Skin Collagen Changes in Postmenopausal Women Receiving Different Regimens of Estrogen Therapy

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Collagen is a widespread body constituent that is affected by estrogen status in women. Its decrease after menopause can be prevented and/or restored by estrogen treatment. We explored the effect of four different hormonal replacement regimens on total skin collagen content by measuring hydroxyproline in skin biopsy specimens taken from postmenopausal women. All regimens showed increases in skin collagen levels proportionate to the levels at the start of the treatment. Estrogen replacement therapy is shown to be prophylactic in women who have higher skin collagen levels and both prophylactic and therapeutic in women with lower skin collagen levels. ***(Obstet Gynecol 70:123, 1987)***

Collagen constitutes approximately one-third of the total mass of the body.[1] Despite this, little work has been done to establish the relationship between collagen and sex hormones. Collagen is a major constituent of the dermis; therefore the skin has been used as a model for determining overall collagen changes associated with sex hormones. Brincat et al[2] reported that the skin collagen content in postmenopausal women who had subcutaneous estradiol (50 mg) and testosterone (100 mg) implants for between two and ten years was higher than in an untreated control group of age-matched postmenopausal women. It has been shown that skin collagen and skin thickness decreased proportionally with time after menopause, and that this decrease was prevented with sex hormone therapy. This finding suggests that the collagen decrease after menopause is due to estrogen deficiency,[3] and that the decrease is not simply arrested but is reversed by estrogen therapy.

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Because the connective tissue of bone and skin is similar and 33% of dried bone is composed of a collagenous matrix,[4] it is possible that any decline in bone organic matrix paralleling a postmenopausal decline in dermal connective tissue contributes to postmenopausal osteoporosis. If the decrease in skin collagen content can be prevented or even reversed by treating postmenopausal women with estrogen, there would be important implications concerning not only the health of the skin, but maybe also the etiology and prevention of organic matrix loss in postmenopausal bones.

To test the hypothesis that skin collagen decrease can be prevented postmenopausally and to establish the optimum treatment, four groups of postmenopausal women were given four different regimens of sex hormone therapy, and their skin collagen levels were established at various times during the study. The study considered overall collagen changes rather than specific types of collagen, because 90% of the collagen in the body is type I,[5] and the relationship between total collagen content and hormones has not yet been established.

Materials and Methods

Seventy-eight postmenopausal women who had not received any previous hormone treatment were recruited from the Dulwich Menopause Clinic. The patients were prescribed one of four regimens of sex hormone replacement.

The first group of 16 patients applied topically 1.5 mg of estradiol gel (in 2.5 g of base) (Oestrogel; Besins, Paris) to the lower abdomen every night for one year. This method of estrogen administration maintains a constant 1:2 physiologic ratio of estrone and estradiol, once the wide variations that occur among women in

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Table 1. Patient Data for Four Groups of Postmenopausal Women Receiving Different Regimens of Sex Hormone Therapy*

Group	Estradiol gel	50-mg estradiol implant	50-mg estradiol and 100-mg testosterone implant	100-mg estradiol implant
N	16	22	20	20
Age (yr)	48.9 ± 4.85	48.85 ± 8.3	50.17 ± 8.42	52.49 ± 5.37
Menopausal age (yr)	3.47 ± 4.06	4.41 ± 5.0	4.8 ± 5.49	4.89 ± 6.52
Weight (kg)	66.6 ± 10.45	63.5 ± 12.2	62.62 ± 9.77	66.87 ± 10.2
Patient (m)	1.6 ± 0.05	1.6 ± 0.06	1.62 ± 0.09	1.6 ± 0.06

* Data (except *N*) are expressed as mean ± SD.

the first three days disappear.[6] The other patients were put on three different regimens of subcutaneous implants (Organon, UK).

