The Effects of an Estrogen and Glycolic Acid Cream on the Facial Skin of Postmenopausal Women: A Randomized Histologic Study

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A prospective, randomized, double-blind study was conducted to determine if estradiol and glycolic acid creams produced a significant reversal of epidermal and dermal markers of aging and if the cumulative effect of the creams was greater than either alone. Sixty-five patients applied a cream containing 0.01% estradiol or 15% glycolic acid, alone or in combination, to one side of the face, and a vehicle cream to the opposite side, for 6 months. A 2-mm punch biopsy was obtained from the hairline of each patient and processed for analysis. The estradiol treatment produced a 23% increase in epidermal thickness (P=.00458); the glycolic acid, a 27% increase (P=.00467); and the combination, a 38% increase (P=.000181). All groups showed a statistically significant improvement in reversing markers (rete peg pattern, epidermal thickness) of skin aging. Although not statistically significant (P=.1), a cumulative effect was seen when estradiol and glycolic acid creams were used in combination.

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Studies have shown α-hydroxy acids (AHAs) can help to reverse many signs of skin damage. In 1974, Van Scott and Yu[1] reported that AHAs can have profound effects on disorders of keratinization by diminishing corneocyte cohesion immediately above the stratum granulosum. In 1996, Ditre et al[2] reported that AHA helped to improve markers of skin aging by thickening the epidermis, reversing basal cell atypia, dispersing melanin pigmentation, and maintaining a more normal rete peg pattern. Like AHA, estrogen compounds have been proven effective for the treatment of aging skin. In 1996, Schmidt and colleagues[3] reported that women treated with 0.01% estradiol and 0.03% estriol compounds were found to have skin that was markedly improved in wrinkle depth, pore size, elasticity, and firmness. AHAs and estrogen compounds both have been reported to have significant beneficial effects on the quality of aging facial skin.[4-8] Our study was conducted to clarify by histologic analysis the effects of these creams used alone and in combination to reverse skin-aging markers.

Methods

Sixty-five postmenopausal women aged 39 to 83 years with moderate to severe markers of skin aging were randomly assigned to 1 of 3 groups (22 to estradiol, 21 to glycolic acid, and 22 to a combination of the estradiol and glycolic acid). Patients were volunteers from a private gynecologic practice. They were given a questionnaire for data collection and to exclude any patient with a history of breast cancer, skin cancer, or severe skin sensitivities. Smokers and users of hormone replacement therapy were not excluded. Those who qualified were randomly assigned to a group by a nurse blinded to the identity of the creams. Each woman was instructed to apply the creams to the right and left sides of her face in the morning and at night. Those in the combination group applied the estradiol cream in

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Table 1.

Epidermal Thickness Measured in $\mu m^2/\mu m$

	Vehicle (SE)	Treated (SE)	Difference (SE)	% Change	*P* value
Estradiol (n=15)	57.3 (4.0)	70.5 (3.7)	13.2 (3.7)	23	.0045
Glycolic acid (n=15)	54.2 (2.9)	68.8 (3.2)	14.6 (2.9)	27	.0046
Combination (n=20)	54.8 (3.3)	75.4 (5.8)	20.6 (3.3)	38	.00018

