Effects of Estrogen Replacement and Lower Androgen Status on Skeletal Muscle Collagen and Myofibrillar Protein Synthesis in Postmenopausal Women

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Our aim was to determine synthesis rate of myofibrillar and collagen proteins in 20 postmenopausal women, who were either nonusers (Controls) or users of estrogen replacement therapy (ERT) after hysterectomy/oophorectomy. Myofibrillar and muscle collagen protein fractional synthesis rate (FSR) were determined in a nonexercised leg and 24 hours after exercise in the contralateral leg. A significant interaction between treatment and mechanical loading was observed in myofibrillar protein FSR. At rest, myofibrillar protein FSR was found to be lower in ERT users than in Controls. Exercise enhanced myofibrillar protein FSR only in ERT users. Similarly, muscle collagen FSR tended to be lower in ERT users compared with Controls. In ERT participants, the androgen profile was reduced, whereas estradiol and sex hormone–binding globulin were higher. In conclusion, at rest, myofibrillar protein FSR was lower in hysterectomized/oophorectomized women using ERT compared with healthy postmenopausal women. Nevertheless, resistance exercise in combination with ERT seems to have a counteracting effect on myofibrillar FSR in hysterectomized/oophorectomized women.

Key Words: Human—Hormone replacement therapy—Aging—Extracellular matrix.

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Sarcopenia is characterized by a decline in skeletal muscle protein mass, a relative accumulation of intramuscular connective tissue and fat, and a reduction in muscle strength and power (1). These changes contribute to increased risks of comorbidities related to falls in and diminished ability to perform physical daily living activities (2). The mechanisms behind muscle loss and collagen accumulation are receiving increasing attention and need further elucidation. In women, the loss of muscle mass and strength are accelerated around the time of menopause (3–7), where concentrations of the ovarian hormones estradiol and progesterone decrease. Estrogen receptors (α and β), which act as ligand-activated transcription factors, are known to be expressed and present at protein levels in human striated muscles (8). Similarly, androgen receptors have been localized in connective tissue cells and striated muscle cells (9). This suggests that sex hormones may influence muscle protein turnover. This is further supported by a recent systematic meta-analysis, which concluded that postmenopausal women on hormone replacement therapy (HRT) were stronger than nonusers of HRT (10).

Animal studies and muscle cell culture studies have shown diverging results when it comes to the effect of estrogen on muscle mass, muscle protein synthesis, and degradation (11–16). Cross-sectional data have shown a positive relation between serum estradiol concentration and muscle mass and strength in postmenopausal women (17). However, no final causal link has been established between age-dependent decline in estrogen and loss of strength and muscle mass. Moreover, a study by Ronkainen and colleagues (18) including 15 monogygotic postmenopausal twin pairs discordant for HRT strongly supports a positive effect of HRT on skeletal muscle mass and muscle function. In line with these observations, a number of intervention studies support a positive effect of HRT on skeletal muscle mass in postmenopausal women (19–23), whereas others report no significant effect (24–26). The discrepancy in the literature may be explained by several methodological differences between the studies, such as the hormonal status of the women, the age of the participants, and the type and dose of HRT.

Recently, Dieli-Conwright and colleagues (27) reported that the myogenic gene expression profiles at rest and in response to resistance exercise seem to be more anabolic in postmenopausal women who were given HRT compared with controls. In rats, estrogen administration enhances satellite cell activation following exercise (28,29), and estrogen has a
positive influence on muscle mass during loading (12–14). These observations suggest a positive interaction between estrogen and exercise on skeletal muscle protein turnover. Based on this, we hypothesized that the blunt response to resistance exercise training, which has been observed in elderly participants compared with young participants (30) and in postmenopausal women compared with age-matched men (31–33), may be counteracted by enhancing circulating estrogen levels in elderly women to a level comparable with young women.

Insulin-like growth factor-I (IGF-I) is positively associated with muscle collagen fractional synthesis rate (FSR) (34). However, oral administration of estrogen has been reported to reduce the levels of both circulating IGF-I and local tissue IGF-I (35,36). Based on this, we hypothesized that oral estrogen administration by lowering the level of free IGF-I indirectly inhibits muscle collagen FSR, which may reduce accumulation of muscle connective tissue in elderly women.

