Collagen Synthesis in Postmenopausal Women During Therapy With Anabolic Steroid or Female Sex Hormones

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The effect of anabolic steroid therapy and estrogen-progestogen substitution therapy on serum concentration of procollagen type III aminoterminal peptide (PIIINP), a measure of collagen synthesis, in postmenopausal women was studied in two double-blind studies: (1) 39 women allocated to treatment with either 50 mg nandrolone decanoate as an intramuscular depot or placebo injections every third week for 1 year, and (2) 40 women allocated to receive either 2 mg 17β-estradiol plus 1 mg norethisterone acetate daily or placebo tablets for 1 year. Serum PIIINP was measured every 3 months during the study. Anabolic steroid therapy resulted in a more than 50% increase ($P < .001$) in serum PIIINP at 3 months, which thereafter decayed but remained significantly increased throughout the study period. Serum PIIINP showed the same pattern during estrogen-progestogen therapy, but to a lesser degree. We conclude that anabolic steroids stimulate type III collagen synthesis in postmenopausal women, while estrogen-progestogen therapy may have such an effect, but only to a lesser degree.

ANABOLIC STEROIDS and female sex hormones have been used for treatment of osteoporosis in postmenopausal women.[1,2] Recent studies have demonstrated that these steroids also affect soft tissue body composition in postmenopausal women.[3-5] Treatment with anabolic steroids results in an increased lean body mass and a decreased fat mass.[3] Estrogen replacement therapy also seems to decrease the amount of body fat slightly.[4,5] Furthermore, Brincat et al[6-8] have shown that both estrogen monotherapy and combined testosterone-estrogen therapy increase skin collagen content in postmenopausal women. Whether this is caused by increased formation of new collagen or decreased breakdown of old collagen is not known.

Type III collagen is present in dense and loose connective tissues throughout the body. During the conversion of procollagen type III to collagen type III, an extension-peptide (procollagen type III aminoterminal peptide [PIIINP]) is cleaved off in a stoichiometric fashion and released into the extracellular fluid.[9,10] Serum PIIINP may therefore reflect collagen synthesis. In two double-blind placebo-controlled studies, we have investigated the effect of nandrolone decanoate (anabolic steroid) therapy, as well as combined estrogen-progestogen therapy on collagen type III synthesis in postmenopausal women by using serum PIIINP as a marker thereof.

MATERIALS AND METHODS

Subjects

All participants in the present study had previously suffered a forearm fracture or a vertebral (wedge or compression) fracture, but were otherwise healthy postmenopausal women age 55 to 75 years. The participants were selected by the following procedures: (1) All spinal x-rays (4,900) of women age 55 to 75 years taken during the preceding 5 years at Glostrup Hospital were reassessed. Two hundred nineteen women were found to have at least one vertebral fracture. Of these, 81 were excluded because of malignant, gastrointestinal, metabolic, or rheumatic disease, or drug intake. Of the remaining 138 patients, 44 consented to enter the study. After the initial examination five patients dropped out. (2) All patients referred to Glostrup Hospital because of distal forearm fracture during the preceding 5 years were approached and the same exclusion criteria were applied. Of 316 women, 133 were excluded. Eighty of the remaining 183 agreed to participate.

The patients from these two groups were randomly allocated to a anabolic steroid trial or a female sex hormone trial. For further details, see reference 1.

Anabolic steroid trial. Thirty-nine women were blindly allocated by random sampling numbers to treatment with either 50 mg nandrolone decanoate (Deca-Durabolin, Organon, The Netherlands) (n = 20) as an intramuscular depot or placebo injection (n = 19) every 3 weeks for 1 year.[3] Thirty-six women (92%) completed the 1 year of treatment (19 active-treated and 17 placebo-treated). Three women dropped out, one from the active group (hoarseness and depression) and two from the placebo group (without specific reasons). Furthermore, six women (three active-treated and three placebo-treated) failed to collect a 24-hour urine sample either initially or at 1 year.

Female sex hormone trial. Forty women were blindly allocated by random numbers to receive either 2 mg 17β-estradiol plus 1 mg norethisterone acetate daily (n = 20) or placebo tablets (n = 20) for 1 year (5). Thirty-one women (78%) completed the 1 year of treatment (16 active-treated and 15 placebo-treated). Nine women dropped out, four from the active group (one benign mammary tumor, one mastalgia, one aggravation of heart disease, one killed in a traffic accident) and five from the placebo group (one aggravation of heart disease, one aggravation of eczema, one died of unknown cause, two for personal reasons).