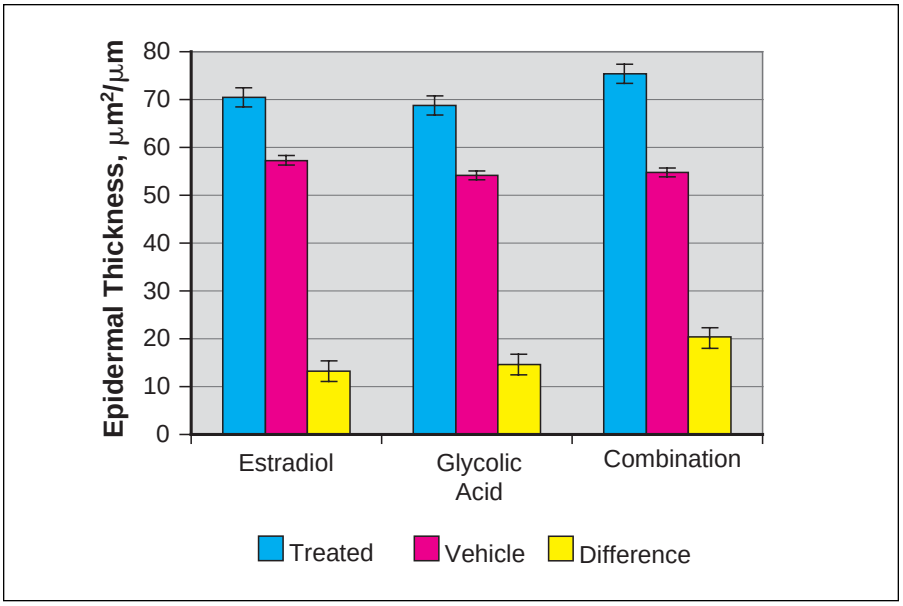

Figure 1. Average epidermal thickness of the estradiol, gycolic acid, and combination groups vs vehicle.

the morning and the glycolic acid cream at night on one side of the face, and the vehicle cream on the other side in the morning and at night. The estrogen and glycolic acid groups applied the treatment cream in the morning and vehicle cream at night on one side of the face, and the vehicle cream in the morning and at night on the other side. Patients were specifically instructed to apply the cream to the temple hairline. All patients were blinded to the identity of the creams. The pathologists were blinded to treatment versus vehicle sides of the face.

The vehicle cream was purchased from Professional Compounding Centers of America in Houston, Texas. The cream contains anhydrous ointment, polysorbate 80, purified water, butylated hydroxytoluene, and sodium denzoate. The estradiol cream was compounded using the same base with US Pharmacopeia (USP), micronized, 0.01% estradiol added. The cream was not altered in its formulation (NeoStrata® 15% AHA face cream) and contains water, glycolic acid, caprylic/capric triglycerides, stearic acid, propylene glycol, isopropyl palmitate, glyceryl stearate, PEG-100 stearate, cetyl alcohol, isostearic acid, cholesterol, ammonium hydroxide, sorbitan stearate, hydroxyethylcellulose, dimethicone, stearamidopropyl dimethylamine, magnesium aluminum silicate, tetrasodium ethylenediamine tetraacetic acid, and butylated hydroxytoluence. The pH level of this cream is 3.7. All creams are stable at room temperature.

The patients continued application for 6 months, after which time a 2-mm punch biopsy was obtained from the hairline of each woman's left and right temple. The specimens were labeled and placed in a 10%-buffered formalin. The cases were assigned numbers and transferred into tissue cassettes. The cassettes were then placed in a VIP Tissue Tex™ processor for further fixation, dehydration, and paraffin infiltration. The processed specimen was then oriented and placed in a paraffin block. This block was trimmed, and

Table 2.

Rete Peg Pattern Length, μm

	Vehicle (SE)	Treated (SE)	Difference (SE)	% Change	*P* value
Estradiol (n=15)	81.6 (5.2)	92.9 (5.4)	11.3 (5.3)	14	.01
Glycolic acid (n=15)	76.1 (4.9)	92.5 (4.2)	16.4 (4.2)	22	.001
Combination (n=20)	92.6 (4.6)	110.9 (5.7)	18.3 (4.6)	20	.04

