Effects of transdermal estrogen on collagen turnover at rest and in response to exercise in postmenopausal women


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Pingel J, Langberg H, Skovgård D, Koskinen S, Flyvbjerg A, Frystyk J, Kjær M, Hansen M. Effects of transdermal estrogen on collagen turnover at rest and in response to exercise in postmenopausal women. J Appl Physiol 113: 1040–1047, 2012. First published July 5, 2012; doi:10.1152/japplphysiol.01463.2011.—Menopause is associated with loss of collagen content in the skin and tendon as well as accumulation of noncontractile tissue in skeletal muscle. The relative role of hormones and physical activity on these changes is not known. Accordingly, in a randomized, controlled, crossover study we investigated effects of transdermal estrogen replacement therapy (ERT) on type I collagen synthesis in tendon and skeletal muscle in 11 postmenopausal women. Patches with estrogen (Evorel) were placed on the skin above the patellar tendons and compared with no patch postmenopausal women. ERT was associated with enhanced synthesis of type I collagen in the front of the patellar tendons and in the vastus lateralis muscle of both legs at days 3 and 5. The collected dialysate was analyzed for procollagen type I NH2-terminal propeptide (PINP), insulin-like growth factor I (IGF-I), and interleukin-6 (IL-6). Neither loading (Rest leg vs EX leg) nor treatment (control vs ERT) influenced peritendinous PINP, whereas combined exercise and ERT enhanced muscle PINP after 72 h (interaction between loading and treatment P = 0.008). In neither skeletal muscle nor peritendinous fluid were IGF-I and IL-6 influenced by treatment or exercise. In conclusion, ERT was associated with enhanced synthesis of type I collagen in the skeletal muscle in response to acute exercise. In perspective, this indicates that the availability of estrogen in postmenopausal women is important for repair of muscle damage or remodeling of the connective tissue within the skeletal muscle after exercise.

estradiol; insulin-like growth factor I; hormone replacement therapy; menopause; connective tissue

Menopause is associated with a loss of muscle and bone mass. In addition, the content of type I collagen, the most abundant structural protein in tendons and ligaments, is reduced (31, 33). These alterations may be partly explained by the postmenopausal hormonal milieu, which includes reductions in sex hormones, growth hormone, and insulin-like growth factor I (IGF-I) and increases in proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).

Estrogen receptors are localized throughout the skeletal muscle tissues, in the nuclei of muscle fibers, in their capillaries (39), in ligaments in the synovial lining, and in fibroblasts (27, 29). Furthermore, tendons express transcripts for estrogen receptors (19). Nevertheless, knowledge concerning any potential direct effect of estradiol on human skeletal muscle connective tissue and tendon tissue is sparse. In ovariectomized women using oral estrogen replacement therapy (ERT), a higher tendon collagen synthesis rate and a reduced relative tendon stiffness has been observed compared with healthy aged-matched postmenopausal women (15). Furthermore, in arcus tendineus fasciae pelvis, a lower type I collagen content has been observed in postmenopausal women compared with young women, which was not was present in postmenopausal women using hormone replacement therapy (31). These observations suggest an effect of estrogen on tendon and skeletal muscle collagen turnover.

The effect of estrogen on tissue turnover and composition may be direct, but estrogen may also exert its effects indirectly via changing levels of hormones and cytokines known to influence collagen turnover, e.g., IL-6 and IGF-I. For example, the positive inhibiting effect of ERT on bone turnover in postmenopausal women may partly be mediated though a reduction of IL-6 (38). Fibroblasts synthesize IL-6, and results have shown a stimulating effect of IL-6 on collagen synthesis in skin (8) and tendon (2). Nevertheless, the interaction between IL-6 and estradiol in tendon and muscle collagen turnover remains to be elucidated. In addition, IGF-I stimulates collagen synthesis rate in tendon and muscle (1, 7). However, oral ERT reduces the hepatic synthesis and secretion of IGF-I (6, 28) and lowers the concentration of IGF-I in the interstitial fluid surrounding the patellar tendon (15). Of note, transdermal estradiol patches exert no effect on circulating IGF-I (5, 6), whereas their effect on the local synthesis of IGF-I in the skeletal muscle and tendon tissue remains unknown.

