

Influence of Sex and Estrogen on Musculotendinous Protein Turnover at Rest and After Exercise

Mette Hansen¹ and Michael Kjaer²

¹Section of Sport Science, Department of Public Health, Aarhus University, Aarhus, Denmark; and ²Institute of Sports Medicine, Bispebjerg Hospital, and Centre of Healthy Aging, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

HANSEN, M. and KJAER, M. Influence of sex and estrogen on musculotendinous protein turnover at rest and after exercise. *Exerc. Sport Sci. Rev.*, Vol. 42, No. 4, pp. 183–192, 2014. *Women differ from men with regard to muscle and tendon, most likely because of sex differences in estrogen. The present experimental findings suggest the hypothesis that estrogen has an anabolic effect on muscle primarily by lowering the protein turnover and enhancing sensitivity to resistance training. Furthermore, estrogen may reduce the stiffness of tendons, an effect that may be modified by physical training.* **Key Words:** tendon, ligaments, strength, female hormones, collagen, women

INTRODUCTION

Healthy adult women have both absolutely and relatively less muscle mass than men. Furthermore, sex differences in tendon and ligament structure and biomechanical properties have been reported (21), and these may have implications for the adaptation to training and the risk of developing sports injuries. The size and composition of muscle and tendons are determined by the balance between synthesis rates and breakdown rates of the structural proteins in the specific tissues. The net protein balance is influenced by external stimuli such as feeding and exercise but also by the hormonal profile.

As women generally have a longer life expectancy than men and experience a rapid decline in muscle mass and strength around the time of the menopause (27), they are vulnerable to age-related frailty and morbidity. In addition, aging is associated with the accumulation of fat and collagen-rich connective tissue in the skeletal muscle tissue, which reduces muscle quality (maximal voluntary strength per cross-sectional area (CSA) (27)). These changes may at least partly be related to age-dependent hormonal changes during aging.

Regular physical exercise is recommended as part of a healthy lifestyle, but adaptations in the skeletal muscle system are needed to prevent injuries during loading. Sex differences in injury risk are reported, which may be explained by anatomical/biomechanical differences or differences in training status or loading exposure. However, sex hormones also may influence tissue composition and function and, thereby, the risk of injuries.

Estrogens are a class of steroid molecules of which women have about four times the amount compared with men until menopause. Estrogen receptors (ER) have been localized within skeletal muscle tissue but also in tendons and ligaments. The important actions of the endogenous estrogens are mediated by ER, which are synthesized in many cell types in two protein forms, ER-alpha and ER-beta, that function as transcription factors once bound to their ligand.

The purpose of this review is to highlight sex differences in musculotendinous protein turnover, with a specific focus on the influence of endogenous and exogenous estrogen. We hypothesize that estrogen influences the turnover of skeletal muscle and connective tissue proteins at rest in the postabsorptive phase, along with enhancing the sensitivity to anabolic stimuli (Fig. 1).

A number of studies have been conducted to elucidate the role of sex and/or female hormones on skeletal muscle, tendon, and ligament collagen protein turnover, composition, and biomechanical properties. These experiments involve laboratory studies in cell culture, animal studies, and human studies comparing men and women, women across the phase of their menstrual cycles, and women ingesting exogenous female hormones. In the following article, the focus primarily is on data from human studies in which protein synthesis rate

Address for correspondence: Mette Hansen, Section of Sport Science, Department of Public Health, Aarhus University, Aarhus, Denmark. Dalgas Ave. 4, 8000 Aarhus, Denmark (E-mail: kontakt@mettehansen.nu).

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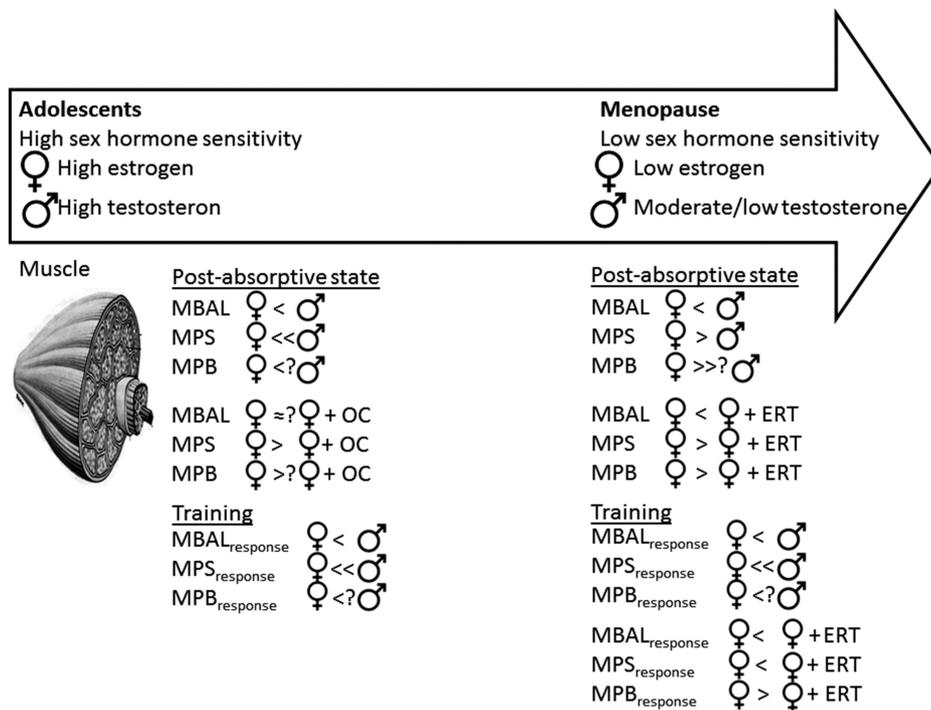


Figure 1. Overview of the hypothetical influence of sex, estrogen, and estrogen administration on skeletal muscle protein turnover. MBAL indicates muscle protein balance; MPS, muscle protein synthesis; MPB, muscle protein breakdown.

has been measured. To substantiate the influence of sex or female hormones on skeletal muscle, additional data (gene and protein expression, biomechanical properties, etc.) will be included. Estrogenic influence on skeletal muscle in men is inferred rather than discussed directly.