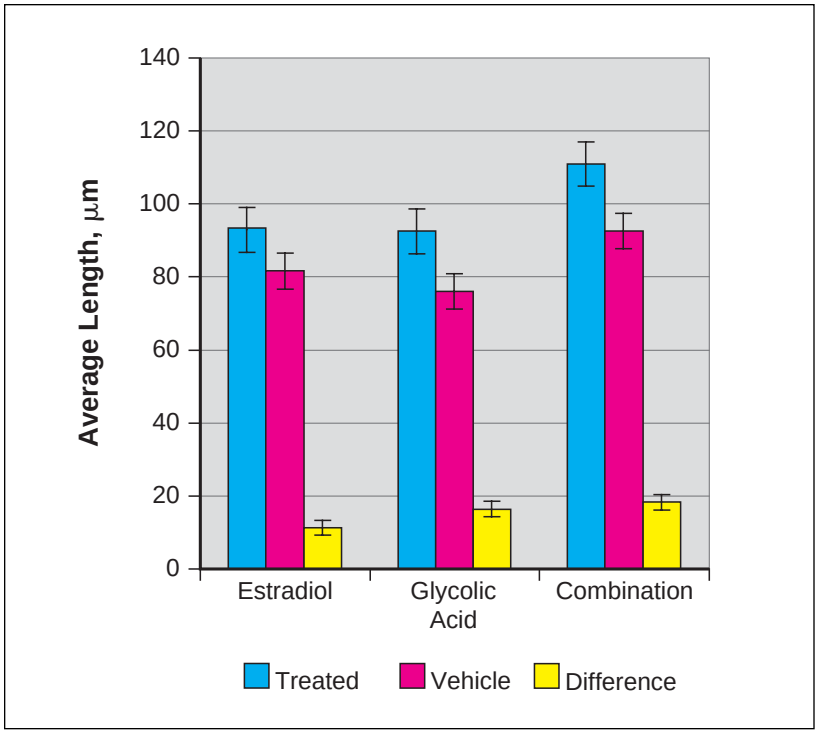

Figure 2. Average rete peg pattern lengths measured at ×40 magnification in the estradiol, glycolic acid, and combination groups vs vehicle.

4 micron sections were placed in a 1×3-inch precleaned microscope slide. Hematoxylin-eosin, Gomori's blue trichrome, and elastic von Giesen stains were made from each specimen.[9] The hematoxylin-eosin sections then were evaluated with the Cell Analysis System™ image analyzer, using a micrometer program to take measurements.

Epidermal thickness was measured at ×40 magnification. Here, a measured line was drawn parallel to the epidermis. The corresponding area was measured from one end of the measured line to the other end. The corresponding area was divided by the value of the measured line. The result was then reported as area in $\mu m^2/\mu m$.

At a ×40 magnification, rete peg patterns were measured. The measurement was taken as perpendicular as possible from the edge of the granular layer to the basal layer. After taking 3 measurements, the average was reported. The vascular area then was examined by selecting a random field in the papillary dermis. A rectangular field was drawn and the corresponding area was determined. The vascular channels within the field were measured, and all the areas were added.

Semiquantitative measurements of other features were made independently by 3 pathologists. Average scores for each feature were calculated for each patient by totaling the scores given by each observer and dividing by 3.

Criteria for nuclear atypia chromaticity were graded on a scale of 0 to 3. The criteria examined were nuclear-cytoplasmic ratio, nuclear chromatism, and irregularity of the nuclear contour. No change was graded as 0, mild atypia as 1, moderate atypia as 2, and

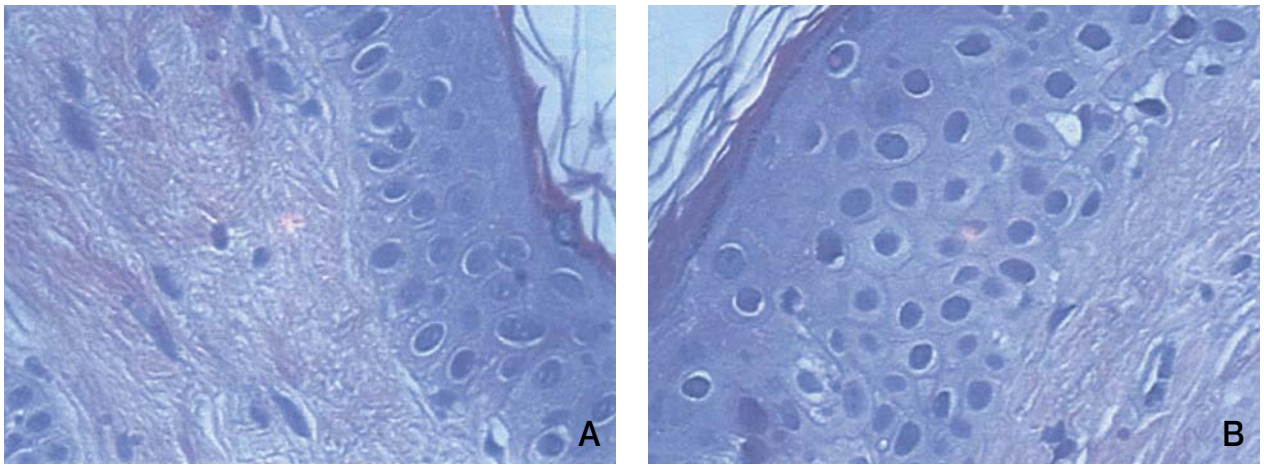

Figure 3. Epidermal thickness with vehicle (A) and combination treated (B) specimens (H&E, original magnification ×40).