Increases in collagen synthesis rates in tendon and skeletal muscle connective tissue have been observed after acute resistance exercise in some (18, 20) but not all studies (15, 17). The stimulating effect of exercise on collagen synthesis may be explained by increases in local concentrations of IL-6 and IGF-I, which are reported to increase in response to exercise (25, 32) and to have a direct stimulating effect on tendon collagen synthesis (14). In ovariectomized women who use oral ERT, a negative association has been observed between circulating estradiol and the response to exercise in tendon but not in skeletal muscle collagen synthesis rate (15, 18). In support of this, tensile loading of cultured porcine anterior cruciate ligament (ACL) fibroblasts attenuates estrogen-stimu-
lated collagen synthesis (27). Still, it has not been elucidated whether the negative interaction between estradiol and exercise on tendon collagen synthesis observed in oral ERT users is direct or indirectly caused by inhibition of stimulating agents, e.g., IGF-1 and IL-6.

The primary aim of the present study was to determine the short-term effect of transdermal ERT on a marker for type I collagen synthesis, procollagen type I NH2-terminal propedipite (PINP), in tendon and skeletal muscle at rest and in response to exercise. Transdermal estrogen patches were used to bypass the effect oral ERT on the hepatic IGF-1 generation. Therefore, a secondary aim was to elucidate whether transdermal ERT has any influence on local concentrations in IGF-1, but also IL-6 in the interstitial tissue in the skeletal muscle and peritendinous tissue surrounding the patellar tendon.

**MATERIALS AND METHODS**

**Subjects.** Eleven healthy, nonsmoking postmenopausal women (>60 yr) were enrolled in the study, which was approved by the local Ethics Committee of Copenhagen and Frederiksborg Communities (KF 11 2006–2386). The study was carried out at Institute of Sport Medicine, Bispebjerg Hospital, Copenhagen, Denmark. Prior to inclusion, informed written and oral consent was obtained from each subject, in accordance with the Declaration of Helsinki. Each subject underwent a medical evaluation, including routine blood tests, a submaximal bike test, and measurements of height and weight. Exclusion criteria for participation in the study were diabetes, obesity, smoking, skeletal muscle diseases, hypermobility, previous or present knee injuries, medicine with a potential effect on connective tissue, or screening blood samples values outside normal range. Baseline subject characteristics were age, 65 ± 2 yr; weight, 66 ± 4 kg; height, 1.69 ± 0.03 m; body mass index, 24 ± 2 kg/m²; maximal oxygen uptake (V\textsubscript{O\textsubscript{2max}}), 2.10 ± 0.2 l O\textsubscript{2}/min.

**Study design.** The study was designed as a randomized, controlled, crossover study comparing effects of transdermal ERT for 5 days on parameters related to collagen metabolism in tendon and skeletal muscle in postmenopausal women. This subject group was chosen to achieve low baseline values of estrogen in the control period. The subjects were randomized to start with either the ERT period or the control period with 30 days washout between the two experimental periods. During ERT, estrogen patches were applied on the skin in front of the patellar tendons for 5 days. At day 2, in both periods (control and ERT), the subjects performed one-legged resistance exercise to study the effect of exercise on type I collagen synthesis (with or without ERT) by comparing the results from the nonexercised leg (Rest leg) with the leg that had performed exercise (EX leg). At days 3 and 5 (1 and 3 days postexercise), the subjects arrived to the laboratory, and local concentrations of PINP, as a marker for type I collagen synthesis, IL-6, and IGF-1 within the skeletal and tendon were measured by microdialysis technique. Microdialysis catheters were inserted in front of the patellar tendon of each leg after initial preparation of incision sites with local anesthetic (lidocaine 1%) as previous described (26). The inflow tube of the microdialysis catheter was connected to a high-precision syringe pump with an infusion rate of 2 μl/min. The catheters were perfused with a Ringer acetate solution. A sample vial was placed at the end of the outflow tube, and after 30 min perfusion of the catheter, the dialysate was collected in four 1-h periods and stored at −80°C until subsequent analyses. After each hour of collection the weight of the sample vial was measured to check that the outflow rate of dialysate corresponded with the inflow rate of perfusate (120 μl/h). If the weight of the dialysate was below 100 mg, the sample vial was discarded. The collected dialysate was analyzed for PINP, a marker for type I collagen synthesis, and analyzed for IL-6 and IGF-1.