EFFECTS OF FEMALE HORMONES ON MUSCLE PROTEIN TURNOVER AND MUSCLE MASS

Sex Differences in Muscle Protein Turnover in the Postabsorptive State Is Age Dependent

Changes in muscle mass are determined by the balance between synthesis and degradation of structural contractile muscle proteins, the myofibrillar proteins. Sex differences in body composition are evident from birth but are attained primarily during the teenaged years. During adulthood, muscle mass is fairly stable in both sexes until the age of about 60 yr, after which it declines. Nevertheless, increase in muscle mass can be induced by resistance training, and muscle loss is experienced in response to immobilization and/or disease. For ethical reasons, myofibrillar protein synthesis rate has, to our knowledge, not been measured in young girls and boys to document the establishment of a sex difference in muscle mass. The progressive increase in muscle mass in teenage boys, in contrast to teenage girls, is associated with a surge in secretion of testosterone, which suggests a causal link between testosterone and muscle growth at least in young boys. Nevertheless, in the elderly, a lower muscle protein synthesis rate has been observed in men compared with age-matched postmenopausal women, even though the testosterone level is still approximately 10-fold higher in men compared with women (18,35,36). The latter observation may be explained hypo-

thetically by long-term exposure to testosterone that reduces the anabolic sensitivity in men (18). However, it is more likely that the sex difference in muscle protein synthesis in the elderly is explained by the marked decline in estrogen in women after menopause. Muscle protein synthesis rate is not only higher compared with age-matched elderly men but also compared with premenopausal women (37). An inhibition of estrogen on muscle mass is supported by some animal studies (40). Although muscle protein synthesis rate is enhanced in elderly women, they still experience an accelerated loss of muscle mass around menopause (27). This can be explained by a higher protein synthesis rate in postmenopausal women, which is counteracted by an upregulation of protein breakdown. Both an upregulation of stimulatory and inhibitory muscle growth regulatory genes in postmenopausal women compared with premenopausal women have been reported (37). Furthermore, a decline in estrogen around menopause may have a negative effect on muscle protein balance, and thereby muscle mass, by reducing the sensitivity to anabolic stimuli such as feeding and resistance exercise (discussed later).

Age-Dependent Sex Differences in Response to Anabolic Stimuli

Whether there is a sex difference in how muscle protein turnover is affected by anabolic stimuli in teens is not clear, but the establishment of sex difference in muscle mass indicates this. In young and middle-aged adults, there seems to be no detectable sex difference in the relative muscle growth in response to training. Furthermore, no sex difference in myofibrillar protein synthesis in response to ingestion of 25 g of whey protein or strenuous resistance exercise coupled with ingestion of 25 g of whey protein was observed between young men (n = 8) and women (n = 8), regardless of the fact that

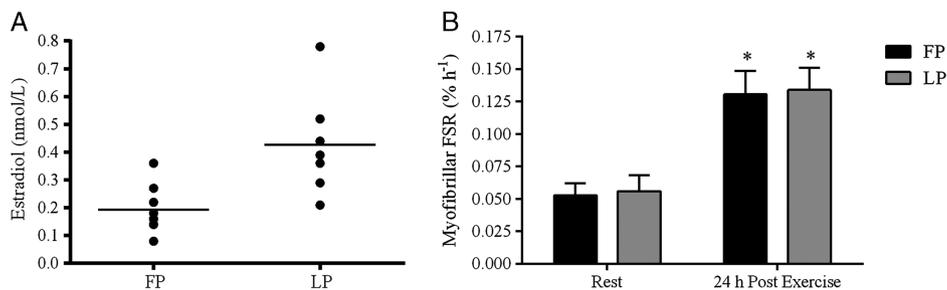


Figure 2. Plasma estradiol (A) and resting and postexercise fractional synthesis rates (FSR) of muscle myofibrillar protein (B) in follicular phase (FP) and luteal phase (LP) of the menstrual cycle. *Straight line* in (A) represents the mean value of subjects. *Significantly different from resting value at $P < 0.05$ (23). (Reprinted from (23). Copyright © 2006 The American Physiological Society. Used with permission.)