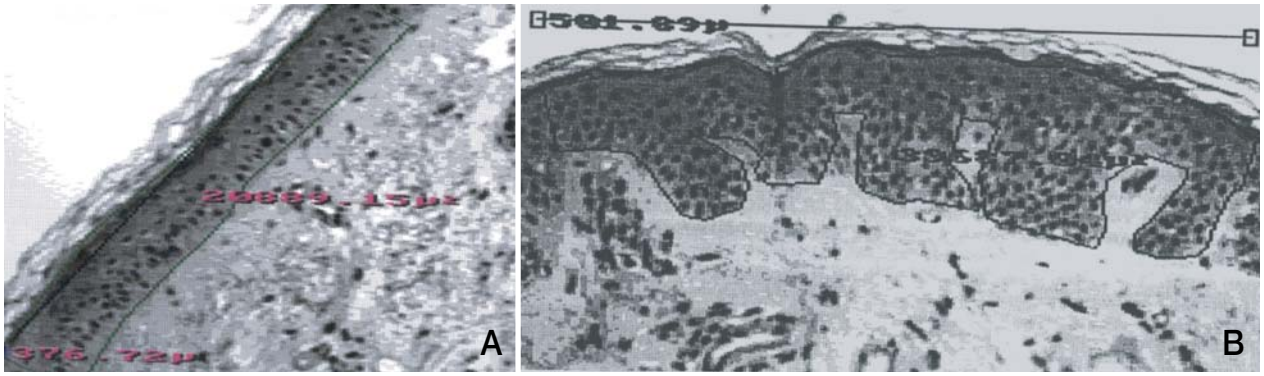

Figure 4. Rete peg pattern with vehicle (A) and combination treated (B) specimens. The treatment group shows a return to a more undulating rete peg pattern (H&E, original magnification ×40).

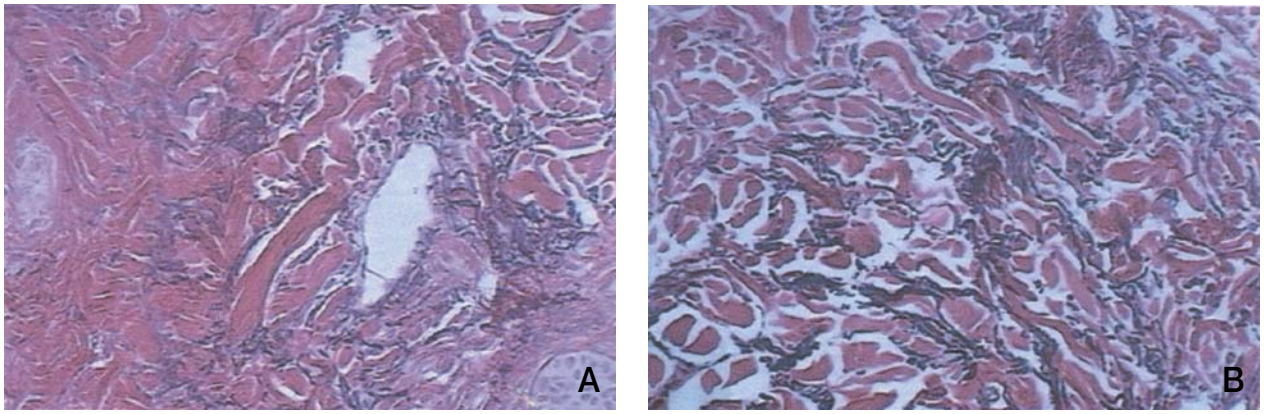

Figure 5. Elastic fibers with vehicle (A) and combination treated (B) specimens. Elastic fibers in all treated areas tended to be longer, thicker, and less fragmented compared with vehicle specimens (von Gieson, original magnification ×40).

severe atypia as 3. Keratosis was graded from 0 to 3 based on the thickness of the stratum corneum. Within normal limits was graded as 0, mild density as 1, moderate density as 2, and heavy density as 3.