These routes were chosen because both implants and percutaneous estradiol gel bypass the intestine, avoiding the first pass effect of the hormone on liver metabolism, and preventing the unphysiologic ratio of estradiol to estrone found wih oral preparations.[8] Unlike implants and estradiol gel, oral preparations also reduce liver metabolism of clotting factors and lipids.[9]

The three different implant regimens comprised one group on 50 mg estradiol ($N = 22$), one group on 50 mg estradiol and 100 mg testosterone ($N = 20$), and one group on 100 mg estradiol ($N = 20$). All three groups received only one implant which lasted for six months.

All implant patients with a uterus received 5 mg norethisterone daily for seven days each cycle to prevent endometrial hyperplasia. The women on percutaneous estradiol who had a uterus were given 300 mg progesterone orally for ten days in each cycle, for the same reason.

Punch biopsy specimens of skin, 3 mm in diameter, were taken from the right thigh 5 cm below the greater trochanter. Hydroxyproline was extracted from these biopsy specimens and measured using Woessner's method.[12] The collagen content was then calculated as described by Neuman and Logan.[13]

The results were expressed in relation to the surface area of the skin biopsy specimen ($\mu g/mm^2$) so that the skin mass would not be changed because of other independent variables, such as water content, which would affect the collagen content.

Thigh biopsy specimens were taken at the beginning of the study, at three months, and at six months from all patients treated with hormone implants. In addition, those patients on estradiol gel also had lower abdominal 3-mm skin biopsy specimens taken at these times, and abdominal and thigh skin biopsy specimens taken at one year.

Table 1 summarizes the data on the patients in all four groups.

The paired Student's *t* test was used to calculate changes in collagen content during the study. Pearson's correlation test (*r*) was used to calculate all correlations. The distribution of the data was normal according to the Kolmogorov-Smirnov one-sample test, which was available on the SPSS-X program.

Results

Estradiol Gel

Of the sixteen women in this group, three dropped out after their six-month visit because they obtained insufficient relief of their climacteric symptoms (1.5 mg estradiol daily) and desired alternative therapy. All of the other patients had satisfactory symptomatic relief from the estradiol gel and continued the treatment. In one other patient, the collagen extraction of one thigh biopsy specimen at 12 months was unsuccessful.

Figure 1 shows the abdomen and thigh skin collagen changes after one year of estradiol gel therapy. The skin collagen content in the thigh increased steadily

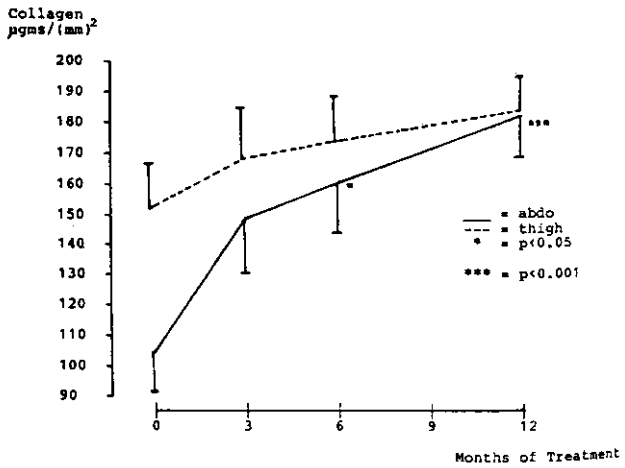

Figure 1. Changes in the mean ± SE abdominal and thigh skin collagen levels of 16 postmenopausal women applying estradiol gel (Oestrogel) daily for one year.

during the study period from a mean of 151.9 $\mu g/mm^2$ to a mean of 180.9 $\mu g/mm^2$, but this increase did not reach significant levels. The skin collagen content in the abdomen showed statistically higher values as compared with baseline values at both six and 12 months after starting therapy, rising from 107.7–180.9 $\mu g/mm^2$ after one year.

Figure 2 shows the changes in skin collagen content after one year of therapy as compared with the original collagen levels. Both the abdomen and the thigh data showed a significant negative correlation between the change in skin collagen content and the collagen content at the start of the study (abdomen: $r = -0.68$, $P < .005$, $N = 13$; thigh: $r = -0.72$, $P < .005$, $N = 12$).