the exercise-induced area under the testosterone curve was 45-fold greater in men than women in the first hour of the recovery period after exercise (42). Similarly, no significant sex difference in mixed skeletal muscle protein synthesis rate in response to hyperinsulemia-hyperaminoacidemia in middle-aged and nonobese elderly women and men has been reported, but the response typically was attenuated in the elderly compared with middle-aged subjects, especially in women (36). In contrast, a significant sexual dimorphism in response to mixed meal ingestion has been observed in 65- to 80-yr-old obese adults (35). Mixed skeletal muscle protein synthesis rate increased in men in response to feeding, whereas no significant change was detected in women (35). In line with this, protein translation initiation seemed to be stimulated by feeding in men (increased phosphorylation of muscle eIF4Eser²⁰⁹ and eIF4E-BP1^{thr27/46}) but not in women (35). This suggests that elderly women compared with men experience a reduction in the ability to respond to the anabolic stimuli feeding after the menopause when estrogen is reduced. Further investigation is needed to clarify whether the effect depends on the degree of adiposity. In support of a sex difference in response to anabolic stimuli, a blunted response to resistance exercise training has been observed in postmenopausal women compared with age-matched men after 26 wk of knee extensor training three times a week (1). Furthermore, we detected no difference in myofibrillar protein fractional synthesis rate (FSR) in postmenopausal women between the leg that had performed strenuous resistance exercise and the contralateral control leg 24 h after finishing the exercise (16). In contrast, we observed a significant increase in the myofibrillar protein synthesis rate in response to resistance exercise in elderly women who had an estrogen level comparable to that of young women because of use of estrogen replacement therapy (ERT) (16). The latter observation highlights the importance of a more thorough investigation of the effect of estrogen on skeletal muscle protein turnover in groups of females with varying levels of estrogen, especially because women and men differ in so many ways, which complicates the clarification of the isolated effect of the sex hormones. The rise in testosterone in response to training may play an important role for the adaptation to resistance training in men; whereas in premenopausal women, estrogen may enhance the sensitivity to anabolic stimuli. If this is the case, no sex difference in muscle protein turnover during adulthood may obscure differences in the underlying mechanisms leading to comparable skeletal muscle protein synthesis

rates and maintenance of the sex difference in muscle mass induced during the teenage years.

Influence of Endogenous Female Hormones on Skeletal Muscle in Young Women

Animal and muscle cell culture studies have shown diverging results when it comes to the effect of estrogen on muscle mass and skeletal muscle protein synthesis and breakdown. For instance, in ovariectomized, young, growing rats, the administration of estrogen inhibited muscle protein synthesis and muscle growth (40); whereas in young steers, implants of 17 β -estradiol enhanced muscle hypertrophy probably by increasing the activation and proliferation of satellite cells (26). The diverging results may be related to the route of administration of the estrogen compounds and the design of the trials. In addition, the effect of estrogen may be dependent on the species, and the transferability of data from animal trials to human beings is in general problematic because the menstrual cycles and sex hormonal profiles differ substantially between species. Focus in the following is on the effect of female hormones on skeletal muscle protein turnover in human subjects.

The blood concentrations of female hormones change during the menstrual cycle, with low estrogen and progesterone during the early follicular phase (FP), followed by a peak in estrogen just before ovulation, and high concentrations of estrogen and progesterone during the luteal phase (LP). Measurements in both phases of the menstrual cycle should therefore allow for the detection of effects of female hormones on skeletal protein turnover. In a cross-sectional trial, we measured myofibrillar protein FSR in eight young females 2 to 3 d after the onset of menses (FP) and seven females 4 d after a positive ovulation test (LP) (23). Although there was, on average, a twofold difference in circulating estrogen and a marked difference in progesterone between menstrual phases, we were not able to detect any significant difference between groups in the postabsorptive phase or in response to strenuous exercise. This may be related to overlap in the individual estrogen levels between phases and the cross-sectional design (Fig. 2) (23). Finally, estrogen and progesterone may have divergent effects on the muscle protein synthesis rate that may counteract each other. This is supported by *in vitro* animal data (see review by Oosthuysen and Bosch (25)). However, the separate effect of the endogenous circulating female hormones on skeletal muscle protein turnover in premenopausal women is still not clarified. Further studies measuring skeletal muscle

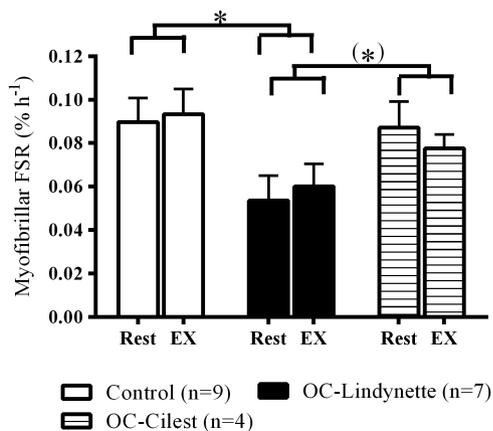


Figure 3. Myofibrillar fractional synthesis rate (FSR) at rest and 24 h after exercise in users of oral contraceptives (OC) or non-users of OC (Control). Values are mean \pm SEM. Two-way analysis of variance (one repetition) $*P < 0.05$ control versus OC Lindynette users. $*P = 0.098$ OC Lindynette users versus OC Cilest users (14). (Reprinted from (14). Copyright © 2009 John Wiley and Sons. Used with permission.)

protein turnover across the menstrual cycle in the postabsorptive state, the fed state, and in response to exercise are warranted.

Oral Contraceptives Disturb the Regulation of Skeletal Muscle Protein Turnover

Use of oral contraceptives (OC) is widespread among young fertile women for contraception and menstrual regulation and to decrease acne or dysmenorrhea. OC has been reported not to change lean body mass. Nevertheless, evidence is limited in relation to the effect of OC on muscle protein turnover and how the use of OC interacts with the response to acute exercise and regular training. In a clinically controlled trial, we observed a lower myofibrillar protein FSR in users of third-generation OC compared with users of second-generation OC and controls when the subjects were fed a commercial clinical nutrient drink according to their individual determined fat-free mass each 30 min during a subsequent 5-h period where myofibrillar protein FSR was determined (Fig. 3) (14). The type of synthetic gestagens varies between second (norgestimate)- and third (gestoden)-generation OC and, thereby, differs in the androgenic properties in general. This may explain the differential effect of OC on myofibrillar FSR. But clarification is needed with regard to the isolated effects of synthetic estradiol and different types of synthetic gestagens on skeletal muscle protein turnover at rest and in response to exercise in premenopausal women. The preliminary results suggest that the use of a certain type of OC is associated with lower myofibrillar protein FSR, but the effect on myofibrillar protein breakdown rate and, thereby, the overall net protein turnover and protein balance is not elucidated.

