Induction of Collagen by Estradiol

Difference Between Sun-Protected and Photodamaged Human Skin In Vivo

Laure Rittie´, PhD; Sewon Kang, MD; John J. Voorhees, MD; Gary J. Fisher, PhD

Objective: To evaluate the effectiveness of topical estradiol in stimulating collagen I and III production in naturally aged and photoaged human skin of postmenopausal women and age-matched men.

Design: Vehicle-controlled treatment followed by biochemical and immunohistochemical analyses of skin biopsy specimens.

Setting: Academic referral center.

Participants: Seventy healthy volunteers (40 postmenopausal women with a mean age of 75 years, and 30 men with a mean age of 75 years) with photodamaged skin.

Interventions: Topical application of estradiol, 0.01%, 0.1%, 1%, or 2.5% or vehicle on aged or photoaged skin, with biopsy specimens taken after last treatment.

Main Outcome Measures: De novo synthesis of collagen by quantitative polymerase chain reaction, immunohistochemistry, and enzyme-linked immunosorbent assay.

Results: Topical estradiol increased procollagen I and III messenger RNA and collagen I protein levels in sun-protected aged hip skin in postmenopausal women and, to a lesser extent, in age-matched men. Surprisingly, no significant changes in production were observed in women or men after 2-week estradiol treatment of photoaged forearm or face skin, despite similar expression of estrogen receptors (ER-α, ER-β, and GPR30) in aged and photoaged skin. Estradiol treatment induced the estrogen-responsive gene GREB1, indicating that penetration of topical estradiol and genomic response to estrogen were similar in the 3 anatomic sites.

Conclusions: Two-week topical estradiol treatment stimulates collagen production in sun-protected hip skin, but not in photoaged forearm or face skin, in postmenopausal women and aged-matched men. These findings suggest that menopause-associated estrogen decline is involved in reduced collagen production in sun-protected skin. Interestingly, alterations induced by long-term sun exposure hinder the ability of topical 2-week estradiol to stimulate collagen production in aged skin.

Trial Registration: clinicaltrials.gov Identifier: NCT00113100

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Skin aging is associated with reduced skin function, increased skin fragility, and compromised wound healing.1 On areas of the body that are often not covered by clothing (ie, face, nape of the neck, hands, and forearms), long-term UV exposure from the sun damages the skin, causing it to look prematurely old. Photaging is the main process responsible for the worsening appearance of aged skin.

Clinical signs of natural aging include fine wrinkles, skin laxity, and sagging, while photoaged skin appears dry, with coarse wrinkles and uneven pigmentation. Histologically, epidermis is thin in aged skin and rather irregular in photoaged skin.2 However, both processes share major biochemical features such as reduced collagen content in the dermis, resulting from increased collagen degradation and decreased procollagen synthesis.3 Collagen I is the major structural protein of the dermis, and it provides strength and resiliency to the skin. Collagen I is primarily produced by dermal fibroblasts. Collagen production is regulated by a variety of mediators including growth factors, cytokines, hormones, and mechanical tension.4 In naturally aged skin, the combination of reduced fibroblast number, reduced fibroblast metabolic activity, and loss of mechanical tension results in a 70% decrease in new collagen (procollagen) production by fibroblasts.5 In photoaged skin, loss of mechanical tension appears to be primarily responsible for reduced procollagen production.6-8 This loss of mechanical tension is due to accumulation of fragmented collagen, which is generated by repeated exposures to UV radiation (reviewed by Rittie´ et al9). Overall, decreased quantity and quality of collagen fibers in the dermis are associated with aged appearance of human skin. Hence, antiaging therapies include drugs (such as tretinoin) or pro-

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topical estradiol treatment increased procollagen I and III expression in sun-protected hip skin in a dose-dependent manner. Surprisingly, 2-week estradiol treatment had no effect on procollagen synthesis in photoaged skin (forearm or face). We found that ERs were expressed at similar levels in sun-protected and photoaged skin and that estradiol penetration and genomic activity were similar in all skin sites, as assessed by quantification of estrogen target gene expression. Taken together, our results demonstrate that estradiol-mediated collagen stimulation is indirect and that alterations induced by long-term sun exposure hinder the ability of topical estradiol to stimulate collagen production in aged human skin in vivo.