Dermal fibrosis was evaluated with a trichrome stain. The collagen fibers were graded on a scale of 0 to 3. The criterion considered by the evaluators was the density of the collagen bundles represented by the intensity of the blue staining reaction. Less dense fibers were graded as 1, and dense fibers were graded as 3. Also examined was the homogeneity and thickness of the fibrous bundles. The thinner

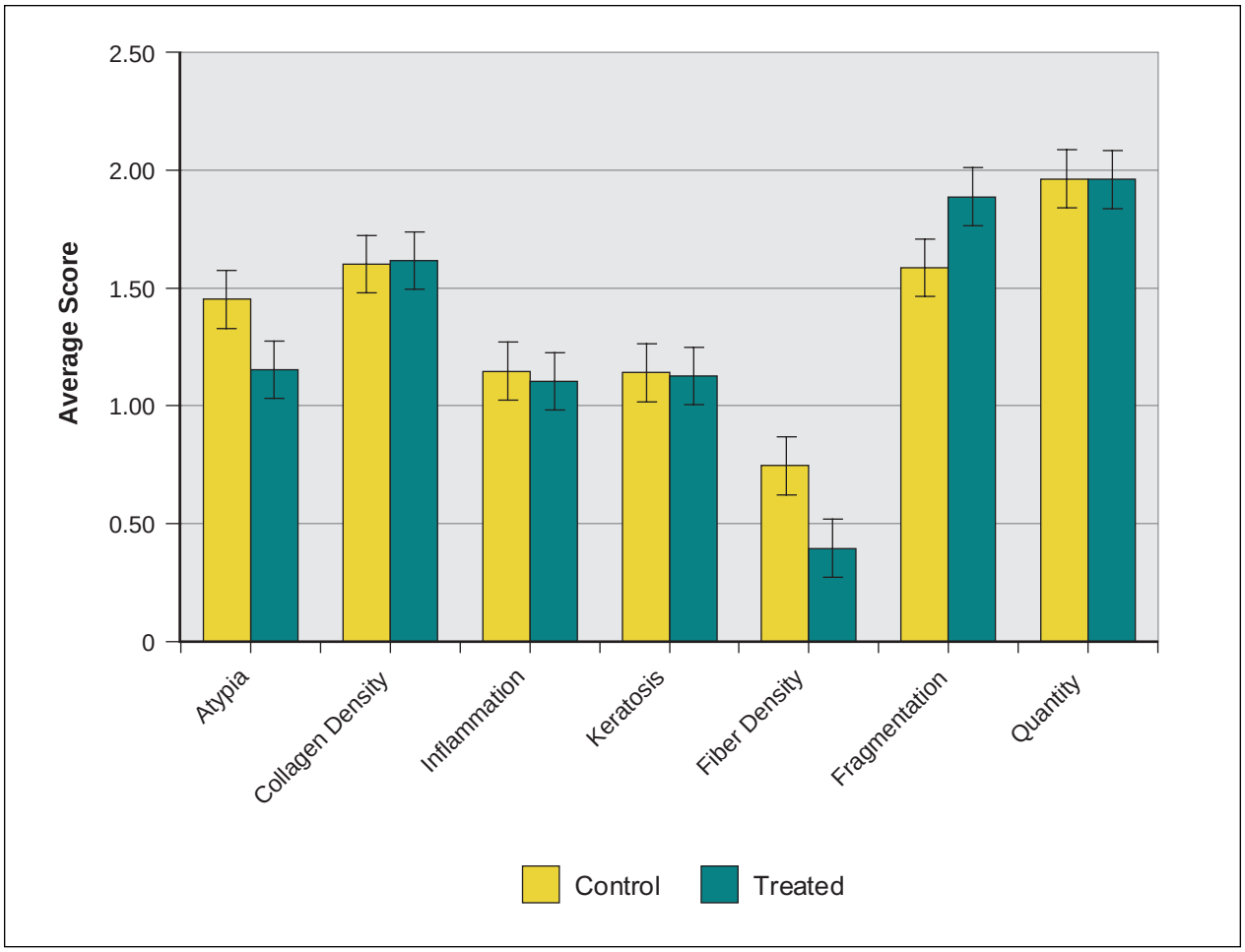

Figure 6. Semiquantitative data for the estradiol group vs vehicle (average score of 3 pathologists).

bundles were graded as 1, and the thickest bundles were graded as 3. Collagen bundles in the reticular dermis also were evaluated.

In addition, the elastic fibers in the papillary dermis were evaluated. The criteria used were thickness, degree of fragmentation, and number of elastic fibers. All areas were graded on a scale of 0 to 3. Inflammation also was graded on a scale of 0 to 3. No inflammation was graded as 0, mild inflammation as 1, moderate inflammation as 2, and severe inflammation as 3.