ERT Reduces Muscle Loss in Postmenopausal Women

Menopause is the cessation of a woman's reproductive life, where the estrogen level is reduced to a negligible level. This typically occurs in women during their late forties or early fifties. Based on cross-sectional data, it has been hypothesized that there is a link between the accelerated decline in muscle mass, strength, and physical function around the time of menopause and the reduction in estrogen (27). However, no

causal link has been established between the age-dependent decline in estrogen and loss of strength and muscle mass. Nevertheless, positive associations between serum estradiol concentrations and muscle mass and strength have been observed in postmenopausal women (41).

A decline in muscle mass is induced by a net negative in muscle protein balance. In a recent study, transdermal administration of estradiol to postmenopausal women to enhance circulating estradiol to a level comparable to young women did not change the skeletal muscle protein synthesis rate (37). Furthermore, the skeletal muscle protein synthesis rate is enhanced after menopause compared with premenopausal women (37), even though the skeletal muscle mass is declining. It seems counterintuitive but may be explained by a marked increase in skeletal muscle protein breakdown rates at menopause, which led to a net loss of muscle proteins. This hypothesis is supported by oral administration of hormone replacement therapy (HRT) to postmenopausal women counteracting postmenopausal-related enhancement of protein degradation through the ubiquitine-proteosome pathway (29). Furthermore, in line with a hypothetically enhanced muscle protein turnover in postmenopausal women, new data indicate an upregulation of both stimulatory and inhibitory muscle growth regulatory genes in postmenopausal women compared with premenopausal women (37). Furthermore, in a randomized double-blind trial, transcriptional change in the ubiquitine-proteosome system was observed in controls after 1-yr intervention in the early postmenopausal years, which was not observed in subjects receiving HRT (29). In agreement with this observation, lean body mass was reduced in the control group but enhanced in the HRT group after the 1-yr intervention (29). Actually, the majority of randomized controlled trials have reported that HRT helps to maintain or even increase muscle mass, muscle strength, and muscle function when used in the beginning of the postmenopausal period (41). A twin trial including 13 pairs of monozygotic postmenopausal twin pairs, where only one of each pair had been taking HRT, showed that long-term HRT treatment is associated with greater muscle power and higher walking speed compared with control (30).

HRT contains not only estrogen but also synthetic progestagen, which complicates the identification of the distinct effects of synthetic progestagens and estrogen on musculotendinous tissue in HRT. There are data to suggest that progesterone replacement therapy enhances muscle protein synthesis in postmenopausal women (37). But if progesterone replacement therapy enhances skeletal muscle mass or at least helps maintain muscle mass in postmenopausal women, this has not been studied to our knowledge. The isolated effect of estrogen replacement has been studied in a limited number of human trials (16,37). Many hysterectomized women receive ERT containing only estradiol. Therefore, we included hysterectomized women using oral ERT and age-matched postmenopausal women who were characterized by very low levels of estrogen (16). The myofibrillar protein FSR was lower in the postabsorptive state in the ERT users compared with age-matched postmenopausal women (Fig. 4) (16). This may be related to the estrogen in ERT users being raised to a level corresponding to that in premenopausal women because a higher postabsorptive muscle protein synthesis rate in postmenopausal women compared with premenopausal women

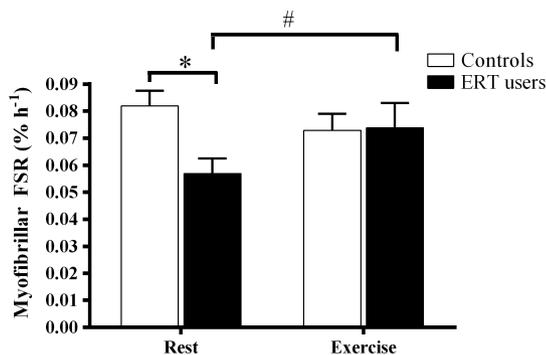


Figure 4. Myofibrillar protein fractional synthesis rate at rest and 24 h after exercise in women who use estrogen replacement therapy (ERT) and in women who do not use ERT (Controls). Values are means \pm SEM. Two-way analysis of variance with repeated measures in one factor: ERT users versus Controls, $P = 0.18$; rest versus exercise, $P = 0.34$; and interaction between rest or exercise and Controls or ERT, $P = 0.005$. * $P = 0.015$ ERT versus Controls. # $P = 0.010$ ERTrest versus ERTexercise (16). (Reprinted from (16). Copyright © 2012 Oxford University Press. Used with permission.)

has been observed, as mentioned earlier (37). If this is the case, it suggests that oral ERT inhibits skeletal muscle protein synthesis in the postabsorptive state. However, we cannot exclude that the lower myofibrillar protein FSR in ERT users was related to the androgen profile being reduced in the hysterectomized women (4-androstenedione, calculated free androgen index, and s-free testosterone) (16). In a recent trial, transdermal estrogen replacement did not change skeletal muscle protein synthesis (37). Therefore, it can be suggested that the main beneficial effect of exogenous administration of estrogen on muscle mass in elderly women may be to turn down the enhanced skeletal muscle protein breakdown rate and enhance the sensitivity to anabolic stimuli (the latter is discussed later). In line with this, oral estrogen administration reduced leucine oxidation in men compared with placebo treatment and induced an improvement in protein balance calculated as the difference between whole-body protein synthesis and breakdown (9). Furthermore, in a randomized double-blind trial, Pöllänen *et al.* (29) observed that transcriptional changes in the ubiquitin-proteasome system at menopause were counteracted partially by HRT. Whether exogenous estrogen administration also will help reduce muscle loss in other catabolic situations, such as during illness and immobilization, needs to be clarified in future studies.