To determine the relative recovery (RR) over the membrane during each dialysate sampling period, the Ringer-acetate solution was mixed with a small amount of radioactively labeled glucose ([3-14]H)glucose in aqueous solution steriapar, Perkin Elmer Life and Analytical Science, Boston, MA; Net 331A, lot 3559–801) (35). The total amount of radioactivity that the patients received was <0.001 mSv. From each dialysate vial 3 μl dialysate was pipetted into a counting vial and mixed with 3 ml scintillation fluid (Ultima Gold, Perkin Elmer) and analyzed in a beta counter. The relative recovery over the microdialysis membranes was 0.32 ± 0.02 and 0.36 ± 0.02 in control tendon and ERT tendon, respectively (P = 0.09) and 0.35 ± 0.01 and 0.34 ± 0.02 in control muscle and ERT muscle, respectively (P = 0.69). The individual relative recovery measured in each sample was used when estimating the interstitial concentrations of PINP, IL-6, and IGF-1.

**Analysis of blood, urine and dialysate.** Urine and blood samples were collected before exercise and in the morning at days 3 and 5. Furthermore blood samples were collected immediately after the end of the exercise protocol. After separation by centrifugation, serum and plasma were stored at −80°C until analysis. All analyses were performed blinded with regard to treatment.

Tendon dialysate analyses for estradiol, estrone, and estrone sulfate and analysis for serum estradiol were quantified by a chemiluminescent competitive immunoassay (Estradiol, Diagnostic Product Corpora- tion, Los Angeles, CA) on Cobas e 601. The detection limit for estradiol was 0.10 nmol/l in serum and <0.40 pmol/l in dialysate. Estrone sulfate was determined since 17β-estradiol has a very short half-life. To prolong the half-life of estrogen in vivo 17β-estradiol can

**Microdialysis measurements.** In the morning at days 3 and 5 corresponding to 24 and 72 h postexercise, the subjects arrived to the laboratory after an overnight fast. Urine and blood samples were collected and a standard protein drink (Fortimel Extra, Nutricia A/S NV, 10 g protein, 14.7 g carbohydrate, 3.5 g fat) was served. Ethylene oxide-sterilized microdialysis catheters with high-molecular-mass cutoff (3,000 kDa, membrane length 30 mm, ID 0.50 mm) were inserted into the vastus lateralis muscle and in the peritendinous tissue in front of the patellar tendon of each leg after initial preparation of incision sites with local anesthetic (lidocaine 1%) as previous described (26). The inflow tube of the microdialysis catheter was connected to a high-precision syringe pump with an infusion rate of 2 μl/min. The catheters were perfused with a Ringer acetate solution. A sample vial was placed at the end of the outflow tube, and after 30 min perfusion of the catheter, the dialysate was collected in four 1-h periods and stored at −80°C until subsequent analyses. After each hour of collection the weight of the sample vial was measured to check that the outflow rate of dialysate corresponded with the inflow rate of perfusate (120 μl/h). If the weight of the dialysate was below 100 mg, the sample vial was discarded. The collected dialysate was analyzed for PINP, a marker for type I collagen synthesis, and analyzed for IL-6 and IGF-1.

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be reversibly metabolized to estrone sulfate by binding of a sulfate group.

Serum and tendon and muscle dialysate were analyzed for PINP using a sandwich ELISA method that purified the alpha-1 chain-specific rabbit antibodies (donated by Teisner B, Dept. of Medical Microbiology, Univ. of Southern Denmark, Odense, Denmark) (26). The within-assay (double determination) and between-assay variation coefficients (CV) were on average 2.2% and 4.9%, respectively.