To study the effect of estrogen on skeletal muscle protein turnover, we included both women with high and low levels of estradiol and measured collagen and myofibrillar protein synthesis rates at rest and after exercise. The low and high levels of estradiol were achieved by recruiting postmenopausal women with a natural low level of estradiol and age-matched women who were users of estrogen replacement therapy (ERT) after hysterectomy/oophorectomy. The latter group was chosen because we wanted to study the isolated effect of estrogen replacement instead of the combined effect of estrogen and gestagens in the most common HRT products.

**Materials and Methods**

**Design**

A cross-sectional design was used to determine differences in skeletal muscle protein FSR in Controls and ERT users measured by a stable isotope technique. One leg was exercised while the other leg rested. Measurements of skeletal muscle protein FSR in both legs were performed 24 hours after the unilateral exercise. Thereby, results from one leg represented resting conditions, whereas the other leg represented the response to exercise. In the present study, tendon collagen FSR, tendon fibril characteristics, and tendon biomechanical properties were also measured. These results have been published previously (35). Participant characteristics will be repeated in the present article to illustrate the comparability between the two groups in body composition, physical activity level, and fitness status.

**Participants**

Twenty healthy postmenopausal women participated in the study. They were all nonsmokers and had no history of metabolic disorders or orthopedic leg injuries. Written informed consent was obtained from the participants. The protocol was approved by a local ethical committee in Copenhagen, Denmark (KF-01-032/04).

Many hysterectomized women receive ERT containing only estradiol instead of HRT, which contains both estradiol and one kind of synthetic progesterone and which complicates identification of the distinct effects of estradiol and the synthetic progesterone. Therefore, in the present study, we aimed at including hysterectomized women who by use of ERT had a circulating level of estradiol comparable to that of young women. Ten women were long-term ERT users following hysterectomy (treatment for 16.1 ± 3.0 years [mean ± standard error of the mean], range 2–30 years). Nine of the 10 participants had undergone oophorectomy at the same time as hysterectomy. All participants were supplemented daily with 2 mg oral 17β-estradiol produced by different companies (n = 5, Oestradiol; n = 3, Oestrofem; n = 1, Femestane; or n = 1, Pygynon). The remaining 10 postmenopausal women (Controls) had not been menstruating for the last 7.2 ± 1.4 years (range 2–15 years). Preferentially, a control group with hysterectomized/oophorectomized women without ERT would have been optimal but that was not logistically possible. In Denmark, almost all hysterectomized women use some kind of HRT. Therefore, similar-aged postmenopausal women with intact organs and naturally low estradiol levels were used as Controls (C). Seven years earlier one control participant used ERT for less than 3 months. Otherwise, none of the Controls had used ERT during their postmenopausal periods. To maximize the estrogen difference (endogenous secreted and exogenous administrated estrogens) between the groups during the period in which samples were obtained, the ERT users were tested in the early hours after the last pill ingestion. The two groups were otherwise comparable based on age, height, weight, body mass index, and body composition quantified by dual-energy x-ray absorptiometry (Table 1).

**Screening**

All participants visited the laboratory 1–3 weeks before the study to receive detailed information about the experiment.

### Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 10)</th>
<th>ERT Users (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>60 ± 4</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64 ± 4</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 6</td>
<td>162 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 2.4</td>
<td>24.3 ± 3.2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34 ± 5</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>43 ± 4</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>PAL</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>5 RM&lt;sub&gt;estimated&lt;/sub&gt; (kg)</td>
<td>31 ± 9</td>
<td>27 ± 9</td>
</tr>
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</table>

**Note:** Values are means ± standard error of the mean. BMI = body mass index (weight/height²); ERT = estrogen replacement therapy users; LBM = lean body mass; PAL = physical activity level (daily EE<sub>estimated</sub>/basal metabolic rate<sub>estimated</sub>); RM = repetition maximum during one-legged dynamic knee extension strength test (kg). The participant characteristics are repeated from the previous article from this study (35).
and to undergo a health examination. Furthermore, blood samples were taken and analyzed for hemoglobin, serum ferritin, serum transferrin, serum leucocytes, serum thyroid-stimulating hormone, plasma creatinine, plasma cholesterol, plasma C-reactive protein, plasma aminotransferase, plasma aspartate aminotransferase, plasma alkaline phosphatase, and plasma albumin. All results were within the normal range (data not shown).

