacy regulation by the US Food and Drug Administration (FDA). However, the FDA does not recognize the designation "cosmeceuticals" and instead considers cosmeceutical products as cosmetics. One of the greatest sources of new cosmeceutical ingredients comes from the plant kingdom [34, 35, 80, 81]. Plants are rich in antioxidants because they must survive continual ultraviolet radiation exposure. Botanicals are also thought to be safe, which meets the FDA's criteria of substances that can be put into topical and over-the-counter formulations. Flowers, seeds, stems, leaves, roots, twigs, and fruits like berries, grapes, etc. from all over the world are being incorporated into cosmeceuticals [49, 62]. In choosing an effective cosmeceutical(s) regimen, it is critical to match patients and their skin needs with the appropriate active ingredients.

In this regard, many studies have examined phytochemicals of the polyphenolic class that are also known as phytoestrogens, which act as SERMs where many possess ERβ-agonist properties [7, 49, 51, 80, 81]. Notably, topical isoflavones effects on the skin in postmenopausal women have been reviewed [34]. Creams, gels, and lotions containing phytoestrogens and isoflavones or genistein alone in 12–24 week clinical studies showed improvement in skin dryness, thickness, facial wrinkles, fibroblast viability, increased hyaluronic acid levels, and type I and III collagen production [34]. In these studies, no significant adverse effects were detected after topical usage of the formulations using the phytochemical active ingredients.

The anti-aging properties of the well-known resveratrol compound that can be derived from grapes has been available for over a decade and reviewed [49, 82–84]. More recent studies report the skin benefits of resveratrol which include anti-inflammatory, antioxidant properties that protect against UV radiation, oxidative stress by Nrf2 activation by reducing the expression of activator protein 1 (AP-1) and NF-kB factors, proliferation of fibroblasts to increase collagen (types I, II, and III), inhibition of melanogenesis, and activation of sirtuin 1 (SIRT 1, the anti-aging factor) [49, 81–83]. Importantly, NF-kB signaling has been reviewed, emphasizing how free radicals activate this key factor involved in skin aging [85].

To increase the effectiveness of resveratrol in topical skin applications, due to the activity of phase I and phase II enzymes in skin such as cytochrome P450, esterases, and transferases, respectively [86, 87], resveratrol analogs have been generated and tested [49, 88]. For instance, a report examined the resveratrol analog, resveratrol triacetate that demonstrated increased stability, can lighten human skin without skin irritation [88], and is well-known that esterase and dehydrogenase activity plays an important role in the skin metabolism of retinyl palmitate to retinol (vitamin A) during percutaneous absorption [89].

Thus, one of our laboratories tested several resveratrol analogs in preliminary studies to determine whether human skin benefits were obtainable, and the most potent was 4′-acetoxy resveratrol (4AR) [49, 90] where via human gene expression analysis of this polyphenolic compound increased: (a) gene expression of the anti-aging factor, SIRT 1 by over 3.3-fold, extracellular matrix proteins collagen III, IV, elastin and tissue inhibitors of metalloproteinases (TIMP 1), (b) the anti-oxidants, CAT, lysyl oxidase (LOX), superoxide dismutase (SOD 1, 2), metallothioneins (MT1H, MT1H), (c) skin aging biomarkers fibrillin (FBN1), laminin (LAMB1), proliferating cell nuclear antigen (PCNA), and (d) skin growth factors [heparin-bind EGF-growth factor (HBEGF), insulin-like growth factor 1 (IGF1), nerve growth factor (NGF), and transforming growth factor (TGF)]. 4AR also decreased gene expression of inflammatory and skin-aging molecules [interleukin (IL-1, IL-6, IL-8), cyclooxygenase-2 (COX-2), tumor necrosis factor receptor super family (TNFRSF)] and the S100 calcium binding proteins A8, A9, suggesting that 4AR 2 has potential for topical treatment and prevention of dermal aging, especially in estrogen-deficient skin. In general, the human skin gene analysis results for 4AR displayed significantly greater efficacy compared with all-trans resveratrol [49].

Subsequently, 4AR was tested in a single center clinical study (by an independent company) that examined 36 female subjects for 12 weeks, the demographics and results of the self-assessment questionnaire are shown in Table 2 [91]. Across eight skin attributes (from firmness to hydration) the subjects reported significant improvements after 12 weeks of topical 4AR application, suggesting that this resveratrol analog maybe effective in treating estrogen-deficient skin [91].