Plasma IL-6 was measured before and after exercise at day 2 and in the morning at days 3 and 5 was analyzed using an ELISA high-sensitivity quantikine HS ELISA kit (R&D Systems, Minneapolis, MN) after dilution (1:1) (CV, 6.9–7.8%). To minimize the impact caused by the insertion procedure, IL-6 was measured in dialysate collected from 3.5 to 4.5 h after insertion of the catheters (26). IL-6 in the dialysate was analyzed with a DuoSet ELISA Development kit (R&D Systems). Samples for analysis of IL-6 were serially diluted with an appropriate Reagent Diluent (R&D Systems) to obtain values within the dynamic range of the assay. Muscle dialysate was diluted 1:10 and tendon dialysate was diluted 1:5 or 1:3.

IGF-I was determined in serum, and muscle and tendon dialysate by time-resolved immunofluorometric assay (TR-IFMA) after acid-ethanol extraction, as previously described (9). All samples were measured in the same assay run (intra-assay CV < 5%).

Urine collected in the morning at days 2, 3, and 5 was analyzed for degradation products of COOH-terminal telopeptides of type I collagen (CTX-I, Urine CrossLaps ELISA, Nordic Bioscience Diagnostics A/S, Herlev, Denmark) and used as a bone resorption marker. Unfortunately, at day 2, five of the urine samples in the ERT period and four of the urine samples in the control period were missing. Therefore, only CTX-I results from days 3 and 5 of the experimental periods are reported.

Statistical analysis. Data are presented as means ± standard error of the mean SE. Normal distribution was assessed by a normality test (Shapiro-Wilk) and inspection of histogram, and an equal variance test was used to test for equal variance. If the test failed, data were log transformed. Data for serum PINP, plasma IL-6, serum IGF-I, and IGF-I obtained at day 3 and day 5 were analyzed separately using a two-way, repeated-measures ANOVA (2-factor repetition: time and treatment periods). Furthermore, muscle and tendon dialysate parameters (PINP, IL-6, and IGF-I) obtained at day 3 and day 5 were analyzed using a two-way, repeated-measures ANOVA [2-factor repetition: treatment periods and loading (Rest leg vs. EX leg)]. When a significant difference in the ANOVA tests was detected, a post hoc analysis (all pairwise multiple comparison procedure: Holm-Sidak method) was performed. SigmaPlot version 11 was used for the statistical analysis. The level of significance was set at P < 0.05.

RESULTS

Estrogens. Serum estradiol increased significantly following transdermal ERT (P = 0.009, Table 1), whereas in the control period, serum estradiol was predominantly below the detection limit in most of the individuals (Table 1). Irrespective of ERT, the tendon dialysate levels of estradiol and estrone were below the detection level (<0.40 pmol/l), and estrone sulfate was only detectable in ~20% of the samples (data not shown).

Markers for whole body collagen synthesis, and circulating IGF-I and IL-6. Overall, no significant effect of time (P = 0.48) or treatment (control vs. ERT; P = 0.35) in serum PINP was observed. However, the interaction between time and treatment was significant (P = 0.05). Post hoc analysis showed that serum PINP was significantly higher at day 5 of the ERT period compared with day 2 preexercise (P = 0.04), whereas no difference was detected between these time points in the control period (P = 0.72). Furthermore, serum PINP at day 5 was higher in ERT than control at day 5 (P=0.01) (Fig. 1A).

Plasma IL-6 was not significantly changed by ERT (P = 0.70), and no interaction between time and treatment was observed (P = 0.93). However, plasma IL-6 did change over time (P = 0.03). Post hoc analysis showed a significant difference from baseline (preexercise) to immediately postexercise in both groups (ERT: P = 0.001; control: P = 0.004). Furthermore, plasma IL-6 at day 5 was higher than at day 2 before the exercise (P = 0.004) (Fig. 1B).

Serum IGF-I was measured before and after exercise at day 2 and in the morning at day 3 (Fig. 1C). Serum IGF-I was not influenced significantly by treatment (n = 10, treatment P = 0.18, interaction between time and treatment P = 0.98). In contrast, a tendency toward an effect of time was observed (P = 0.06), and post hoc analysis showed a higher serum IGF-I postexercise compared with preexercise (P = 0.02).