In addition, all subjects in both trials received a daily oral supplement of 500 mg calcium (Calcium, Sandoz, Switzerland). Informed consent was obtained from each participant according to the Helsinki Declaration II, and the trial was approved by the ethical committee of Copenhagen County. In the calculations, we only included data from women who had the parameter in question measured at all observation times.

Methods

Blood samples were taken in the morning after an overnight fast, and stored at −20°C until analyzed. The concentration of intact and high molecular weight PIIINP antigens were determined by a commercial available radioimmunoassay[11] (PIIINP, RIA kit, Far-

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Table 1. Initial Data (mean ± SD)

	Nandrolone Decanoate Study		Estrogen-Progestogen Study	
	Active	Placebo	Active	Placebo
N	19	17	16	15
Age (yr)	66 ± 6	68 ± 4	64 ± 5	65 ± 6
Height (cm)	156 ± 6	161 ± 7	160 ± 9	159 ± 6
Weight (kg)	64 ± 8	64 ± 14	60 ± 8	65 ± 10
PIIINP (ng/mL)	3.7 ± 2.0	2.8 ± 0.7	3.1 ± 1.1	3.5 ± 1.6

mos Diagnostica, Oulunsalo, Finland), modified to be a sequential saturation assay. To ensure a high constancy, we have prepared a long-life tracer for the assay, using the iodogen method.[12]

The antigen used for iodination is highly purified human PIIINP,[13] kindly donated by Dr Juha Risteli, Oulu, Finland. Twenty-four–hour urinary hydroxyproline excretion was measured by a spectrophotometric method.[14] The subjects were on free diet.

Serum PIIINP was measured every 3 months in all subjects during the study. Twenty-four–hour urinary hydroxyproline was measured initially and at 1 year in 30 of the subjects in the nandrolone decanoate trial (16 active- and 14 placebo-treated).

Only parametric statistics were used (Students' t test). The cumulative change in PIIINP was calculated in each individual at each measurement time, t_i, as the area under the curve from the plot of the difference between the measured value and the initial value versus time:

$$\text{Cumulative change in PIIINP}(t_i) = 3 \times \sum_{i=1}^{4} [(\text{PIIINP}(t_i) + \text{PIIINP}(t_{i-1})) \times \tfrac{1}{2} - \text{PIIINP}(t_0)] \text{ ng/mL} \times \text{months}.$$

RESULTS

The initial data of the subjects are given in Table 1. Both active groups were comparable with their respective placebo group. The changes in serum PIIINP in the four groups during the study are shown in Fig 1. Compared with placebo, treatment with nandrolone decanoate resulted in a more than 50% increase ($P < .001$) in serum PIIINP at 3 months, which thereafter decayed but remained significantly ($P < .01$) increased throughout the study period. Serum PIIINP showed the same pattern during estrogen-progestogen therapy, but to a much lesser degree, with the difference between active and placebo being nonsignificant at the end of the study. The cumulative changes in serum PIIINP increased during the study in both active groups.

The 24-hour urinary excretion of hydroxyproline was not significantly affected by the nandrolone decanoate therapy (0.13 ± 0.07 mmol/24 h initially, and 0.14 ± 0.06 mmol/24 h at 1 year in the actively treated group; and 0.13 ± 0.04 mmol/24 h initially, and 0.12 ± 0.06 mmol/24 h at 1 year in the placebo-treated group [mean ± SD], difference NS).

DISCUSSION

Type III collagen is present in connective tissue throughout the body. During its synthesis, PIIINP is cleaved from procollagen and released into the extracellular fluid.[9,10] The present study showed that serum PIIINP was increased by nandrolone decanoate therapy, and to a lesser degree by estrogen-progestogen therapy. Assuming unchanged degradation of PIIINP, this finding indicates that these steroids stimulate collagen synthesis.