In another study, one of our laboratories examined the isoflavonoid compound, equol, a relatively new phytochemical used as an ingredient for human skin applications, which has a polyphenolic chemical structure found in plant and food sources [7, 16, 49]. It is also classified as a phytoestrogen, having selective estrogen receptor modulator (SERM) characteristics that yield an enhanced/sustained topical delivery up to 28 h into the dermal skin layers by binding to ERβ in keratinocytes [62, 92], which inhibits dermal aging and enhances facial attractiveness [7, 16, 49, 62]. Additionally, it has been reported in a double-blind study that oral supplementation of equol on skin aging in postmenopausal women in Japan for 12 weeks of treatment resulted in significant reductions in wrinkles (crow's feet) compared with the placebo group [93]. Subsequently, other investigators reported that topical equol after 8 weeks improved structural and molecular skin parameters (roughness, texture, smoothness, firmness, elasticity, and decreased methylation and telomere length in skin cells) [93]. Also, the women did not show a significant difference in topically applied equol verses micro-encapsulated equol, suggesting the delivery was not enhanced by microencapsulation, confirming prior results of sustained topical delivery via percutaneous dermal penetration [93, 94]. From recent human skin gene analysis studies, equol's efficacy was greater than astaxanthin for antioxidants, extracellular matrix integrity and breakdown, growth factors and inflammatory biomarkers, including the significant stimulation of the anti-aging factor, SIRT 1 [95].

Notably, equol has a chiral carbon, resulting in two isomers or mirror image molecules (R-equol and S-equol). Both equol isomers exhibit antioxidant, anti-inflammatory, skin protectant (against ROS/oxidative stress) and specifically anti-androgen hormonal actions by binding free 5α-dihydrotestosterone (5α-DHT) as a selective androgen modulator (SAM) and blocking the 5α-reductase type I enzyme in dermal cells to protect fibroblast viability [7, 16, 49, 62].

In a clinical study, equol was tested in a single-center investigation by an independent company that examined 59 female subjects for 12 weeks, the demographics and results of the self-assessment questionnaire are shown in Table 2 [96]. Across eight skin attributes (from firmness to hydration) the subjects reported significant improvements after 12 weeks of topical equol application, suggesting that this isoflavonoid compound may be effective in treating estrogen-deficient skin [96].

Table 2 Self-Assessment Questionnaire analysis from randomized, single-center 12-week clinical studies comparing equol, 4′-acetoxy resveratrol (4AR)

Efficacy: percent improvement over baseline (parameters 1–8)	Equol	4' Acetoxy resveratrol
1. Skin firmness (around eyes)	78	68
2. Skin Smoothness	63	71
3. Even skin tone	70	83
4. Frown lines/wrinkles	72	77
5. Radiance/brightness	73	72
6. Pore size	52	63
7. Skin spots/discoloration	56	73
8. Hydration	71	72
Number of subjects	59	36
Mean age (years ± SEM)	56.1 ± 7.8	53.5 ± 6.2
Age range (years)	40–70	34–60
Caucasian (number of subjects)	30	24
Asian (number of subjects)	29	12
Amenorrheic for at least 2* or 3** years (% of total number of subjects)	76%**	56%*
Glogau aging II and II (wrinkling)	Mild to moderate	Mild to moderate

For each parameter (1–8), there was a significant increase (percent improvement over baseline), $P < 0.05$. In both studies, the female subjects reported the tolerance of the morning and evening treatment was "excellent." Both clinical studies were performed at the same contract research organization (location) with the same endpoint parameters. The concentration of the 4AR was 1.0% while the concentration of equol was 0.3% [91, 96]

SEM standard error of the mean

*indicates percentage of women in the 4AR study that were amenorrheic for at least 2 years, while **indicates percentage of women in the Equol study that were amenorriheic for at least 3 years

When comparing the clinical parameters of the 4AR with the equol technology, results showed that: (a) in general, the percent improvement in the eight skin areas for both treatment were similar, (b) the slightly higher percentages for some of the skin parameters for the 4AR versus the equol technology may be due to the difference in the number of post-menopausal women, where the equol study had 20% more female subjects that were amenorrheic for at least 3 years compared with the 4AR subjects and, (c) the concentration of the 4AR treatment was more than three times that of the equol treatment at 1.0% versus 0.3%, respectively. Therefore, 4AR and equol along with many other botanicals may be considered as active ingredients in cosmetic topical and oral applications [34, 49, 79–81, 97, 98].

CONCLUSIONS

Estrogens play major roles in maintaining physiological functions in the human body. Menopause represents an inflection point, after which the skin undergoes conspicuous decline in appearance and function. This is especially true in exposed areas (face, neck, and hands) and carries messages of age-related decline. Women with estrogen-deficient skin seek cosmetic and medical treatments to improve dermal health and physical characteristics to

enhance their self-perception and inhibit skin aging, particularly in highly visible body areas.

Early studies showed that traditional MHT prevents or reverses the deterioration of skin aging, however, later in 2016, one rigorous clinical study did not show that systemic treatments with estrogen in doses that treat menopausal symptoms and systemic deterioration can overcome the effects of actinic exposure, smoking, racial makeup, or aging on the skin. However, local applications of estrogenic compounds known as SERM's have been shown to repair and avoid deterioration in the facial area. Many plant-derived compounds (phytoestrogens) have this SERM characteristic without unacceptable adverse effects. These preparations presently play a major role as cosmeceuticals in the skin care industry. Among the steroidal and nonsteroidal SERM's used to enhance aging skin, two phytoestrogen/botanicals, an analog of resveratrol [4′-acetoxy resveratrol (4AR)], and a newer isoflavonoid compound, equol, have peer-reviewed in vitro and novel clinical study results that support the improvement of estrogen-deficient skin are reviewed as potential indicators of future directions in this field.

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