Percutaneous Implants of 50 mg Estradiol

The twenty-two previously untreated postmenopausal women studied in this group showed statistically significant increases in thigh collagen after three and six months of treatment. The thigh skin collagen level (mean ± SD) at the start of the study was 146.55 ± 55.39 $\mu g/mm^2$; at three months, 179.39 ± 49.86 $\mu g/mm^2$; and at six months, 209.79 ± 49.86 $\mu g/mm^2$ (Figure 3).

There was a significant negative correlation ($P < .001$, $r = -0.6239$) between the pretreatment skin collagen content and the skin collagen content after six months of therapy.

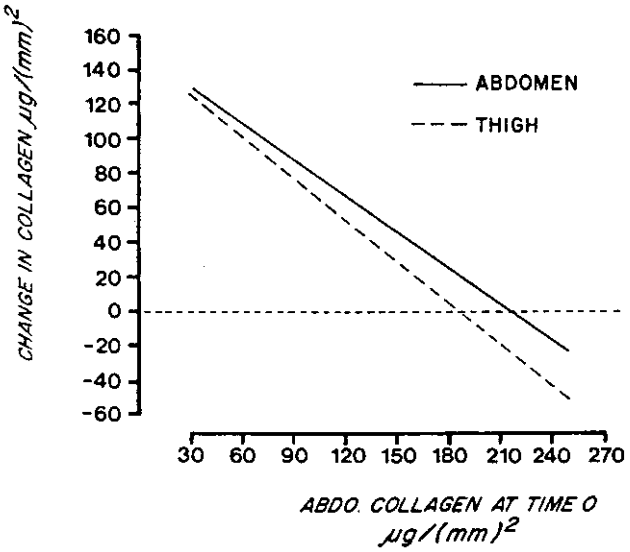

Figure 2. Relationship between the change in abdominal and thigh skin collagen content in postmenopausal women after one year of Oestrogel therapy, and their original level (abdominal and thigh, respectively) of skin collagen content (correlations are given in Table 2).

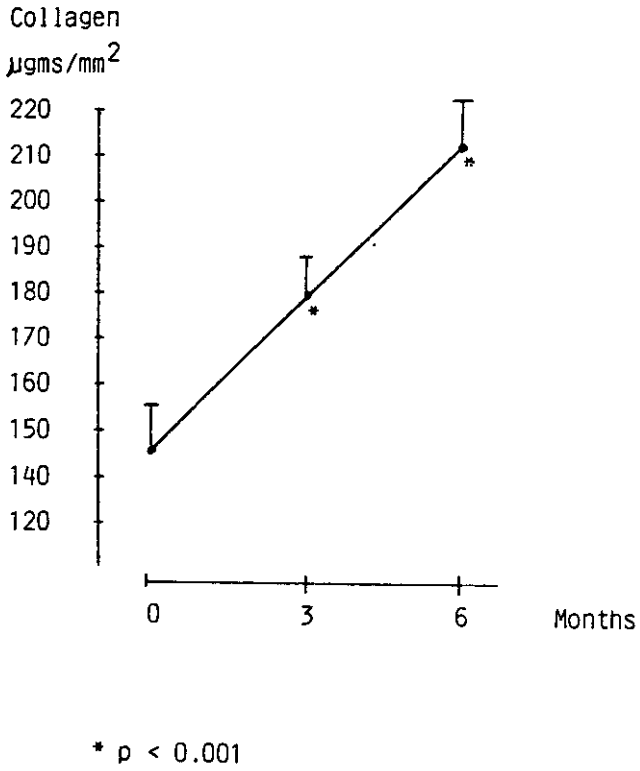

Figure 3. Change in the mean ± SE thigh skin collagen content in 22 women during a six-month period after a 50-mg estradiol implant.

Percutaneous Implants of 50 mg Estradiol and 100 mg Testosterone

Twenty postmenopausal women were recruited for prospective follow-up of their thigh skin collagen content after receiving an implant of 50 mg estradiol and 100 mg testosterone.