PIIINP is primarily degraded in the liver.[15,16] Whether the drugs used in the present study affect this degradation significantly is not known. Liver function evaluated by serum concentration of albumin was unaffected in both trials (data not shown). However, nandrolone decanoate therapy did slightly increase serum aspartate aminotransferase, but all subject remained within the normal range.[3]

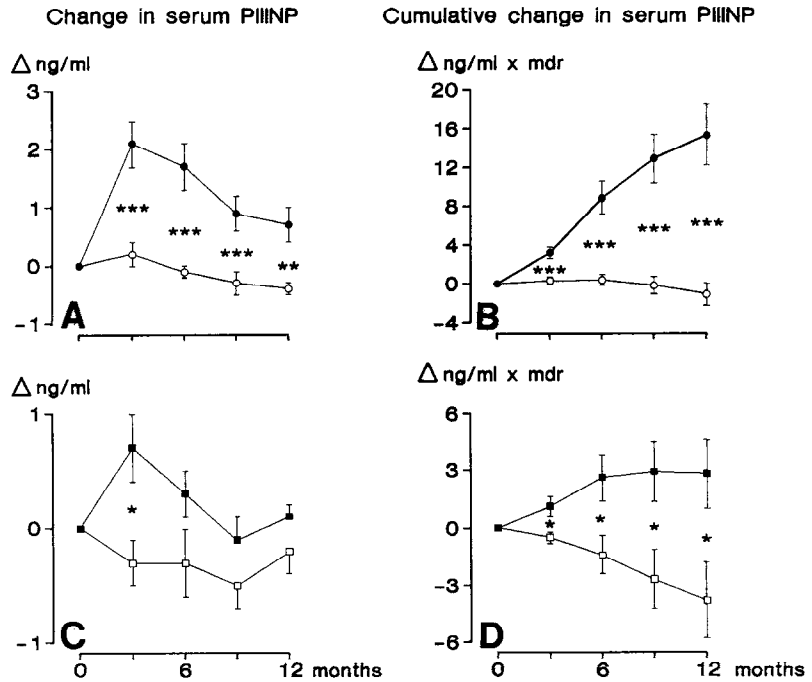

Fig 1. Change in serum PIIINP and cumulative change in serum PIIINP, (A and B) during treatment with Nandrolone decanoate (●), active (n = 19); (○), placebo (n = 17), and (C and D) during estrogen-progestogen substitution therapy (■), active (n = 16); (□), placebo (n = 15). Values are given as difference from the initial values as mean ±SEM. Significance of difference from placebo: *$P < .05$; **$P < .01$; ***$P < .001$.

The serum PIIINP is probably reflecting the formation of new type III collagen going on only at the time the blood sample is taken. The cumulative change in serum concentration of PIIINP (corresponding to the integral value of change in PIIINP) should therefore reflect the absolute change in total body type III collagen. Total body collagen is unfortunately not possible to measure in vivo. However, we have previously shown that nandrolone decanoate therapy increases lean body mass in these elderly women by approximately 10%.[3] This could indicate that total body collagen was increased by the therapy, although it must be borne in mind that collagen is only a small fraction of lean body mass.

Regarding treatment of osteoporosis, the main issue is to increase bone strength. However, increased strength of the supporting soft tissue may also be of some importance. The present data indicate that nandrolone decanoate may affect the latter issue through increased collagen synthesis.

Breakdown of collagen may be evaluated by the hydroxyproline excretion in the urine. Nandrolone decanoate therapy did not significantly affect the 24-hour urinary excretion of hydroxyproline. This is in accordance with Chesnut et al,[17] who found unchanged urinary excretion of hydroxyproline during treatment with the anabolic steroid stanozolol. However, it should be noted that the subjects in both studies were on free diet, but major changes in collagen breakdown during anabolic steroid therapy seem unlikely, assuming that the subjects did not change their diet during the study. We did not measure 24-hour urinary excretion of hydroxyproline in the estrogen-progestogen trial, but several previous investigations have shown a decrease in urinary excretion of hydroxyproline during estrogen-progestogen substitution therapy.[14,18,19] This has generally been attributed to a decrease in bone resorption, but the decrease in urinary hydroxyproline excretion could partly be due to a decrease in collagen breakdown in soft tissue, as proposed by Brincat et al.[6]

Regarding the absolute amount of collagen, Brincat et al have shown that both estrogen monotherapy and combined testosterone-estrogen therapy increased the skin collagen content significantly.[6-8]

In conclusion, both anabolic steroid and female sex hormones affect collagen turnover in postmenopausal women. Nandrolone decanoate therapy seems to increase type III collagen synthesis without affecting collagen breakdown, while estrogen-progestogen substitution therapy both lightly increases type III collagen synthesis and decreases collagen breakdown.

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