Taken together, the preliminary data indicate that the use of oral HRT may help maintain muscle mass and function after menopause by means of the synthetic progestagens enhancing basal skeletal muscle protein synthesis and the estrogen component—reducing basal skeletal muscle protein breakdown rate. The primary missing link in this hypothesis are data on the effect of the exogenous female hormones on myofibrillar protein breakdown rate.

Response to Exercise Is Enhanced by ERT

An important finding in our trial on hysterectomized women was that the use of ERT was associated with an enhanced sensitivity to the anabolic response by exercise compared with age-matched healthy postmenopausal women (16). Only the ERT users experienced an increase in myofibrillar protein FSR in response to the strenuous resistance

exercise (10 sets of 10 repetitions, 10–12 RM), which suggests that enhancing circulating estrogen levels in elderly women to a level comparable to those in young women may counteract the reduction in sensitivity to the resistance exercise-induced anabolic stimuli observed in postmenopausal women (1,16). In support, Dieli-Conwright *et al.* (6) reported that the myogenic gene expression profile in response to high-intensity resistance exercise seems to be more anabolic in postmenopausal women who were given HRT compared with controls because the exercise-induced increase in mRNA expression of follistatin, myogenin, Myf5, and MRF4 was significantly greater. In addition, the decrease in mRNA expression of muscle-specific ubiquitin ligase atrogin-1, MuRF-1, and myostatin in response to the exercise was reported to be significantly more pronounced in postmenopausal HRT users than controls, which is in favor of a positive skeletal muscle protein balance (6).

Animal studies support a positive interaction between estrogen and exercise. In rats, estrogen administration enhances satellite cell activation via ER-related mechanisms after exercise (39). Furthermore, in rats that had undergone 4 wk of unloading, ovariectomized rats failed to regain any of the atrophied muscle mass during 2 wk of reloading and experienced a reduced phosphorylation of Akt and p70s6k (33). In contrast, sham-operated rats regained their muscle mass and experienced an increase in p70s6k activation, which supports an activation of anabolic signaling pathways (33). The importance of estrogen for restoring muscle mass after muscle atrophy is supported by other animal trials, where lack of estrogen during rehabilitation resulted in inadequate muscle restoration (2). Future studies should aim to investigate the synergistic influence of exercise and exogenously administered progestagens and estrogen (separately and combined) on myofibrillar protein turnover, skeletal muscle mass, and physical function in postmenopausal women.

SEX AND ESTROGEN INFLUENCE TENDON AND LIGAMENT COLLAGEN PROTEIN TURNOVER AND COMPOSITION

The transmission of force from the skeletal muscle tissue is dependent on the tendon and ligaments connected to the muscle contractile filaments. Any influence of estrogen on tendon and ligaments will, therefore, indirectly have an impact on the skeletal muscle tissue and its function. In line with this, a reduced risk of muscle damage has been reported in women compared with men (31), which may be related to tendon stiffness being lower in women, thereby reducing the tensile loading of the myofilaments during muscle contractions.

Collagen is the most abundant protein within the entire body and is ubiquitous in tendon and ligaments; in particular, there is a high concentration of the structural protein Type I collagen. The collagen fibril characteristics and the turnover of collagen will influence the biomechanical properties of the structure. Furthermore, the size of the tendon and ligaments impacts the tendon and ligaments' ability to resist tensile stress during loading because a greater CSA allows the load on the tissue to spread over a larger area. Because ligament and tendon injuries are frequent (*e.g.*, tendinopathies and tendon

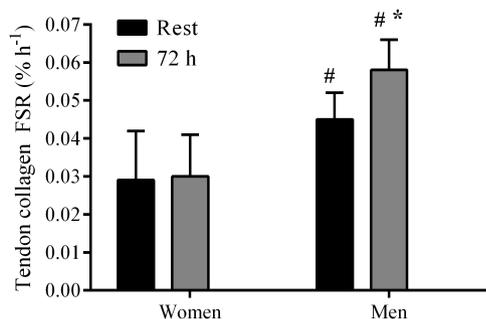


Figure 5. Comparison of patellar tendon collagen fractional synthesis rates (FSR) at rest and 72 h after exercise in women and men. *Significantly different from rest value, $P < 0.05$. #Significantly different from women, $P < 0.05$ (24). (Reprinted (24). Copyright © 2007 The American Physiological Society. Used with permission.)

and ligament ruptures) and costly for both the individual and the community, it is of great importance that our understanding regarding the regulation of tendons and ligaments — also in relation to sex hormones — is improved.