**METHODS**

**HUMAN SUBJECT DESCRIPTION**

All procedures involving human subjects were approved by the University of Michigan Institutional Review Board, and all subjects provided written informed consent. Healthy volunteers, not taking ET, were enrolled in the study with no racial or ethnic distinction. Participants’ age distribution, degree of photodamage, and years after menopause are given in Table 1.

**ESTRADIOL TREATMENT AND TISSUE PROCUREMENT**

Volunteers were treated with estradiol and vehicle (95% ethanol–propylene glycol, 7:3, vol/vol), under occlusion, on sun-protected hip or photodamaged forearm skin sites. Treatment (0.1%-2.5%, ie, 5.4-135 µg of estradiol per square centimeter) was administered 3 times, every other day. Twenty-four hours after the last treatment, 4-mm full-thickness punch biopsy specimens were obtained from each site. For face treatment, estradiol was freshly dissolved in propylene glycol and incorporated into moisturizing cream (Neutrogena; Neutrogena Corp, Los Angeles, California) to a final concentration of 0.2% (wt/vol). Estradiol treatment (570 µL of cream on the whole surface of the face; approximately 5.7 µg of estradiol per square centimeter) was applied twice a day for 14 days (participants washed their face with soap and water before applying the cream in the morning and at bedtime). Biopsy specimens (2 mm) were taken in the crow’s-foot area of the face before and 24 hours after the last treatment. For photoaging studies, all participants were asked to avoid sun exposure at least 48 hours before and during treatment of photaged areas. Face studies (without occlusion) were performed in Michigan between November and February to limit sun exposure during treatment.

Immediately after biopsy, skin samples were embedded in low-temperature embedding medium (Tissue-Tek OCT compound; Miles, Naperville, Illinois), frozen in liquid nitrogen, and stored at −80°C until processing.

**RNA EXTRACTION AND REAL-TIME REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION**

Total RNA was extracted from whole punch skin biopsy specimens by means of a commercial kit (RNasey; Qiagen, Chatsworth, California) and quantified with dye (RiboGreen; Invitrogen, Carlsbad, California). Real-time reverse transcription–polymerase chain reaction (RT-PCR) was performed on 100 ng of total RNA as described previously, with the use of custom primers and probe for collagen I and 36B4, and for collagen III.
Serial frozen sections of 7, 200, and 7 µm were prepared from OCT-embedded skin biopsy specimens. Dermal areas of 7-µm sections were measured with Image ProPlus software (Media Cybernetics, Bethesda, Maryland) and used to calculate the volume of the 200-µm sample. Soluble proteins were extracted from the 200-µm sample in ice-cold extraction buffer (50mM Tris hydrochloride, pH 7.4; 0.15M sodium chloride; 1% Triton X-100; procollagen I antisens primer, 5′-TCT-TGG-TCA-GTC-CTA-TGG-GGA-TA-3′; probe, 5′-CCA-GAA-CCA-TGC-CAA-ATA-TGT-GTC-TGT-GAC-T-3′). All other primer-probe sets were validated by using complementary DNA standards for ER-α (ESR1, UniGene Hs.208124) and ER-β (ESR2, UniGene Hs.660607) (generous gifts from Peter J. Kushner, PhD, University of California, San Francisco). Results were normalized to the level of housekeeping gene 36B4 (UniGene Hs.346285) (internal control). Results are presented as normalized fold change in estradiol-treated vs vehicle or untreated skin sample, or fold vs 36B4 (2-ΔΔCT) in the end point of the real-time PCR reaction.