A 6-μm section then was prepared for estrogen receptor study by the immunoperoxidase technique, using an antibody in the N terminal domain of the estrogen receptor (DAKO). The immunoperoxidase technique was done by an automated method using the DAKO Autostainer™. The tissue was incubated with the appropriate antibody to the appropriate estrogen receptor. The nuclei of the receptor-positive cells were stained. The Cell Analysis System 200™ had 2 sensors. Both stains absorbed at 620 nm, thus providing a mask for all nuclear material. The nuclear threshold was adjusted to isolate the nucleus from the image. All objects below the threshold were ignored as the background. Ethyl green stain is transparent at 500 nm; thus, when the image was seen at 500 nm, the amount of receptor-positive material could be measured. The antibody threshold was used to isolate the antibody from the image. All staining above the threshold was considered positive, and all staining below the threshold was considered as background or nonspecific staining. The amount of positively stained nuclear area was compared with the total nuclear area, and the resulting value was reported as a percentage of positive nuclear area. The prepared sections were interpreted using the Cell Analysis System with the Nuclear Antigen Analysis program. Following this, a paired *t* test was used for statistical analysis. A 2-way analysis of variance was used to compare groups. Institutional Review Board approval was obtained for this study.

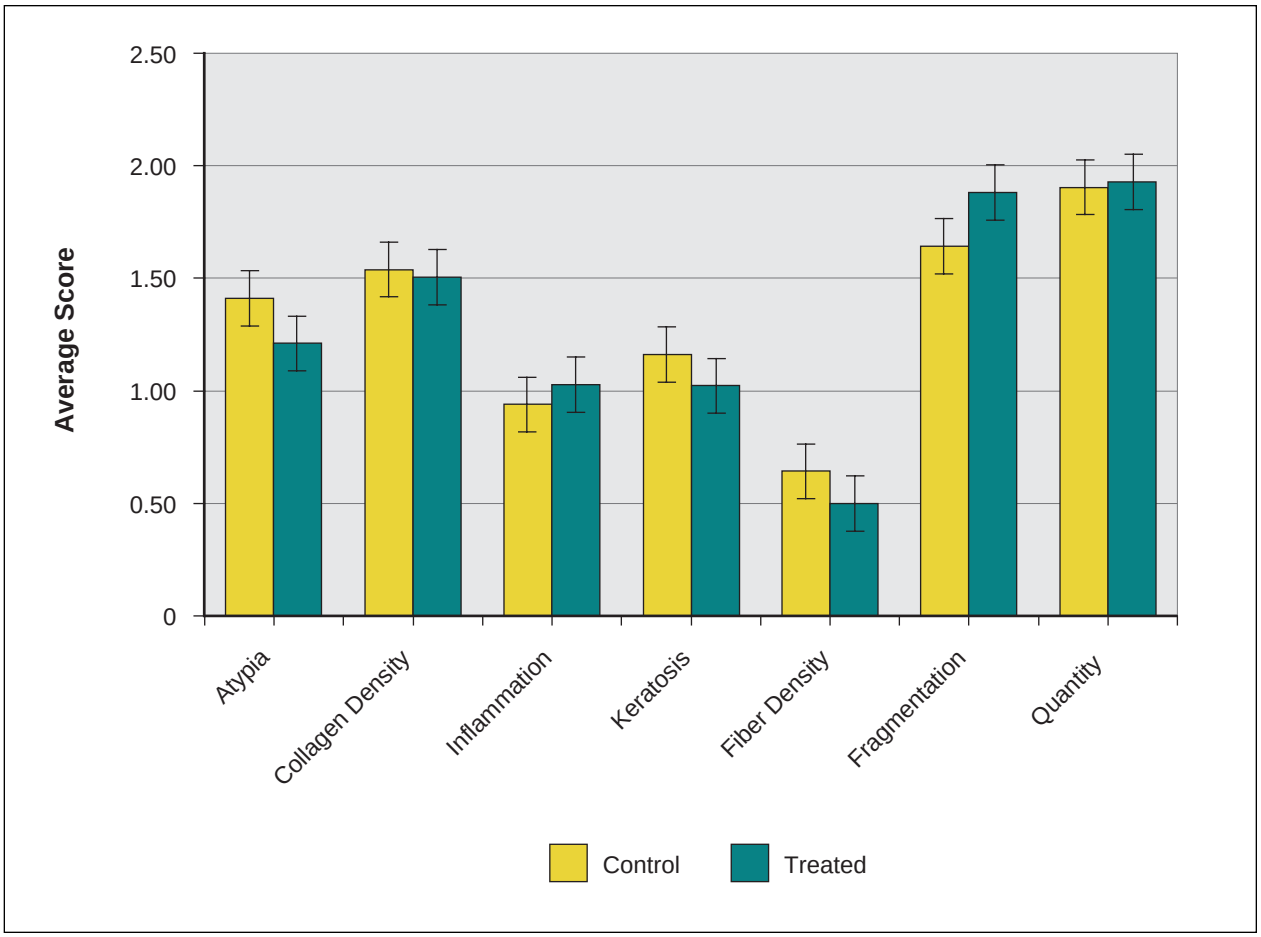

Figure 7. Semiquantitative data for glycolic acid group vs vehicle (average score of 3 pathologists).

Results