Urine-CTX-I was not different between day 3 and day 5 (P = 0.71). Furthermore, CTX-I was not influenced by treatment when urine collected at day 3 and day 5 was analyzed (P = 0.39, interaction between time and treatment, P = 0.64; Fig. 1D).

Marker for local type I collagen synthesis in the skeletal muscle and peritendinous fluid. Peritendinous PINP was not changed by treatment either at day 3 (P = 0.40) or day 5 (P = 0.40) compared

Table 1. Serum estradiol

<table>
<thead>
<tr>
<th>Subjects</th>
<th>D2 pre-EX</th>
<th>D2 post-EX</th>
<th>D3</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
<td>0.11</td>
<td>0.13</td>
<td>0.10</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>7</td>
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<td>9</td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td>11</td>
<td>0.12</td>
<td>0.11</td>
<td>0.14</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE and are serum estradiol levels in nmol/l. ERT, transdermal estrogen replacement period; D2 pre-EX, before exercise at day 2 of the experimental period; D2 post-EX, after exercise at day 2 of the experimental period; D3 and D5, day 3 and day 5 of the experimental period, respectively. ND, not detectable (<0.10 nmol/l). MV corresponds to missing value.
with corresponding time points in the control period (Fig. 2, A and B). Neither was any change in peritendinous PINP detected when samples from the Rest leg were compared with the EX leg at day 3 ($P$ /H11005 = 0.40) or day 5 ($P$ /H11005 = 0.09). No interaction between treatment and loading was observed at day 3 ($P$ /H11005 = 0.76) or day 5 ($P$ /H11005 = 0.94) (Fig. 2, A and B).

At day 3, muscle PINP was not different between ERT and control ($P$ /H11005 = 0.16) or between the Rest leg and the EX leg ($P$ /H11005 = 0.99). Furthermore, no interaction between loading (Rest leg vs. EX leg) and treatment was observed at day 3 ($P$ /H11005 = 0.54) (Fig. 2C). However, at day 5 (72 h postexercise) a significant interaction between treatment and loading was observed ($P$ = 0.008). Furthermore, muscle PINP tended to be higher at day 5 in the ERT period compared with day 5 in the control period ($P$ = 0.053) (Fig. 2D). At day 5 in the control period, the muscle PINP concentration was similar ($P$ = 0.17) in the EX leg compared with the Rest leg, whereas at day 5 in the ERT period, muscle PINP was significantly higher in the EX leg compared with the ERT Rest leg ($P$ = 0.036), but also significantly higher compared with the EX leg in the control period ($P$ = 0.002) (Fig. 2D). Muscle dialysate samples from one subject were omitted due to a relative recovery over the membrane of $<10\%$.

**IL-6 and IGF-1 in the skeletal muscle and peritendinous fluid.** In the peritendinous fluid, neither loading nor treatment affected the IL-6 level at day 3 ($P$ /H11005 = 0.21; loading

![Fig. 1. Procollagen type I NH$_2$-terminal propeptide (PINP) in serum as a marker for type I collagen synthesis in bone (A), plasma IL-6 (B), insulin-like growth factor I (C), and urine concentrations of NH$_2$-terminal telopeptides of type 1 collagen (CTX-I), as a marker for bone resorption (D). ERT, transdermal estrogen replacement therapy. D$_2$ pre, before exercise at day 2 of the experimental period; D$_2$ post, after exercise at day 2 of the experimental period; D$_3$ and D$_5$, day 3 and day 5 of the experimental period, respectively. Values are presented as means ± SE. Two-way ANOVA repeated measurement shows a significant interaction between time and treatment in serum PINP ($P$ = 0.05). Furthermore, a significant effect of time was detected in plasma IL-6 ($P$ = 0.03). No other significant findings were observed.**