PROTEIN EXTRACTION AND PROCOLLAGEN I ENZYME-LINKED IMMUNOSORBENT ASSAY

Serial frozen sections of 7, 200, and 7 µm were prepared from OCT-embedded skin biopsy specimens. Dermal areas of 7-µm sections were measured with Image ProPlus software (Media Cybernetics, Bethesda, Maryland) and used to calculate the volume of the 200-µm sample. Soluble proteins were extracted from the 200-µm sample in ice-cold extraction buffer (50mM Tris hydrochloride, pH 7.4; 0.15M sodium chloride; 1% Triton X-100; procollagen I antisens primer, 5′-TCT-TGG-TCA-GTC-CTA-TGG-GGA-TA-3′; probe, 5′-CCA-GAA-CCA-TGC-CAA-ATA-TGT-GTC-TGT-GAC-T-3′). All other primer-probe sets were validated by using complementary DNA standards for ER-α (ESR1, UniGene Hs.208124) and ER-β (ESR2, UniGene Hs.660607) (generous gifts from Peter J. Kushner, PhD, University of California, San Francisco). Results were normalized to the level of housekeeping gene 36B4 (UniGene Hs.346285) (internal control). Results are presented as normalized fold change in estradiol-treated vs vehicle or untreated skin sample, or fold vs 36B4 (2-ΔΔCT) in the end point of the real-time PCR reaction.

LASER CAPTURE MICRODISSECTION

Laser capture microdissection was performed as previously described46 to separate interfollicular epidermis (without hair follicle infundibulum), dermis, and appendages (whole hair follicles, sebaceous glands, and sweat glands in a defined length of section).

BLOOD ESTRADIOL MEASUREMENTS

Blood estradiol measurements were obtained by radioimmunoassay by the Clinical Chemistry Laboratory at the University of Michigan Hospital.

STATISTICAL ANALYSIS

Data are expressed as mean and standard error of the mean. Comparisons among groups were made with the paired t test. All P values are 2-tailed and were considered significant when less than .05.
0.1%, 1%, and 2.5% estradiol stimulated procollagen I production by 1.53-, 2.33-, and 2.73-fold vs vehicle, respectively (n=6; P < .05 for 1% and 2.5% estradiol).

Collagen-producing cells were localized by immunohistochemistry. As shown in Figure 2, estradiol, 1%, treatment induced a marked increase in procollagen I protein expression throughout the reticular and papillary dermis in sun-protected skin of postmenopausal women in vivo.

Histologic analyses of human skin sections did not show any effect of topical estradiol treatment on epidermal or dermal thickness, as measured by image analysis on hematoxylin-eosin-stained skin sections (Figure 2 and data not shown).

Topical estradiol also increased procollagen expression in sun-protected hip skin of men, although to a lesser extent. As shown in Figure 3A, procollagen I mRNA levels were increased by topical estradiol by 1.69-, 1.58-, and 2.02-fold vs vehicle after 0.1%, 1%, and 2.5% estradiol treatment, respectively (n=12; P < .05 for 0.1% and 1% estradiol). Similarly, procollagen III mRNA levels were increased by topical estradiol by 1.87-, 1.64-, and 2.11-fold vs vehicle after 0.1%, 1%, and 2.5% estradiol treatment, respectively (n=12; P < .05 for all concentrations). Topical estradiol also induced procollagen I protein (Figure 3B) by 1.25-, 1.3-, and 2.3-fold vs vehicle after 0.1%, 1%, and 2.5% estradiol treatment, respectively (n=9; P < .05 for 1% and 2.5%).

LACK OF EFFECT OF TOPICAL ESTRADIOL TREATMENT ON PROCOLLAGEN PRODUCTION IN PHOTOAGED FOREARM SKIN

To determine whether the observed lack of effect of estradiol on procollagen collagen production in photoaged skin, we next treated postmenopausal women on photodamaged forearms with vehicle or estradiol for 1 week and measured procollagen I and III mRNA levels in skin biopsy specimens by real-time reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. NS indicates nonsignificant vs vehicle. * P < .05 vs vehicle. †Nonsignificant vs estradiol, 1%.