At the start of the study, these twenty postmenopausal women had a thigh skin collagen level of 197.35 ± 75.25 $\mu g/mm^2$. Given the considerable individual variation that has been noted in these studies, this high mean must have been caused by individuals with high skin collagen levels. At three months, the thigh skin collagen level was 224.71 ± 76.1 $\mu g/mm^2$, while at six months it was 192.81 ± 43.82 $\mu g/mm^2$. There was no overall significant improvement in thigh skin collagen content, either at three or at six months.

When we compared the change in skin collagen content after six months with the thigh skin collagen content at the start of the study, we obtained a significant negative correlation ($P < .0001$, $r = -0.8329$) (Figure 4).

Percutaneous Implants of 100 mg Estradiol

When the study began, the twenty postmenopausal women in this group had a mean skin collagen level of

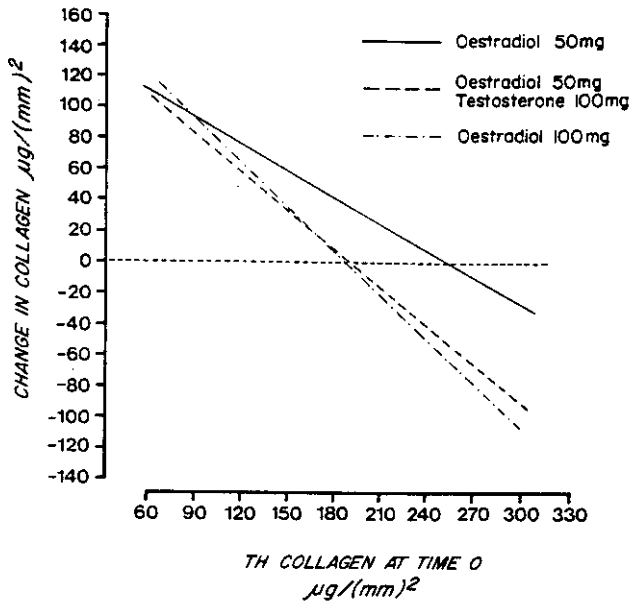

Figure 4. Relationship between the change in thigh skin collagen level and the original thigh skin collagen level in the groups of postmenopausal women six months after receiving implants of 50 mg estradiol, 50 mg estradiol and 100 mg testosterone, or 100 mg estradiol (correlations are given in Table 2).

164.74 ± 47.45 $\mu g/mm^2$. At three months it was 158.67 ± 32.67 $\mu g/mm^2$, and at six months it was 181.45 ± 31.93 $\mu g/mm^2$. This increase is not statistically significant.

Again, there was a significant negative correlation ($r = -0.7848$, $P < .0001$) between the skin collagen levels at the start of the study and after six months (Figure 4).

In summary, Table 2 shows that all four treatment regimens exhibited a significant negative correlation between collagen levels at the start of the study and those at the end. This finding indicates that the level of skin collagen at the end of the study depended on the initial level of skin collagen. The higher the original collagen level, the smaller the increase; the lower the original collagen level, the larger the increase.

Table 2. Correlation Between Baseline Thigh Collagen Level and Changes in Thigh Skin Collagen Level in Four Groups of Postmenopausal Women After Treatment With Different Hormone Regimens

	r	*P*
Estradiol gel (12 m) thigh collagen ($N = 12$)	−0.72	<.003
Estradiol gel (12 m) abdominal collagen ($N = 13$)	−0.68	<.005
50-mg estradiol implant (6 m) thigh collagen ($N = 22$)	−0.62	<.001
50-mg estradiol and 100-mg testosterone implant (6 m) thigh collagen ($N = 20$)	−0.83	<.001
100-mg estradiol implant (6 m) thigh collagen ($N = 20$)	−0.78	<.001

Discussion

This study showed that the dermal collagen content in postmenopausal women changed with four different parenteral treatment regimens: 1) estradiol gel, 2) implants of 50 mg estradiol, 3) implants of 50 mg estradiol and 100 mg testosterone, and 4) implants of 100 mg estradiol.

Estradiol gel apparently has a greater local than a distant effect on dermal collagen, because the abdominal skin collagen showed a significant change with therapy. Thigh collagen, although it showed an increase over the study period, did not achieve statistically significant changes. It could be that such differences would disappear with longer use.