![Fig. 2. PINP in the peritendinous tissue (A and B) and interstitial tissue in the skeletal muscle (C and D) after 3 or 5 days of transdermal estrogen replacement therapy (ERT) or control, respectively. Values are presented as means ± SE. Two-way ANOVA repeated measurement shows a tendency toward a difference in muscle PINP between ERT vs. control at day 3 ($P$ = 0.053). Furthermore, for muscle PINP the interaction between treatment and loading (Rest leg vs. EX leg) was significant at day 5 ($P$ = 0.008). Post hoc test showed a significantly higher level in muscle PINP in the EX leg compared with the Rest leg at day 5 during the ERT period (*$P$ = 0.04) and compared with the EX-leg in the control-period (**$P$ = 0.002).**
No significant differences in peritendinous IGF-I or muscle IGF-I were detected between the Rest leg and the EX leg at day 3 (tendon $P = 0.50$; muscle $P = 0.99$) or day 5 (tendon $P = 0.76$; muscle $P = 0.16$). Similarly, no significant difference between ERT and control was observed at day 3 (tendon $P = 0.33$; muscle $P = 0.69$) or day 5 (tendon $P = 0.82$; muscle $P = 0.62$). Furthermore, the interaction between treatment and loading was insignificant at day 3 (tendon $P = 0.81$; muscle $P = 0.66$) as well as at day 5 (tendon $P = 0.77$; muscle $P = 0.91$). One tendon dialysate sample and one muscle dialysate sample were excluded from the analysis of IL-6 and IGF-I as the relative recovery over the microdialysis membrane was below 10%.

**DISCUSSION**

This study investigated acute effects of topical estrogen administration during exercise on collagen synthesis in tendon and muscle in postmenopausal women. The novel finding in the present study was that exercise combined with ERT enhanced muscle PINP significantly compared with in the Rest leg, as well as compared with in the EX leg in the control period. This indicates that estrogen in combination with exercise stimulates collagen synthesis in skeletal muscle in postmenopausal women.

**Effect of ERT on muscle and peritendinous PINP.** The synergistic stimulating effect of exercise and ERT on muscle collagen synthesis may either be a direct or an indirect effect induced by the synergistic stimulating effect of exercise and ERT on muscle growth (18). Myofibrillar muscle protein synthesis was not determined in the present study. However, recently we observed a significant increase in myofibrillar fractional synthesis rate in response to resistance exercise in ovariectomized women using oral ERT, whereas age-matched postmenopausal women not using ERT did not experience a change in myofibrillar fractional synthesis rate (18). The latter observation indicates that the availability of estrogen may be crucial to obtain the beneficial anabolic effect of resistance exercise in postmenopausal women. Furthermore, to achieve an optimal adaptation in the skeletal muscle, an increase in muscle fiber cross-sectional area should be coupled to an associated remodeling of the surrounding collagen-rich connective tissues around the muscle fibers and the muscle fascia. Chronic muscle compartment syndrome is an example of a painful condition where there is a mismatch between the increase in muscle volume and the necessary adaptation of the surrounding muscle fascia (11). Thus the higher muscle collagen synthesis rate in EX leg compared with Rest leg during the ERT period in the present study can therefore be expected to be part of a beneficial adaptation to anabolic exercise within the skeletal muscle. Another possibility is that the increase in collagen synthesis reflects an initiation of a repair process within the skeletal muscle tissue due to muscle damage induced by the strenuous exercise bout (24).

ERT did not stimulate PINP in the peritendinous fluid significantly, despite the fact that the ERT patches were placed on the skin just above the patellar tendon to enhance the local exposure to estrogen. The lack of effect of ERT on tendon collagen synthesis contrasts with earlier findings that showed a higher tendon collagen fractional synthesis rate in oral ERT.