RT-PCR. As shown in Figure 4A, 1% estradiol treatment did not significantly alter procollagen I or III mRNA levels in photodamaged forearm skin of postmenopausal women in vivo (n=18). In contrast, estradiol treatment of sun-protected hip skin increased procollagen I mRNA levels by 3.54 (0.56)–fold (mean [SEM]) vs baseline (n=18; P < .05, Figure 1A). Similarly, 2.5% estradiol did not alter procollagen I mRNA levels in photodamaged skin of postmenopausal women (1.61 [0.44]– and 1.37 [0.16]–fold vs vehicle for procollagen I and III, respectively; n=6). Because type I collagen regulation includes multiple posttranslational steps, procollagen I protein levels were measured in estradiol- and vehicle-treated forearm skin samples of postmenopausal women. As shown in Figure 4B, estradiol treatment had no significant effect on procollagen I protein levels (n=12).

Results obtained in photoaged forearm skin of men were similar to those obtained in postmenopausal women. Topical treatment with 1% or 2.5% estradiol did not alter procollagen I or III mRNA expression (Figure 4C; n=12) or procollagen I protein (Figure 4D; n=6) compared with vehicle.

Lack of effect of estradiol on collagen levels in photodamaged forearm skin was confirmed by immunohistochemistry in postmenopausal women (Figure 5) and age-matched men (not shown).

LACK OF EFFECT OF ESTRADIOL TREATMENT ON COLLAGEN I PRODUCTION IN PHOTOAGED FACE

To determine whether the observed lack of effect of estrogen on collagen synthesis in photoaged forearms is related to anatomic site or photoaging, we evaluated the effect of topical estradiol on procollagen synthesis in the photoaged face. To this end, 5 postmenopausal women and 3 age-matched men with clinical photoaging were treated on the face with 0.2% estradiol cream twice daily
for 2 weeks. The treatment regimen matched the quantity of estradiol per surface area that was used on forearms and hips. Levels of procollagen were compared in skin samples obtained before and after estradiol application. No change in procollagen mRNA (Figure 6A) or protein (Figure 6B) expression was observed after 2-week treatment of photoaged faces of men or postmenopausal women. In parallel, blood estradiol concentrations were measured before and after treatment. Topical estradiol treatment significantly increased estradiol blood levels in all treated subjects (Figure 6C). The increase in estradiol blood levels was greater in postmenopausal women than in men (12- vs 2-fold, respectively; \( P = .006 \) and \( P = .001 \), respectively). These results demonstrate that the 0.2% estradiol penetrated the skin and was chemically stable throughout the treatment.

**ESTROGEN RECEPTOR LEVELS IN NATURALLY AGED AND PHOTOAGED SKIN**

To determine whether lack of response to estradiol treatment in photoaged skin was due to a lower level of ERs, we quantified ER-\( \alpha \), ER-\( \beta \), and GPR30 (GPER; UniGene Hs.20961) mRNA levels in vehicle- and estradiol-treated skin samples from hip, forearm, and face of postmenopausal women and age-matched men. The ER-\( \alpha \), ER-\( \beta \), and GPR30 mRNA levels were readily detected in human skin of all tested subjects (Figure 7). We did not find any differences in the levels of expression of ER-\( \alpha \) (Figure 7A), ER-\( \beta \) (Figure 7B), or GPR30 (Figure 7C) between sun-protected hip and photoaged forearm or face skin samples, in women or men, nor were these levels affected by estradiol treatment. Interestingly, men had significantly less ER-\( \alpha \) in hip skin (−49%) and less ER-\( \beta \) in forearm skin (−49%) than did site-matched women (\( n = 6; P < .05 \)). Direct comparison of Figure 7A, B, and C shows that ER-\( \alpha \) mRNA levels were approximately 10 times more abundant than those of ER-\( \beta \) or GPR30 in both men and women.