Sex Differences in Tendon and Ligament Injury Risk

Sex differences in tendon and ligament injury risk exist. Nevertheless, discrepancies seem to depend on the anatomical location. Women are at greater risk than men of sustaining an anterior cruciate ligament (ACL) rupture. In contrast, women are at lower risk compared with men of sustaining an Achilles rupture until menopause; hereafter, the risk is similar in women and men. In support of an influence of the female hormones, pathological changes in the Achilles tendon seem to be reduced by HRT after menopause (5). Hence, female hormones may have a protective effect on tendons and ligaments, but sexual dimorphism increases ACL injury risk only. This discrepancy in the risk of the different types of injuries within tendon and ligaments is not clarified but may be related to sexual dimorphism in anatomical/biomechanical features that enhance the risk of ACL injuries in women. The influence of estrogen also may differ between anatomically different tendons and ligaments because of differences in tissue composition, loading profile, and the distribution and numbers of different ER, which may have divergent effects. In summary, no single explanation for the sex disparity in risk has been outlined.

Sex Difference in Tendon and Ligament Collagen Turnover and Tissue Composition

Compared with the ACL, the patellar tendon represents a relatively easily accessible collagen-rich tissue for the study of connective tissue turnover in an *in vivo* setting in humans. To investigate sex-based differences in tendon collagen protein turnover, we measured the patellar tendon collagen FSR at rest using stable isotope and microdialysis techniques in young men and eumenorrheic women (24). A lower tendon collagen FSR was observed in women compared with men, both at rest and in response to exercise (24). Tendon collagen breakdown rate was not measured because of methodological difficulties. Nevertheless, Lemoine *et al.* (20) observed a significantly lower patellar tendon dry mass in women than men and a tendency ($P = 0.08$) toward a lower collagen content

per tendon wet weight in women. In addition, transmission electron microscopy analysis of cadaveric ACL adjusted for body size showed reduced collagen fibril density per area in women, and the collagen fibril density was significantly positively correlated to ACL stiffness both before and after adjustment for tendon size (20). The reduced stiffness in women indicates less resistance to deformation during loading, which has been confirmed *in vivo*, showing higher stress and deformation during loading of the patellar tendon in women compared with men (4). Part of this sex-based difference in tendon stiffness of the patellar tendon and ACL also may be explained by a higher expression of Type III collagen being observed in the patellar tendon in women compared with that in men (38). Type III collagen enhances the elastic properties of the connective tissue. Taken together, the sex dimorphisms in tendon collagen synthesis rate, structure, and mechanical properties may explain sex differences in the susceptibility to rupture in tendon and ligaments. In line with this, mechanical testing of single isolated ACL collagen fascicles in our laboratory showed that the ultimate stress before rupture was significantly greater in fascicles from young men compared with that from young women, indicating a reduced ACL quality and strength in women (21).

The majority of ACL ruptures occur in sports. Cross-sectional data and intervention studies have shown that tendon collagen FSR is enhanced after loading in men, and regular loading of tendon enhances tendon stiffness (absolute and adjusted for tendon size). Furthermore, heavy loading induces tendon hypertrophy and, thereby, reduces the stress on the tissue during loading. Nevertheless, the ability to adapt to training seems to be sex specific. We observed an enhanced tendon collagen synthesis rate compared with at rest in young men 72 h after a 1-h one-legged kicking exercise, whereas no difference was observed in young women between values obtained in the resting state and after exercise, even though the relative intensity of the loading was similar (Fig. 5) (24). Furthermore, in a cross-sectional trial, we observed no difference in Achilles and patellar tendon CSA between untrained and trained female runners; whereas in well-trained male runners, CSA was significantly greater compared with untrained men but also compared with untrained and trained women, even though training profile and training history did not differ between sexes (Fig. 6) (21). In combination, the data support a reduced ability to adapt to training in women compared with men (Fig. 7).

Effect of Circulating Sex Hormones on Tendon and Ligament Collagen Turnover

Based on our observations of a higher tendon collagen synthesis rate in young men compared with young women, we were not able to conclude whether this was caused by a stimulating effect of testosterone or an inhibiting effect of female hormones or other hormones, which differ in concentrations between the sexes.

The understanding of the effect of testosterone on tendons and ligaments is to our knowledge almost nonexistent. Hama *et al.* (8) observed that the collagen content within the hip joint capsule of rats was significantly greater in males than in females after sexual maturation, and testosterone administration to orchietomized male rats enhanced collagen content and fibril diameter in the hip joint capsule. This suggests that

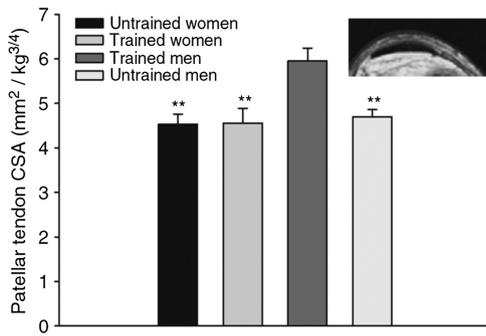


Figure 6. The magnetic resonance imaging (MRI) determined patellar tendon cross-sectional area (CSA) for trained and untrained men and women normalized to body mass. Trained men had a greater CSA than untrained men ($P < 0.01$); however, note that trained women had a similar CSA compared with untrained women. An MRI of the patellar tendon (21). (Reprinted from (21). Copyright © 2007 John Wiley and Sons. Used with permission.)

the difference between sexes may be explained at least partly by a higher testosterone level in men, but there is a lack of human data to support this hypothesis. Nevertheless, a training study in elderly men and women gives some support for the testosterone level influencing training adaptation in tendons (35). After 12 wk of alpine skiing training, the change in tendon stiffness was blunted in postmenopausal women compared with elderly men (32), who are characterized by markedly higher circulating testosterone levels than women, whereas estrogen is comparable between the sexes after menopause (35). Future studies are needed to clarify the regulatory role of testosterone in tendons and ligaments.