![Fig. 3. Intersitial IL-6 in the peritendinous tissue (A and B) and interstitial tissue in the skeletal muscle (C and D) after 3 and 5 days transdermal estrogen replacement therapy (ERT) or control, respectively. Values are presented as means ± SE.](http://jap.physiology.org/)
users compared with control subjects (15). There may be several methodological and physiological explanations for this disagreement. We have to point out that the tendon dialysate levels of estradiol were undetectable despite the ERT patch. This suggests that the attempt to administer estrogen to the targeted patellar tendon tissue may have been ineffective, which thereby limits the interpretation of the tendon data. However, the microdialysis catheters were inserted peritendinously, and accordingly, the dialysate originated from the peritendinous tissue and not from the tendon itself; clearly this reduces the possibility of determining events within the tendon. Furthermore, PINP only represents a marker for type I collagen synthesis. Although type I collagen is the major component of the tendon extracellular matrix (ECM), in vitro findings suggest that estradiol stimulates type III collagen synthesis, but not type I collagen synthesis (21). Thus the higher tendon collagen fractional synthesis rate observed in the previous study in postmenopausal women using oral ERT compared with control may be a result of an increased type III rather than type I collagen synthesis (15). An additional methodological concern is that in the ERT period we observed a tendency toward a greater loss of glucose over the microdialysis membrane from the perfusate to the peritendinous area compared with in the control period ($P = 0.09$). This observation indicates an enhanced local tissue blood perfusion, which will enhance the disappearance rate of glucose as well as PINP away from the peritendinous area, thereby resulting in an underestimation of the peritendinous concentration of PINP during the ERT period. Finally, the differential findings between the previous (15) and presents findings may be explained by the circulating levels of estradiol, which were markedly higher in the aforementioned study (range 0.2–0.6 nmol/l) (15) than in the present study (<0.3 nmol/l). In addition, we only studied acute effects of transdermal ERT (5 days) compared with long-term use of ERT in the previous study (17 yr) (15). To conclude, peritendinous PINP was not significantly higher in the ERT period as hypothesized based on earlier findings (15). This observation may be explained by physiological and methodological limitations in the study design (sample size, and dose and duration of ERT) and by changes in the diffusion over the microdialysis membrane induced by ERT.

The effect of strenuous exercise on peritendinous PINP was measured 24 and 72 h postexercise. We observed no significant change in peritendinous PINP in the EX leg compared with the Rest leg regardless of the strenuous exercise protocol. No significant change in tendon collagen synthesis in postmenopausal women in response to exercise has previously been reported (15). These observations may point to a reduced sensitivity to anabolic stimuli in tendon tissues, and thereby a reduced ability to adapt to training in postmenopausal compared with young women (3, 22, 36, 37). If the sensitivity to anabolic stimuli is reduced in postmenopausal women this would suggest that a more strenuous exercise would enhance tendon collagen synthesis. Nevertheless, it should be noted that the strenuous exercise bout in the present study was already markedly above what is normally performed in this age group. The observation of no response to exercise in tendon collagen synthesis may also be explained by the chosen time points for measurements. Based on earlier observations in young men (26, 30), we chose to study the response to loading 24 and 72 h postexercise. Thus we cannot exclude that by including more time points we would have been able to detect change in tendon collagen synthesis in response to loading.

**Effect of ERT on IGF-I and IL-6.** We measured circulating and local concentrations of IGF-I and IL-6, which both have a stimulating effect on collagen synthesis (1, 2, 7, 25). In contrast to oral ERT, transdermal ERT does not reduce circulating IGF-I (5), which was confirmed in the present study. Similarly, transdermal ERT reduced local IGF-I neither in tendon nor muscle, in contrast to earlier findings in young women where estrogen administration lowered circulating levels of IGF-I markedly and at the same time reduced tendon collagen synthesis (16, 17). Thus the present findings indicate that the observed effects on muscle collagen synthesis were a result of estrogen itself and not an indirect effect of ERT on the IGF-I level within the muscle.