Despite readily detectable ER mRNA levels, we were not able to reliably detect the presence of ER protein in skin samples by immunohistochemistry, using various different antibodies (data not shown). We therefore used laser capture microdissection to localize ER mRNA expression in normal human skin. Expression of each of the 3 genes was extremely low in the epidermis (Figure 7D). The ER-\( \alpha \) and GPR30 were primarily expressed in dermal cells, while expression was near the limit of detection in appendages. In contrast, ER-\( \beta \) expression was localized in appendages and, to a lesser extent, in dermal cells. These data indicate that all ERs are expressed in the dermal compartment of human skin in vivo.
Figure 3. Effect of topical estradiol treatment in men on sun-protected hip skin with vehicle or estradiol for 1 week. Mean (SEM) levels of procollagen I (COL1A1) and III (COL3A1) messenger RNA (mRNA) (A; n=12) and procollagen I protein (B; n=9) were quantified in skin biopsy specimens by real-time reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. NS indicates nonsignificant vs vehicle. *P<.05 vs vehicle.

Figure 4. Effect of topical treatment in postmenopausal women (A and B) and men (C and D) on photodamaged forearms with vehicle or estradiol for 1 week. Mean (SEM) levels of procollagen I (COL1A1) and III (COL3A1) messenger RNA (mRNA) in women (A; n=18) and men (C; n=12) and procollagen I protein in women (B; n=12) and men (D; n=6) were quantified in skin biopsy specimens by real-time reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. NS indicates nonsignificant vs vehicle.
Figure 5. Skin specimens showing effect of topical treatment in postmenopausal women on photodamaged forearm skin with vehicle or estradiol, 1%, for 1 week. Skin samples were stained for procollagen I by immunohistochemistry. Positive staining appears red, and hematoxylin counterstaining appears blue (original magnification ×10; insets ×40). White squares in A and B indicate areas shown in insets. Insets in C and D show the deepest part of the dermis. Specimens are representative of 6 experiments.

Figure 6. Effect of topical treatment in postmenopausal women and men on photodamaged face skin with estradiol, 0.2%, twice daily for 2 weeks. Mean (SEM) levels of procollagen I (COL1A1) and III (COL3A1) messenger RNA (mRNA) (A) and procollagen I protein (B) were quantified in skin biopsy specimens before and after treatment by real-time reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent assay, respectively (n=5). NS indicates nonsignificant vs vehicle. C, In parallel, estradiol blood levels were determined before and after treatment (to convert estradiol to picomoles per liter, multiply by 3.671). *P<.05 vs before treatment.
LACK OF EFFECT OF TOPICAL ESTRADIOL TREATMENT ON ESTROGEN-METABOLIZING ENZYME GENE EXPRESSION

Sex steroid concentrations in human tissues are tightly regulated, and excess estrogens are biologically inactivated by specific enzymes. To determine whether lack of response to estradiol treatment in photoaged skin was due to increased estrogen catabolism, we measured the levels of estrogen inactivating enzymes in human skin treated with vehicle or estradiol.

Estrogen-inactivating enzymes can be subdivided into 3 groups: (1) 17β-hydroxysteroid dehydrogenases (HSD17B) 2, 4, and 8 catalyze oxidation of the biologically active estradiol into weaker estrone; (2) sulfo-transferase (SULT) 1A1, SULT2A1, and SULT1E1 catalyze sulfation of estradiol, preventing it from binding to, and thereby activating, ERs; and (3) cytochrome P450 1B1 (CYP1B1) is the major CYP450 enzyme inactivating estradiol by 4-hydroxylation. We first assessed gene expression of each of these enzymes in hip, forearm, and face skin in vivo. The HSD17B4 was the most highly expressed (Table 2).

As shown in Table 2, mRNA levels of estradiol metabolizing enzymes were similar in hip and forearm skin and were not altered by estradiol treatment in postmenopausal women. Expression of HSD17B2 tended to be higher in face skin than in hip skin, although this difference (approximately 10-fold) did not reach statistical significance (P = .09 and .06 for vehicle- and estradiol-treated sites, respectively; n = 5-6). In addition, CYP1B1 mRNA levels were 2.7-fold higher in face skin than in hip skin (P = .04). The level of HSD17B8 was approximately 4 times lower in face skin than in hip skin of postmenopausal women (P < .001).