Daily physical activity level was assessed by a validated questionnaire developed by Aadahl and Jørgensen (Table 1; (37)). One-legged five repetition maximum was measured in a Technogym leg extension R.O.M machine. Physical activity level and dynamic muscle strength (five repetition maximum) were not significantly different between the two groups (Table 1).

**Experimental Protocol**

Participants were instructed to avoid strenuous physical activity for at least 2 days before and during the experiment. Weighed dietary records were obtained 2 days before and on the exercise day. No difference between the groups was observed in energy intake or macronutrient composition (all p > .20; Table 2).

At Day 1, the participants performed 10 sets of 10 repetitions of one-legged knee extension at 10 repetition maximum. A new set was started every 3 minutes. Participants fasted overnight (12 hours) and reported to the laboratory at 8 AM the following day. Upon arrival, two cannulae were inserted into veins on opposite forearms, one for tracer infusion and the other for blood sampling. Blood samples were obtained for measurement of background isotope enrichments and hormone concentrations. Afterward, the isotope protocol was initiated to measure muscle collagen FSR and myofibrillar protein FSR by infusion of a flooding dose of stable isotopelabeled amino acids. After 2 hours, muscle biopsies were taken (for details about the procedures, see later). Muscle FSR was measured 24 hours postexercise because earlier findings in men have observed the highest response to acute exercise in myofibrillar FSR and muscle collagen FSR at that exact time (38).

**Myofibrillar Protein and Muscle Collagen FSRs**

Measurement of myofibrillar protein FSR was performed according to previously applied approaches (38,39). Briefly, a skin biopsy (10 mg) was obtained under local anesthesia (lidocaine 1%) from the posterior hip, just below the waist, for determinations of background enrichment of \([^{13}C]\)-proline. A flooding dose of proline (3.75 g total; 0.75 g labeled L-\([^{13}C]\)-proline >99 atoms\% \([^{13}C]\)-proline; Cambridge Isotope Laboratories, MA), 3.0 g L-\([^{13}C]\)-proline [AppliChem, Darmstadt, Germany]) mixed in 0.9% NaCl was infused intravenously over 3 minutes. Blood samples were drawn at 0, 10, 20, 30, 45, 60, 75, 90, 120, and 150 minutes after flooding to determine the area under the plasma \([^{13}C]\)-proline enrichment curve (Figure 1). Two hours after the proline flood, muscle biopsies were taken from the vastus lateralis (50–100 mg) of each leg after having prepared incision sites with local anesthetic (lidocaine 1%). The biopsies were obtained using a 5 mm Bergström needle with suction. Biopsies were freed from visible adipose and connective tissue and blood, frozen in liquid nitrogen, and stored at −80°C until further analyses. The precise time point for each biopsy was noted and used in the calculation of FSR for each leg.

**Proline enrichment (%)**

*Figure 1. Plasma enrichment of \([^{13}C]\)-proline after flooding dose of 1\(\text{-}\)[\([^{13}C]\)]-proline (tracer-to-trace ratio) in women, who use estrogen replacement therapy (ERT) and in women, who do not use ERT (Controls).*
fractions were then precipitated and washed with ethanol, hydrolyzed in 6 mol/L HCl at 110°C overnight (4). Before derivatization as their N-acetyl-n-propyl (NAP) ester (41), the amino acids were purified over disposable columns using resin (Acidic cation exchanger, Dowex AG-50W; Bio-Rad, Sundbyberg, Sweden). Tracer incorporation was determined by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS; Delta Plus XL; Thermo Finnigan, Bremen, Germany) using a CP-SIL 19 CB 60 m x 0.32 mm, coating 0.25 µm column (ChromPack; Varian, Palo Alto, CA).