Circulating IGF-I was higher in the morning at day 3 compared with day 2 preexercise when data from both treatment periods were pooled. We do not know whether local IGF-I also was higher at day 3 compared with day 2 or whether tendon and muscle collagen synthesis rates were changed. Nevertheless, serum PINP remained unchanged during this time period, suggesting that the change in circulating IGF-I probably did not have a major physiological impact on the collagen synthesis rates within the body. Acute increases in serum, muscle (34), and peritendinous (25) IL-6 have been reported after exercise. After a 36-km run, a more than 100-fold increase in peritendinous IL-6 was observed, and the level remained significantly elevated for at least 48 h postexercise compared with preexercise (25). In the present study, circulating IL-6 was also significantly higher immediately after exercise compared with before exercise. Nevertheless, we observed no difference in local IL-6 in the skeletal muscle tissue and the peritendinous area 24 and 72 h postexercise when comparing the Rest leg and the EX leg. This may be explained by the fact that the present exercise bout was not as strenuous as a 36-km run (25). Nevertheless, local IL-6 may have been enhanced immediately after exercise. Unfortunately, the local response to exercise in IL-6 was not measured at day 2 postexercise. We cannot rule out that locally, IL-6 was increased in both the EX leg and Rest leg subsequently to the systemic increase in circulating IL-6 postexercise. However, previous results have shown that the local increase and concentration of IL-6 in the peritendinous tissue after running is markedly higher than the changes in circulating IL-6 (25), which suggests that the local response to exercise in the EX leg compared with the Rest leg is not overruled by the systemic changes in IL-6. One reason for not inserting catheters before and after the exercise at day 2 in addition to the catheters at days 3 and 5 was that we did not want to enhance the risk of an effect of repeated insertion of catheters. Still, we cannot exclude that the enhanced circulating IL-6 at day 5 may be related to a delayed effect of the insertion of the catheters at day 3 (23).

In bone, estrogen suppresses IL-6 expression and thereby counteracts the IL-6-mediated increase in osteoclast activity and bone loss (12, 13, 38). In contrast, IL-6 seems to have a beneficial role in other collagen-rich tissues. In the skin, IL-6 seems to be an important paracrine/autocrine regulator of dermal fibrotic repair by increasing the production of collagen and glycosaminoglycans (8). Furthermore, IL-6 has previously been shown to increase tendon synthesis in healthy tendons and to be important for healing of injured tendons and ligaments.
(2). We hypothesized that if transdermal ERT reduced local IL-6, similarly to what has been observed in bone, we would expect that collagen synthesis in the skeletal muscle and tendon would be lower during the ERT than control period. In the present study, peritendinous IL-6 did not seem to be influenced by ERT. In contrast, interstitial IL-6 in skeletal muscle tended to be lower during the ERT period as hypothesized (day 3, \( P = 0.07 \); day 5, \( P = 0.13 \)). Nevertheless, the stimulating synergistic effect of ERT and exercise on muscle collagen synthesis may have overruled the potentially negative impact of a reduced IL-6 level on collagen synthesis within the muscle.

**Effect of transdermal ERT on whole body collagen turnover.** Serum PINP is predominately a marker for type I collagen synthesis in bone. Overall, serum PINP was not different during the ERT period compared with the control period, but a significant interaction between time and treatment was observed, and the results indicate a higher bone collagen synthesis rate in the end of the ERT period compared with day 2 of the ERT period. No significant effect of ERT on bone breakdown (urine CTX-I) was detected. These results disagree with the majority of studies in the literature, which report that ERT reduces collagen turnover and bone mass in postmenopausal women (4). Nevertheless, our observations may be related by temporal changes in collagen turnover in the initial phase of ERT use before the beneficial effect of ERT on bone mass is achieved (10) or to an insufficient statistical power related to the number of subjects included in the present study. Alternatively, the increase in circulating estradiol was insufficient even though we applied double the recommended dose of transdermal ERT (2 times 50 μg/24 h). The patches were placed on the skin in front of the patellar tendon. A higher circulating level of estradiol would probably have been achieved if the patches had been applied on the skin at the lower back or thigh where the blood flow to the skin area is greater than in the knee area. Furthermore, estradiol from the patches may have been metabolized and/or stored in the fat pad close to the patellar tendon before reaching the circulation. Our observations underline the importance of a suitable application site to reach the desired effects on bone.

**Conclusion.** Short-term transdermal ERT combined with resistance exercise enhanced PINP within the skeletal muscle compared with ERT or exercise alone. The result indicates that ERT combined with exercise stimulates collagen synthesis in human skeletal muscle, which may be important for repair of muscle damage or remodeling of the connective tissue within the skeletal muscle after strenuous anabolic exercise.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**