All of the estradiol metabolizing enzymes that were expressed in women were also expressed in men, and their levels were not altered by estradiol treatment of hip, forearm, or face skin. Interestingly, levels of CYP1B1 mRNA were significantly higher in hip and forearm skin of men.
responsive gene that is directly regulated by ERs.53 As in breast cancer 1; Unigene Hs.467733), an estrogen–
induced induction of GREB1.

To assess topical estradiol biological activity, we measured induction of GREB1 (gene regulated by estrogen in breast cancer 1; Unigene Hs.467733), an estrogen-responsive gene that is directly regulated by ERs.53 As shown in Figure 8, topical estradiol treatment of postmenopausal women induced GREB1 mRNA levels with a 3.6-, 4.7-, and 9.9-fold increase vs control in hip, forearm, and face skin, respectively (P < .05). In men, GREB1 mRNA levels were significantly increased after estradiol treatment in hip skin (2.25-fold; n = 6; P = .005) but not in forearm (n = 12) or face (n = 5) skin (Figure 8).

### EFFECT OF TOPICAL ESTRADIOL TREATMENT ON TRANSCRIPTION OF ESTROGEN-RESPONSIVE GENE GREB1 IN NATURALLY AGED AND PHOTOAGED SKIN

In the present study, we evaluated the effects of estradiol treatment on procollagen production in sun-protected aged and photoaged skin of postmenopausal women and age-matched men. We demonstrated that 1-week topical treatment with estradiol stimulates procollagen I and III expression in sun-protected aged skin of postmenopausal women in vivo. Surprisingly, we found that estradiol has no effect on procollagen expression in photoaged skin (forearm or face) of postmenopausal women, despite similar levels of expression of estrogen receptors in sun-protected and sun-exposed skin sites, and similar levels of expression of estradiol-inactivating enzymes in hip and forearm skin. In addition, we provide evidence that topical estradiol penetration and activity are similar in sun-protected and sun-exposed skin sites, as assessed by quantification of induction of GREB1 gene expression, which is directly regulated by ERs.53 Taken together, these findings support the concept that topical estradiol regulates procollagen I and III production in sun-protected skin in women. Our results also indicate that alterations induced by long-term sun exposure hinder the ability of topical estradiol to stimulate collagen production in aged human skin in vivo.

The study of estradiol responses in male skin yielded interesting differences compared with women. Topical

### Table 2. Gene Expression Levels of Estradiol-Degrading Enzymes in Human Skin In Vivo

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<tr>
<th>Enzyme</th>
<th>Vehicle</th>
<th>Estradiol, %</th>
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<td>HSD17B</td>
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<tr>
<td>Hip</td>
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### Table 2. Gene Expression Levels of Estradiol-Degrading Enzymes in Human Skin In Vivo (cont)

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<tr>
<td>Hip</td>
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<td>SULT1E1</td>
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<td>0.73 (0.28)</td>
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<td>Men</td>
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<td>3.32 (1.55)</td>
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<tr>
<td>Forearm</td>
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<td>1.04 (0.20)</td>
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<td>0.38 (0.06)</td>
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</table>

Abbreviations: CYP1B1, cytochrome P450 1B1; HSD17B2, 4, and 8; SULT1A1 and SULT1E1, sulfotransferase 1A1 and 1E1.

a Postmenopausal women and age-matched men were treated on sun-protected hip or photoaged forearm or face skin with vehicle or estradiol. Levels of estrogen-degrading enzymes (CYP1B1; HSD17B2, 4, and 8; SULT1A1; and SULT1E1) were quantified in skin by real-time reverse transcription–polymerase chain reaction. Values are fold vs Vehicle (2−ΔΔCT) (ΔΔCT = 2Δ(−CTtarget/−CT36B4) × 103), given as mean (SEM) (n = 5-6). (CT indicates cycle threshold).