Free amino acid labeling of proline in the blood.— Plasma proline was prepared as previously described and analyzed as its tert-butyl-dimethyl-silyl (t-BDMS) by gas chromatography–mass spectrometry (GC, Trace GC 2000 series, MS, Automass Multi; Thermo Quest Finnigan, Paris, France; (42,43)) using a CP-SIL 8, CB low Bleed, 30 m x 0.32 mm, coating 0.25 µm column (ChromPack). Plasma 13C-proline enrichment was assumed to represent isotopic enrichment in the true precursor pool, prolyl-transfer RNA.

Calculations.—The rates of myofibrillar and collagen protein synthesis were calculated using standard equations (44); thus, myofibrillar protein and collagen FSR (%/hour) = ΔE(proline) / E(proline) ∙ 1/ t ∙ 100, where ΔE(proline) is the change in enrichment of proline (tracer:tracee) in the muscle tissue samples compared with the enrichment in the initial skin biopsy (assuming basal tissue labeling to be identical to that at the natural abundance in the initial skin biopsy). E(proline) is the mean enrichment over time of the precursor for protein synthesis (taken as venous plasma proline labeling), and t is the tracer incorporation time (hours). No difference between the enrichment curves between the two groups was observed (Figure 1).

Blood Analysis

The procedures for the analyses and the results for serum estradiol, serum testosterone, serum 4-androstenedione, serum IGF-I, and serum insulin-like growth factor binding protein (IGFBP)-1 and IGFBP-3 have been published previously and will only be referred to (35). Sex hormone–binding globulin was analyzed in serum by a commercial ELISA-kit (ALPCO diagnostics, Salem, MA). Serum-free testosterone was calculated using a method validated by Vermeulen and colleagues (45) based on the measured concentration of total testosterone and sex hormone–binding globulin. Free androgen index was calculated as serum total testosterone divided by serum sex hormone–binding globulin x 100.

Statistical Analysis

Data are presented as means ± standard error of the mean. Repeated measures analysis of variance with rest or exercise as within-participants factor and ERT or C as between-participants factor was used to compare myofibrillar FSR and muscle collagen FSR between ERT and Controls in the nonexercised leg and in the exercised leg. Student’s paired t test was used to identify differences in myofibrillar protein FSR between the exercised and nonexercised leg within each group. Student’s unpaired t test was used to identify differences of the remaining parameters between the groups. The level of significance was set at p < .05. The statistical analyses were performed using the statistical software packages: Sigma Plot version 11 (two-way repeated measures analysis of variance) and Prism version 4.01 (GraphPad, San Diego, CA; the remaining tests).

RESULTS

Myofibrillar Protein FSR

Interaction between treatment (rest vs exercise) and groups (ERT vs Controls) was significant (p < .01). At rest, myofibrillar protein FSR was lower in ERT users compared with Controls (p = .015; Figure 2). In contrast, no difference was observed between the groups 24 hours after exercise (p = .93; Figure 2). In ERT participants, myofibrillar protein FSR was significantly higher in the exercised leg compared with the nonexercised leg (p = .01), whereas in Controls, no significant difference in myofibrillar protein FSR was observed between the legs (Figure 2).

Muscle Collagen FSR

Two-way analysis of variance showed a tendency toward lower muscle collagen FSR in general (p = .11, Figure 3). Furthermore, muscle collagen FSR was significantly higher in the exercised leg compared with the nonexercised leg (p = .025). Nevertheless, no significant statistical interaction was observed (p = .6).

Figure 2. Myofibrillar protein fractional synthesis rate at rest and 24 hours after exercise in women, who use estrogen replacement therapy (ERT), and in women, who do not use ERT (Controls). Values are means ± standard error of the mean. Two-way analysis of variance with repeated measures in one factor: ERT users versus Controls, p = .18; rest versus exercise, p = .34; and interaction between rest or exercise and Controls or ERT, p = .005. *p = .015 ERT versus Controls, #p = .010 ERTrest versus ERTexercise.
The present study was conducted to compare muscle protein synthesis rates at rest and in response to resistance exercise in hysterectomized/oophorectomized women using ERT to enhance circulating concentrations of estradiol and healthy postmenopausal women with a low level of circulating estradiol.