Histologic Findings—At the end of treatment, marked improvement of skin-aging symptoms were noted. The mean epidermal thickness for the various groups are shown in Table 1 and Figure 1. The difference in epidermal thickness between the estradiol and vehicle, the glycolic acid and vehicle, and the combination and vehicle were all statistically significant (P=.0045, P=.0046, and P=.00018, respectively). The percentage increase for the combination group was notably greater than either of the other 2 groups alone (P=.1).

The rete peg pattern lengths in all treated specimens was significantly greater and more distinctly undulating than in vehicle specimens (Table 2 and Figure 2). With respect to vascular differences, statistical significance was not seen in either the estrogen or the glycolic acid group.

Treatment is associated with several changes, the most evident being epidermal thickening (Figure 3) and a return to a more undulating rete peg pattern (Figure 4). Elastic fibers in all treated areas tended to be longer, thicker, and less fragmented compared with vehicle specimens (Figure 5).

Figures 6 through 8 summarize the results of treated versus vehicle effects on basal cell atypia, collagen density, inflammation, keratosis, elastic fiber density, fragmentation, and quantity. Because this data was subjectively obtained, it does not lend itself to statistical analysis.

The average percentage of positive nuclear area for estrogen receptor status was 0.74% for the estrogen group, 0.69% for the glycolic acid group, and 0.82% for the combination group.

Observed side effects were minimal. Within the first month of the study, 5 patients withdrew from the study for personal reasons, and 6 patients withdrew secondary to skin sensitivity to the creams. Of these 6 patients, 2 were being treated with estradiol cream, 2 with glycolic acid, and 2 with a combination of both. The results of 3 biopsies were not satisfactory for analysis because the epidermis was not present in the sample. All irritation resolved with discontinuation of the cream. No increase in