b P < .05 in forearm or face vs hip.

c P < .05 in estradiol- vs vehicle-treated skin.

d P < .05 in men vs women.
estradiol increased procollagen production in naturally aged hip skin of men to a lesser extent than in postmenopausal women. Induction of GREB1 gene expression was also weaker in men than in women. In contrast, topical estradiol did not alter procollagen or GREB1 expressions in photoaged forearm or face skin of men. Differences in estradiol responsiveness between men and women were not due to differences in ER expression. Interestingly, CYP1B1, which inactivates 17β-estradiol by 4-hydroxylation, was significantly more highly expressed in photoaged forearm skin of men than of women, suggesting that reduced responsiveness of men to estradiol may be related to catabolism.

Our data are in agreement with previously published studies that demonstrated that topical estrogen treatment stimulates procollagen production in abdominal skin of postmenopausal women12 and hip skin of elderly men and women.33 We did not observe any correlation between subject age and magnitude of estradiol-mediated induction of collagen mRNA or protein (n = 18).

To our knowledge, the present study is the first to describe a lack of effect of topical estradiol treatment on procollagen production in photoaged skin. It is possible that a treatment time longer than 2 weeks would have demonstrated effects similar to those in sun-protected skin. Additional studies will be necessary to test this possibility. However, GREB1 levels rose significantly and similarly in all treated sites in postmenopausal women, indicating that estradiol penetrated the skin and activated ERs similarly in sun-protected and sun-exposed skin. These results demonstrate that photaging is not associated with a loss of estrogen genomic response, at least in postmenopausal women. Conversely, our results suggest that the effect of estradiol on procollagen is indirect, and that photoaged skin lacks an essential component to the estrogen-mediated collagen response. This hypothesis is in agreement with findings of Meyer et al.34 who showed that 17α-estradiol, which does not activate estrogen receptors, stimulated skin procollagen production in rodent and human skin. However, the mediators of estrogen action on skin collagen remain to be identified.

More recently, it was reported that the combination of ET and topical estradiol increased procollagen immunostaining in facial skin in women.35 The study did not investigate the effects of either ET or topical estradiol alone. It is possible that ET triggers systemic responses that enable topical estradiol to promote collagen production in photoaged skin, or vice versa. This possibility raises interesting questions regarding mechanisms of estrogen actions in human skin.

The presence of estrogen binding sites in skin has been demonstrated by means of radioactive estradiol.56-57 These studies found that thigh skin has approximately 3-fold lower ER levels than does face or abdominal skin (1.5, 4.3, and 4.9 fmol of receptor per milligram of protein, respectively).56,57. In the present study, we did not find any significant difference in the level of ESR1, ESR2, or GPR30 gene expression in face, forearm, or hip skin in postmenopausal women. In addition, we did not find any significant difference in ER expression between vehicle- and estradiol-treated samples, consistent with previous results.57 Detection of ERs in human skin has yielded discordant results. Immunostaining-based studies reported that ER-α was not detected or was weakly detected, whereas ER-β was strongly detected in epidermis, eccrine glands, sebaceous glands, and hair follicles. On the basis of these reports, ER-β is often described as the predominant form of ER in adult human skin,56-60 although the relative abundance of the 2 ER isoforms has not been formally quantified. We quantified ER-α, ER-β, and GPR30 mRNA in human skin and demonstrated that ER-α mRNA levels were approximately 10 times greater than those of ER-β or GPR30. We were unable to detect either protein by immunohistochemistry (using a variety of different antibodies) because of absent or nonspecific staining. Using laser capture microdissection, we found that most of the ER-α and GPR30 mRNA was expressed in dermal cells, whereas ER-β was expressed in both appendages and dermal cells. More studies are needed to precisely identify ER-expressing cells within dermis and appendages.