Myofibrillar Protein Synthesis in the Nonexercised Leg

In the present study, a lower myofibrillar protein FSR was observed at rest in ERT participants than in Controls. In line with this, in vitro findings have shown a lower protein synthesis rate after administration of estradiol in myoblast and myotube cultures (49). Furthermore, mixed muscle protein synthesis rate is lower in young estradiol-substituted ovariectomized (OVX) compared with OVX rats (16). The knowledge about the effect of estrogen administration on muscle protein turnover in humans is sparse. Findings by Dieli-Conwright and colleagues (27) indicate that HRT has a positive influence on the expression of myogenic genes and suggest that HRT may help to preserve muscle mass. However, Toth and colleagues (25) reported no difference in whole-body protein breakdown, synthesis, and oxidation after 2 months of HRT supplementation. Furthermore, Maura and colleagues (50) observed no effect of estrogen delivery on whole-body protein synthesis in the hypogonadal women. The latter findings appear directly in contrast to our observations but may be explained by several factors. First, hormones are known to affect various tissues differently. When determined at the whole-body level, the effect of HRT on skeletal muscle protein may be diluted and thus nondetectable. Second, the women in the previous studies were using HRT, which contains synthetic progesterone besides estradiol (25). The role of the different types of synthetic progesterone in HRT on muscle protein turnover is not clarified, but most likely, they have variable androgenic effects (39). Finally, the women in the previous studies were not hysterectomized, which by itself changes the hormonal profile.

The hormonal profiles in the two groups in the present study were different not only in estrogen but also IGF-I, androstenedione, free testosterone, and calculated androgen index. This observation may be a consequence of either the hysterectomy/oophorectomy or an effect of the estrogen administration, which will be discussed in the following. Serum IGF-I was lower in the ERT group. Suppression of serum IGF-I by oral estradiol administration has been observed by others (51,52). Previous results support that IGF-I induces muscle hypertrophy in animals (53,54). Furthermore, an enhanced net uptake of amino acids has been observed in humans, indicating a stimulation of mixed muscle protein synthesis by IGF-I (55). However, the latter results may be related to other proteins than myofibrillar proteins because the majority of studies in the literature does not support a stimulating effect of IGF-I on skeletal muscle growth in healthy human adults (34,56–60). Therefore, in the present study, we do not regard the difference in IGF-I

Blood Parameters

In the control group, serum estradiol was below the detection level in 8 of 10 participants (<0.10 nmol/L) and above the detection limit in the remaining two Controls (0.11 and 0.21 nmol/L (35)). In the ERT group, serum estradiol was above detection level in all participants (0.35 ± 0.04 nmol/L, range 0.18–0.53 nmol/L (35)).

In the ERT group, serum sex hormone–binding globulin was significantly higher, whereas serum IGF-I (35), serum 4-androstenedione, calculated free androgen index, and s-free testosterone were significantly lower compared with Controls (Table 3). A tendency toward a lower s-testosterone was observed in the ERT participants as compared with Controls (p = .08; (35)). In contrast, serum IGFBP-1 and IGFBP-3 did not differ significantly between the groups (35).

**DISCUSSION**

Menopause has in several studies been associated with an accelerated muscle loss and muscle strength (3,6,7), which has been related to changes in the hormonal profile during aging, for example, the decrease in circulating estrogen, IGF-I, and testosterone (10,46). ERT and resistance exercise training are suggested as effective intervention strategies to prevent muscle loss and thereby the risk of fall-related fractures and decline in muscle function (10,47,48). The

<table>
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<th>Table 3. Blood Parameters</th>
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<tr>
<td>Serum</td>
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<td>----------------------------</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
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<tr>
<td>Free androgen index</td>
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<tr>
<td>4-androstenedione (nmol/L)</td>
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<td>SHBG (nmol/L)</td>
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Notes: Values are means ± standard error of the mean. ERT = estrogen replacement therapy users. SHBG = sex hormone-binding globulin.

*p < .05, **p < .01 ERT users versus Controls.
between the groups to have a major impact on the observed myofibrillar protein FSR.