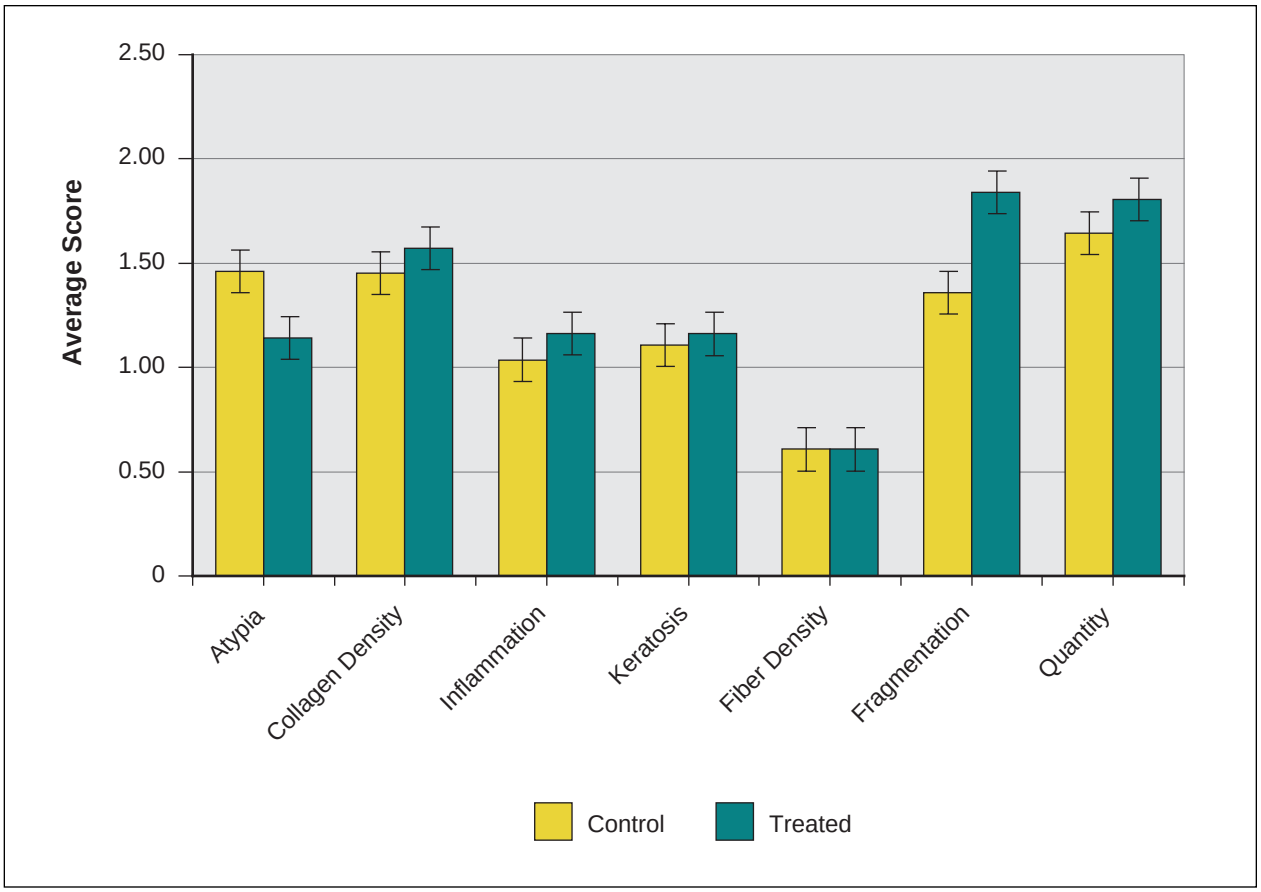

Figure 8. Semiquantitative data for combination group vs vehicle (average score of 3 pathologists).

facial pigmentation was noted in any of the groups. Previous studies have shown no change in follicle-stimulating hormone and estradiol levels with topical facial estrogen.[3,15]

Comment

Both estrogen and glycolic acid compounds were shown to be effective in the treatment of skin-aging symptoms of postmenopausal women. Although not statistically significant, the combined effects of these compounds on epidermal thickness were shown to be considerably greater than the effects of either cream alone (P=.1). A statistically significant increase was noted in all groups for epidermal thickness and rete peg patterns. A return to a more undulating rete peg pattern, typical of younger skin, also was seen in all groups. Elastic fibers were found to be elongated, more unified, and less fragmented—also consistent with younger skin. The semiquantitative data was subjective; therefore, it was not analyzed statistically.

The vehicle used in this study was that of the compounded estradiol cream, which was different from that of the glycolic acid cream. It would have been preferable to have each precise vehicle used with each active formulation, rather than only the estradiol vehicle for both; however, the glycolic acid cream was obtained commercially, so there was not a vehicle base available for this product.

Previous studies have shown that there are varying levels of estrogen receptors throughout the body.[11,12] Estrogen receptor status of the tissue at the temple hairline showed low results; however, results of skin biopsies from around the eye have shown high levels of estrogen receptors. Estrogen receptor status from blepharoplasty skin samples showed an average percentage of positive nuclear area of 29.4% (n=20; S. Hughes, O. Solis, unpublished data, 2002). This should promote the effectiveness of the estrogen cream around the eye.

Previous studies have excluded women on oral estrogens. Of 50 patients in this study, 34 were on oral estrogen replacement, which has been shown to improve skin quality.[13-15] This study showed a further benefit with the topical application of estrogen cream.

Conclusion

This prospective, randomized, double-blind study showed estrogen and glycolic acid creams to be a safe treatment for aging facial skin. The most significant changes occurred in epidermal thickness, rete peg pattern, vascularity, and elastic fiber quality. No inflammation was noted histologically. The combination of estrogen and glycolic acid cream showed a trend toward the greatest increase in skin thickness versus either product alone ($P=0.1$). With a larger sample size, statistical significance likely would have been reached. Estradiol and glycolic acid creams were found to be highly effective in treating skin aging in postmenopausal women.

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