The interest in understanding the effect of estrogen on tendon and ligament composition and protein turnover has been stimulated after the identification of ER in ligaments and tendons (17). In the following, we will provide evidence supporting the belief that estrogen influences tendons and

ligaments (Fig. 7). Our working hypothesis is that long-term exposure to high levels of circulating estrogen influences tendon and ligament collagen turnover, composition, and function, and thereby the susceptibility to injury. In line with this, we have observed a significant negative correlation between circulating estradiol and patellar tendon stiffness ($r = 0.53$, $P = 0.04$) (11). However, knowledge on the effect of endogenous estrogen on tendon and ligament collagen turnover is very limited. Therefore, we presently have to base our understanding on trials that have manipulated the estrogen level by exogenous administration of different types of synthetic female hormones.

Exogenous Estrogen Administration Influences Tendons

How the use of OC influences tendon biomechanical properties and the risk of tendon and ligament injuries is not elucidated, and data are limited (3,11,19,22,28). Nevertheless, in a group of young women who were either long-term (7.2 ± 2.1 yr) users of low-dose OC ($n = 11$) or never users of OC ($n = 12$), we measured tendon collagen FSR at rest and in response to exercise (11). The groups did not differ in age, body composition, or training status. Tendon collagen synthesis rate was measured in the patellar tendon in each leg 24 h after a 1-h one-legged kicking exercise. We used a flooding dose of stable isotope-labeled proline followed by patellar tendon biopsies to quantify tendon collagen FSR. Tendon synthesis also was measured indirectly by measuring changes in NH₂-terminal propeptide of Type I collagen (PINP) in the peritendinous fluid in front of the patellar tendon. PINP is cleaved off during the synthesis of new Type I collagen molecules. Results from the use of both methods indicated that the use of OC is associated with lower tendon collagen synthesis rates. The underlying mechanism is still not elucidated fully. The effect of OC may be related directly

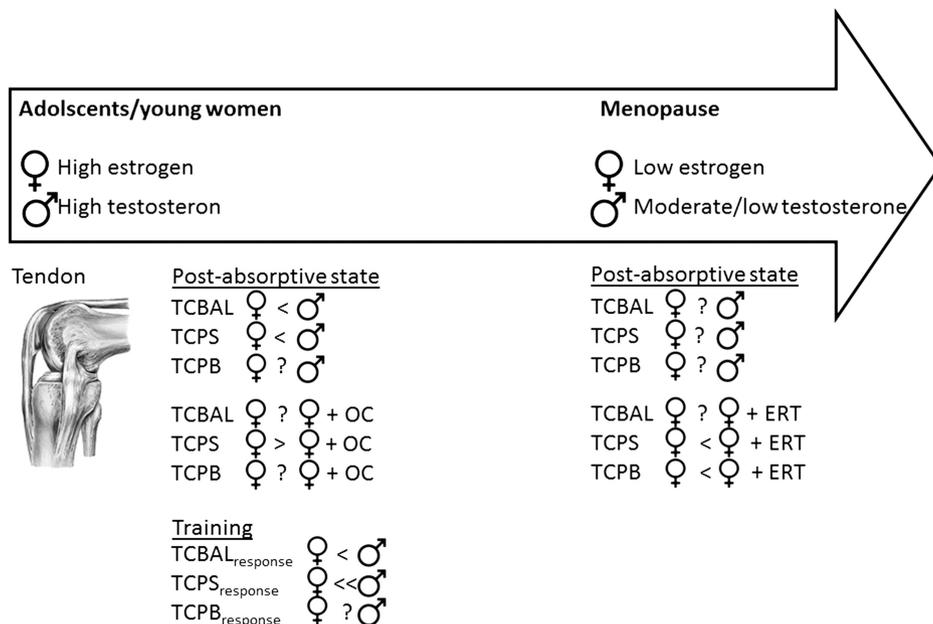


Figure 7. Overview of the hypothetical influence of sex, estrogen, and estrogen administration on tendon collagen protein turnover. TCBAL indicates tendon collagen protein balance; TCPS, tendon collagen protein synthesis; TCPB, tendon collagen protein breakdown. The hypothesis is based on data from human Achilles and patellar tendon.

to total exposure to estrogenic components (endogenous and exogenous estrogen) and the content of synthetic estradiol or synthetic gestagens in OC, either separately or combined. We did not determine the concentration of ethinyl estradiol in the trial but, on the day of the experiment, the concentration of circulating estradiol (s-17 β -estradiol) was low in both groups because of the nonusers being tested in the early FP and the use of OC suppressing the endogenous secretion of estradiol. Thus, the two groups of subjects had contrasting exposure to ethinyl estradiol with minimal levels of endogenous estrogen. We do not know if ethinyl estradiol acts similarly to estradiol within tendons and ligaments, but we believe that our observation of a lower tendon collagen synthesis rate in OC users is caused by secondary indirect effects of OC. Use of OC was associated with markedly lower serum and local peritendinous insulin-like growth factor-I (IGF-I) concentrations compared with controls (15) and, in a subsequent human trial, we have shown that local injections of IGF-I into the patellar tendon enhanced tendon collagen synthesis compared with saline injections in the contralateral tendon (10).