In the present study, the androstenedione concentration was significantly lower in the ERT group. Androstenedione, as a testosterone prohormone, has been marketed as testosterone-enhancing and muscle-building nutritional supplements for the past decades. However, as reviewed by Brown and colleagues (61), there is no convincing evidence for any anabolic effect on muscle mass and strength in healthy individuals introduced by an enhanced concentration of androstenedione (62). Still, we cannot exclude that the present difference in androstenedione between groups could have influenced the results and partly explain the lower myofibrillar protein synthesis rate in the ERT group at rest.

An approximately 50% lower estimated androgen index and free testosterone were observed in the hysterectomized ERT participants compared with Controls. Earlier observations confirm that hysterectomy reduces serum-free testosterone (62). Since it is well known that testosterone stimulates myofibrillar protein synthesis, the lower myofibrillar protein synthesis rate at rest in the ERT group is most likely explained mainly by the hormonal differences in free testosterone induced by the hysterectomy/oophorectomy. In support, low androgen availability is associated with a lower myofibrillar protein FSR.

Androstenedione, as a prohormone, reduces the availability of free amino acids for synthesis by an improved insulin inhibition of myofibrillar protein breakdown. To sum up, the lower synthesis rate at rest in hysterectomized/oophorectomized women using ERT may be caused directly by the enhanced concentration of circulating estradiol and/or indirectly by the changes in the androgen profile.

The lower myofibrillar synthesis rate observed at rest in the postabsorptive phase in hysterectomized/oophorectomized women using ERT does not exclude a muscle-preserving effect of estrogen, as suggested in previous studies (19–23). Estrogen supplementation may have an even more pronounced inhibiting effect on myofibrillar protein breakdown. In line with this hypothesis, earlier findings indicate that ERT counteracts increase in myofibrillar protein breakdown rate at menopause (23,63). Furthermore, ERT in combination with anabolic stimuli, such as feeding and mechanical loading, may counteract the negative effect of hysterectomy/oophorectomy and/or ERT on myofibrillar protein FSR as discussed in the following paragraph.

**Myofibrillar Protein Synthesis in the Exercised Leg**

In contrast to Controls, myofibrillar protein synthesis rate was significantly higher in the exercised leg compared with the nonexercised leg in women using ERT, supporting the observation already discovered in young women (64). The latter observation may support a synergistic interaction between estrogen and exercise on myofibrillar protein synthesis in skeletal muscle. In line with this, HRT has been shown to enhance the anabolic stimulating effect of exercise on transcription of myogenic genes in postmenopausal women (27). Furthermore, in animals, it has been observed that estrogen stimulates muscle growth and muscle protein rebuilding after mechanical loading (12–14). Taken together, it can be hypothesized that ERT enhances the sensitivity to anabolic stimuli and thereby counteract the negative consequences of the hysterectomy/oophorectomy on myofibrillar protein FSR. In line with this hypothesis, cross-sectional data have shown a positive association between circulating estradiol and muscle quality (muscle force per muscle cross-sectional area). In contrast, a high concentration of estradiol within the skeletal muscle in postmenopausal women compared with premenopausal women was associated with accumulation of fat within the muscle tissue, resulting in a decline in muscle quality. These findings indicate that circulating estradiol and local synthesized estradiol have differential effects on muscle quality in postmenopausal women (4).

In Controls, resistance exercise did not lead to any significant difference in myofibrillar protein FSR 24 hours postexercise. The timing of the measurements of myofibrillar protein FSR was chosen based on an earlier study in young male participants, where a peak in myofibrillar protein FSR in response to the acute exercise was observed 24 hours postexercise (38). The time pattern in the response to exercise may differ in postmenopausal women compared with young men. Furthermore, the FSR responsiveness to exercise in Controls and ERT participants may demonstrate a divergent temporal response pattern not reflected by the state at 24 hours. Therefore, we cannot exclude that myofibrillar protein FSR may have been significantly enhanced in Controls at an earlier point after exercise. Nevertheless, the observation of no significant difference in myofibrillar protein FSR in Controls between the nonexercised and exercised leg 24 hours postexercise suggests a blunted response to the anabolic stimulating effect of exercise compared with young women (64) and men (38). Similarly, the capacity for muscle hypertrophy in response to resistance training has been reported to be reduced in elderly women compared with men (31). After an 11-month weight-bearing exercise training program (walking and jogging), it was concluded that HRT did not augment the gains in muscle strength beyond those that occurred in response to exercise alone (26). Nevertheless, the latter study was not designed to induce muscle hypertrophy. Based on our results, it would be interesting to study the effect of ERT on the response to a resistance exercise training program designed to enhance muscle mass.