In summary, our study shows that estradiol stimulates collagen I and III production in sun-protected skin but not in photoaged skin of postmenopausal women and aged-matched men within 2 weeks. Because photaging is superimposed on natural aging in sun-exposed areas of the skin, our results suggest that alterations induced by long-term sun exposure hinder the ability of topical estradiol to stimulate collagen production in aged human skin in vivo.

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Figure 8. Effect of topical estradiol treatment on estrogen-responsive gene GREB1 messenger RNA (mRNA) expression in sun-protected and photoaged human skin in vivo. Mean (SEM) GREB1 transcript levels were measured by real-time reverse transcription–polymerase chain reaction in hip, forearm, and face skin of postmenopausal women and men after treatment with vehicle or estradiol (n=5-12). NS indicates nonsignificant vs vehicle. *P<.05 vs vehicle. †Nonsignificant vs hip.

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40. Rittié L, Varani J, Kang S, Voorhees JJ, Fisher GJ. Retinoid-induced epidermal hyperplasia is mediated by epidermal growth factor receptor activation via spe-


The Nikolsky Sign

In 1894, Russian dermatologist Pyotr Vasiliyevich Nikolskiy, MD (Figure), reported a mechanical phenomenon that would later be internationally recognized by the eponym crediting his name—the Nikolsky sign.1 (His name was originally spelled Nikoloskiy, but it is more commonly referenced as Nikolsky.)1 In 1896, his thesis on pemphigus lioaciaceus described the clinical finding, and his later work suggested that the underlying pathologic process was acantholysis occurring in affected areas as well as in areas with intact, normal-appearing skin.

A review of the literature on the Nikolsky sign interestingly reveals a history of disagreement about its exact definition and method of elicitation. In 2003, Grando et al1 suggested that the existence of the various different interpretations of the Nikolsky sign may be attributed to the difficulty of clinically differentiating between the many types of bullous diseases before immunofluorescence was available and to the inaccurate translation of the Nikolsky sign, along with its subsequent modifications, which were written in Russian, German, and French.1 Therefore, Grando and colleagues returned to the original sources to interpret the Nikolsky sign and other mechanical signs associated with the bullous diseases that are often confused with the Nikolsky sign, including the blister-spread sign (Asboe-Hansen/Lutz sign), perifocal subepidermal separation (Sheklakov sign), and epidermal peeling (pseudo-Nikolsky sign). According to Nikolskiy, the Nikolsky sign can be elicited by the following 3 methods: (1) detaching the stratum corneum far beyond the preexisting erosion and over visibly normal skin by pulling the remnant of a ruptured blister wall, known as the marginal Nikolsky sign, a term that was suggested by Sheklakov2 in 1967;2 (2) displacing or dislodging the stratum corneum to induce an erosion along the periphery of a preexisting erosion by applying lateral pressure with a finger; and (3) displacing or dislodging the stratum corneum to induce an erosion on areas of visibly normal skin by applying lateral pressure with a finger, known as the direct Nikolsky sign. As elucidated by Grando and coauthors, the Nikolsky sign is usually only positive for diseases that involve epidermal acantholysis, including the pemphigus group of disorders and the staphylococcal scalded skin syndrome. The Nikolsky sign is typically negative for diseases with dermoepidermal separation, such as pemphigoid (bullous, cicatrical, and gestationis) and epidermolysis bullosa acquista, as well as for diseases in which exfoliation of dead epidermis occurs, such as erythema multiforme (Stevens-Johnson syndrome) and toxic epidermal necrolysis.

In 2006, Uzun and Durdu3 conducted a study that tested the specificity and sensitivity of the Nikolsky sign in the diagnosis of pemphigus in 123 patients using the elicitation methods described by Grando et al.1 They discovered that the Nikolsky sign offers a moderately sensitive but highly specific tool for the diagnosis of pemphigus. The marginal Nikolsky sign is more sensitive (69%) for the diagnosis of pemphigus, but the direct Nikolsky sign is more specific (100%).

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