In elderly women with low circulating IGF-I, we observed that the use of ERT (containing 17-estradiol) was associated with a higher tendon collagen synthesis rate compared with age-matched postmenopausal women. This observation supports that estradiol has a stimulating effect on tendon collagen synthesis rate but also support the hypothesis that OC indirectly inhibits tendon synthesis rate by reducing the stimulatory effect of IGF-I (Fig. 7). Ten elderly hysterectomized women who were long-term users of ERT (17 ± 3 yr) and 10 postmenopausal women with negligible levels of circulating estrogen were invited to participate in a trial where we measured patellar tendon collagen synthesis rate, structural characteristics, and biomechanical properties (12). The groups were comparable in age, body composition, physical activity level, fitness level, and muscle strength. The results showed that ERT users were characterized by a relatively lower percentage of fibrils with a large diameter, which may impair the resistance to loading because of reduced ability to introduce intramolecular and intermolecular cross-links in small fibrils. In line with this, the use of ERT was associated with a reduced stiffness adjusted for differences in tendon CSA. Furthermore, tendon collagen synthesis rate was significantly higher in ERT users compared with control, and a tendency toward a positive correlation between serum estradiol and tendon collagen FSR was observed in ERT users ($r^2 = 0.41$, $P = 0.06$). Similarly, the indirect marker of synthesis of new collagen molecule PINP correlated positively with circulating estradiol in ERT users ($r^2 = 0.47$, $P < 0.05$). The latter correlation was strengthened by including a subject receiving a double dose of ERT (4 mg d^{-1} estradiol) ($r^2 = 0.68$, $P = 0.01$). The higher tendon synthesis rate coupled with relatively fewer collagen fibrils with a large diameter may suggest an overall higher tendon collagen turnover in ERT compared with controls.

Estrogen may not influence only tendons and ligaments at rest but also influence the response to exercise. Interestingly, in ERT users, changes in tendon synthesis 24 h after a resistance training bout were correlated negatively to s-estradiol. A reduced anabolic response to exercise is supported by our observation of reduced responsiveness to exercise in OC users

compared with non-OC users (13). Whereas tendon synthesis was enhanced after exercise in controls, tested in FP of the menstrual cycle where estrogen and progesterone concentrations are low, OC users did not experience a change in response to the exercise (13). In addition, an increase in muscle collagen synthesis in response to exercise was only experienced in controls (15). Furthermore, Finni *et al.* (7) reported that, in postmenopausal monozygotic twin pairs with a high activity level, Achilles tendon CSA was significantly smaller in HRT users than in co-twins, whereas no difference was observed after including less active twin pairs. Also, cross-sectional data have shown that the use of HRT compared with control was associated with fewer tendon abnormalities and reduced Achilles tendon thickness in active postmenopausal women using HRT, which was not seen in inactive subjects (5). To conclude, the exogenous administration of OC/ERT/HRT seems to inhibit the responsiveness to the anabolic effect of exercise on tendon collagen synthesis and adaptation to regular training (5,7,12,13), similar to when the response in women is compared with that in men (21,24). This may have serious consequences for injury risk in active subjects, which is supported by Slauterbeck *et al.* (34), who experienced that load to failure was significantly lower in ACL from rabbits treated with a high dose of estrogen than in controls.

The research up to now has focused on the ACL, Achilles, and patellar tendons. The future may clarify whether sex will influence tendons and ligaments differently depending on their localization and function. It should be noted that it can be questioned whether the observations can be transferred to other tendons and ligaments within the body because the primary function of the different ligaments and tendons differs (*e.g.*, stabilization or elastic properties).

SUMMARY AND CONCLUSIONS

The scientific interest in the effect of female hormones on human skeletal muscle protein turnover and muscle mass has been increasing in the last decades. The sex-related difference in muscle mass is obvious, but it is still a puzzle how endogenously and exogenously administered sex hormones regulate the protein turnover during different life stages where muscle mass is stable or is changing. The influence of estrogen seems to be most evident in transition phases as during aging (Fig. 1).

The lack of female hormones after menopause seems to be detrimental; muscle protein turnover in the postabsorptive state is enhanced, and there is a net loss of muscle mass and a reduction in muscle function when women enter the postmenopausal state. Nevertheless, administration of oral ERT/HRT seems to counteract these changes by turning down the muscle protein turnover in the postabsorptive state and enhancing the sensitivity to resistance training. Furthermore, long-term use of ERT is associated with changes in the lower limb tendon structure and reduced tendon stiffness compared with age-matched postmenopausal women. Preliminary data suggest that ERT/HRT at least in active postmenopausal women is beneficial and may reduce the risk of lower limb tendon and ligament injuries. How oral ERT/HRT influences the sensitivity to other anabolic and

catabolic stimuli in postmenopausal women, such as feeding and immobilization, needs to be elucidated in future studies.

In young women, certain types of OC are associated with a lower protein synthesis of myofibrillar and tendon collagen proteins, but the effect on protein breakdown, and, thereby, muscle and tendon protein balance at rest and in response to anabolic training, has not been elucidated (Figs. 1, 7). However, cross-sectional data indicate that the use of OC, but also a high level of endogenous estradiol, is associated with a reduced response to exercise in tendons. Future studies are warranted to clarify whether the use of OC enhances the risk of sports injuries in premenopausal women.

In general, estrogen should be perceived as anabolic in relation to maintaining or even enhancing skeletal muscle function. Nevertheless, high levels of endogenously or exogenously administered estrogen in young women may disturb the protein balance in the tendon and ligaments in a nonbeneficial direction in relation to sports injuries.

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