**Muscle Collagen Protein FSR**

Whereas the myofibrillar proteins are responsible for the contractile capability of the muscle, the muscle connective
tissue is essential for force transmission and stability of the skeletal muscle structure (65). In the present study, we observed a tendency toward a lower muscle collagen FSR in ERT participants compared with Controls ($p = .11$). An inhibiting effect of estradiol administration on muscle collagen synthesis is observed in vitro (66) and in animal experiments (12). In OVX rats supplemented with estradiol, a lower expression of type I collagen and a lower percentage of connective tissue have been observed compared with OVX rats (12). Similarly, reduced collagen content in the pelvic supporting connective tissue has been observed in postmenopausal women using ERT participants compared with Controls (67).

The inhibiting effect of ERT on muscle collagen FSR may be indirect. Serum estradiol correlated negatively with IGF-I, which has been reported to be positively associated with muscle collagen FSR in humans (34) and to have a stimulating effect on collagen synthesis in animals (68). Therefore, it can be hypothesized that the observed tendency toward a lower muscle collagen protein FSR in ERT users at rest may be related to a low availability of IGF-I. In perspective, if ERT by inhibiting muscle collagen FSR hinders the relative accumulation of connective tissue in the skeletal muscle during aging, it may help to reduce the age-related reduction in muscle quality and muscle function during physical activities in everyday life.

Muscle hypertrophy brings on a need for remodeling of the connective tissue in the endo-, peri-, and epimysium as well as in the muscle fascia. Therefore, the overall higher synthesis rate of muscle collagen proteins in the exercised leg compared with the nonexercised leg observed in the present study can be regarded as beneficial for adaptation of training in postmenopausal women.

We measured the weighted mean synthesis rate of the total collagen protein pool in the skeletal muscle including all collagen proteins (immature [soluble] as well as mature [insoluble]) in the protein fraction. In a previous study using a similar isolation protocol as in the present study, muscle collagen synthesis rate in young sedentary men at rest in the fasted state ($0.8\%$/hour) was akin to the synthesis rate in Controls ($0.7\%$/hour) (69). Furthermore, a significant increase in muscle collagen protein synthesis rate (2.1 ± 0.2-fold) was reported in the early hours (30 minutes–3 hours) after an exercise protocol comparable to the protocol used in the present study (knee extensions, 10 sets of 8 repetitions at 70% of 1 repetition maximum (69)). We observed only a $0.5 ± 0.3$-fold (mean of all participants) higher muscle collagen FSR 24 hours after exercise (69). Miller and colleagues (38) reported the greatest muscle collagen FSR 6 and 24 hours after acute exercise (no significant difference between the two time points). Taken together, these observations may reflect a blunted response to exercise in postmenopausal women compared with young men (38,69) caused by aging or gender, as in the contractile skeletal muscle tissue as suggested previously. Another explanation may be that the need for remodeling of the muscle connective tissue is diminished in the elderly women caused by a reduced muscle hypertrophy response.

**Conclusion**

A lower myofibrillar protein FSR and a tendency toward a lower muscle collagen FSR were observed in hysterectomized/oophorectomized women using ERT compared with nonhysterectomized postmenopausal women not using ERT. Furthermore, an estradiol level comparable to young women was associated with enhanced synthesis rates of myofibrillar proteins in the exercised leg compared with the nonexercised leg. In contrast, in Controls, myofibrillar protein FSR was not significantly influenced by the anabolic stimuli induced by exercise. The latter observations may indicate that ERT enhanced the sensitivity of the anabolic response to resistance exercise and thereby counteracts the lower myofibrillar protein FSR in hysterectomized/oophorectomized women at rest.

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**References**

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