Estrogen does not simply act on dermal collagen by increasing its quantity. As we have shown, the degree of change is dependent on the initial level of collagen. Thus, if the collagen level was low at the start of the study (as was the case with patients who were several years postmenopausal), the increase was a relatively large one.[3] Those women who had initially high skin collagen levels (who tended to be in the first years after menopause) had much smaller changes or no changes at all, and in some cases actually lost collagen. The "optimum" level of collagen at the start of the study, at which no change occurred ("O" collagen) was remarkably similar in all four groups. Table 3 shows the derived thigh skin collagen levels at the start of the study that would result in no change from hormone therapy ("O" collagen). We derived these levels from the data in Figures 2 and 4 (ie, the levels after twelve months of estradiol gel therapy and after six months of each of the implant therapies).

Both the mean "O" collagen levels of the four treatment groups and the individual values for each treatment group are remarkably similar to the thigh skin collagen level in the group of women treated by Brincat et al[3] for two to ten years (221.12 ± 83.47 $\mu g/mm^2$). This reinforces the hypothesis that there is an optimum thigh skin collagen content, and that there is a stage beyond which further hormone treatment is of no value.

Paradoxically, the therapy (100 mg estradiol) that gave the highest serum levels of estrogen gave a lower

Table 3. Thigh "O" Collagen Levels on Four Different Treatment Regimens*

Estradiol gel	188.45
50-mg estradiol implant	248.27
50-mg estradiol and 100-mg testosterone implant	191.98
100-mg estradiol implant	190.17
Mean ± SD	204.72 ± 25.18

* Data are expressed in $\mu g/mm^2$.

"O" collagen level than the therapy that gave lower serum estrogen levels (50 mg estradiol).[15] The highest "O" collagen levels (248.3 $\mu g/mm^2$) were obtained with the 50-mg estradiol implant regimen. Table 3 shows that the women with implants of 50 mg estradiol and 100 mg testosterone had a similar "O" collagen level as those with implants of 100 mg estradiol, which was over 50 $\mu g/mm^2$ lower than the level obtained with the 50-mg estradiol implant regimen.

This finding suggests than an optimum skin collagen level is obtained by an optimum estrogen (or optimum total of sex hormone) regimen. Doses that are too high, like doses that are too low, give lower "optimum" levels of collagen. Shahrad and Marks[16] and Punnonen[17] reported a similar phenomenon in work on the epidermis. They found that pharmacologically high doses of estrogen thinned the epidermis and reduced epidermal mitotic activity rather than thickening the epidermis and increasing mitotic activity (as happened with lower, more physiologic doses). It is thus clear that the degree of increase in collagen level with estrogen therapy is dependent on the initial level. A lower skin collagen level results in a larger increase, whereas a higher skin collagen level results in a smaller increase, with all values tending toward a common mean.

A previous study[2] showed that the skin collagen level in a group of postmenopausal women treated with estradiol and testosterone for two to ten years was significantly higher than in a similar age-matched group that received no treatment. The skin collagen level in that group was 156.21 ± 78.53 $\mu g/mm^2$, whereas in the present group, the pretreatment skin collagen level of all the women studied was 165.4 ± 52.5 $\mu g/mm^2$. The skin collagen level of the treated patients in that study was 230.89 ± 104.35 $\mu g/mm^2$, a value similar to the one obtained in a later study by the same authors.[3] They obtained a value of 221.12 ± 83.47 $\mu g/mm^2$ in a similar group of women, which also corresponds to the "O" collagen level obtained in our study for the groups as a whole (Table 3). This consistency between the studies reinforces our belief that the lower an individual's skin collagen level, the greater the increase with treatment, until an optimum level is attained, beyond which very little change occurs.

Therefore, we conclude that in women who are several years postmenopausal with low skin collagen levels, estrogens are of therapeutic and, later, prophylactic value, whereas in women in their first postmenopausal years with high skin collagen levels, estrogens are of prophylactic value only.

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