Sex Hormones and Tendon

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Mette Hansen and Michael Kjaer

Abstract

The risk of overuse and traumatic tendon and ligament injuries differ between women and men. Part of this gender difference in injury risk is probably explained by sex hormonal differences which are specifically distinct during the sexual maturation in the teenage years and during young adulthood. The effects of the separate sex hormones are not fully elucidated. However, in women, the presence of estrogen in contrast to very low estrogen levels may be beneficial during regular loading of the tissue or during recovering after an injury, as estrogen can enhance tendon collagen synthesis rate. Yet, in active young female athletes, physiological high concentration of estrogen may enhance the risk of injuries due to reduced fibrillar crosslinking and enhanced joint laxity. In men, testosterone can enhance tendon stiffness due to an enhanced tendon collagen turnover and collagen content, but testosterone has also been linked to a reduced responsiveness to relaxin. The present chapter will focus on sex difference in tendon injury risk, tendon morphology and tendon collagen turnover, but also on the specific effects of estrogen and androgens.

Keywords

Sex hormones • Sex • Gender • Injury risk • ACL rupture • Estrogen • Estradiol • Testosterone • Progesterone • Relaxin • Insulin-like growth factor I (IGF-I) • Collagen • Fascicles • Knee laxity • Joint laxity • Tendinopathy • Cross-links • Biomechanical properties

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Introduction

Sex differences in tendon and ligament injury risk are reported. The relative risk of tearing the anterior cruciate ligament (ACL) is 2–6 times greater in young female compared to male athletes [1–3] even after socioeconomic, health and lifestyle background variables and the level of sports participation have been taken into account [4, 5]. Especially, when circulating estrogen is peaking around the time of ovulation during the menstrual cycle the risk of an ACL-rupture seems to be enhanced [6, 7]. In contrast, the risk of sustaining an Achilles rupture [8] or developing tendon pathology [9] seems to be lower in premenopausal women compared to that in men, whereas this sex discrepancy in risk disappears after menopause [10] where the level of estrogen is comparable between the sexes [11]. Furthermore, the transition to the postmenopausal state characterized by low estrogen levels is associated with a dramatic increase in the prevalence of asymptomatic rotator cuff tears [12]. These observations have led to a search for the underlying mechanism(s) involved in the discrepancy in the risk of injuries between men and women and between young and elderly women. A complex interaction involving several risk factors is probably in play (e.g. ligamentous laxity and size, limb alignment, notch dimensions and decreased neuromuscular control of knee, motion skill level and muscular strength) [13]. However, since estrogen receptors have been localized in tendons and ligaments [14, 15] it has been suggested, that at least part of this gender difference in risk might be explained by sex hormonal differences influencing tendon and ligament structure and biomechanical properties.

Sex Differences in Tendon Structure and Biomechanical Properties

Tendon stiffness has been reported to be lower in young women than men during voluntary isometric contractions using ultrasonography [16, 17]. Similarly, joint laxity has repeatedly been reported to be greater in *post-pubertal* women compared to age-matched men [18–20], whereas no differences seem to exits between pre-pubertal boys and girls [20] and between postmenopausal men and women [21]. Taken together, these observations indicate that sex differences in tendon and ligament biomechanical properties are significant in the period of life where the sex hormonal profile is markedly different between women and men. In line with this, the sex disparity in ACL injury risk is peaking in the teenage years where sex hormonal differences are the greatest [5, 22].

Part of the sex difference in tendon and ligament biomechanical properties is explained by a general greater tendon size in men compared to women. Yet, also after adjustment for body weight and control of activity level Achilles and patellar tendon cross-sectional area (CSA) is greater in young male runners compared to equally trained young female runners [17] (Fig. 13.1). Furthermore, tendon CSA is greater in trained male runners compared to that in untrained men, whereas no difference in tendon CSA is reported between untrained and trained female runners [17]. These cross-sectional data indicate that the ability to adapt to regular training in regards to patellar and Achilles tendon size might be reduced in women in contrast to in men [23]. However, it should be noted that low energy availability is a common phenomenon in endurance running, especially among young female athletes [24, 25]. Therefore, sex difference in tendon adaptation to training may be confounded by hormonal disturbances related to low energy availability such as low estrogen and insulin-like growth factor I (IGF-I) levels as these hormones are coupled to a stimulating effect on tendon collagen synthesis [26, 27]. Actually, other data supports that female handball players seem to be able to adapt in tendon size after long-termed regular strenuous loading of the tissue [28]. Thus, long-term intervention studies are needed to clarify if the is a sex difference in tendon adaptation to training.

Tendon structural quality also seems to differ between young men and women. Mechanical testing of single human patellar tendon collagen fascicles from young men and women have shown that during loading the ultimate stress before rupture is greater in fascicles from males compared to those from females [23]. The sex differences in fascicle biomechanical properties may be related to structural differences. In young

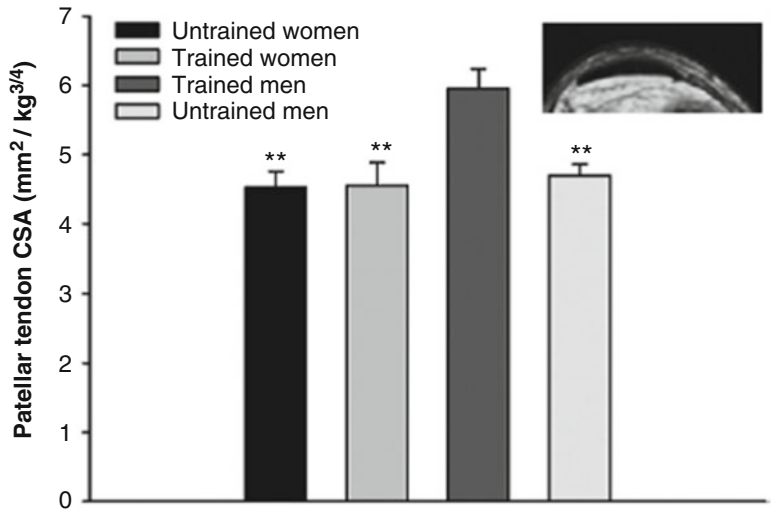

Fig. 13.1 The magnetic resonance imaging (MRI) determined patellar tendon cross-sectional area (CSA) for trained and untrained men and women normalized to body mass. Trained men had a greater CSA than untrained men ($P < 0.01$); however, note that trained women had a similar CSA compared with untrained women. An MRI of the patellar tendon [21]. (Reprinted from [21]. Copyright © 2007 John Wiley and Sons. Used with permission.)

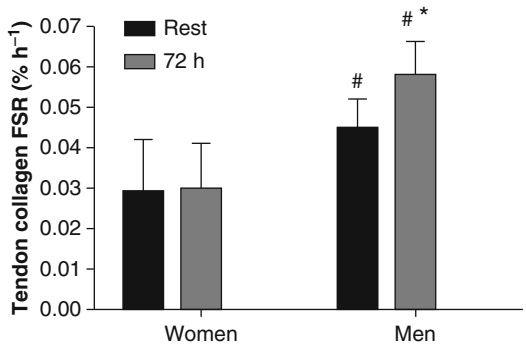

Fig. 13.2 Comparison of patellar tendon collagen fractional synthesis rates (FSR) at rest and 72 h after exercise in women and men. *Significantly different from rest value, $P < 0.05$. #Significantly different from women, $P < 0.05$ [24]. (Reprinted [24]. Copyright © 2007 The American Physiological Society. Used with permission.)

women, expression of Type III collagen in the patellar tendon is higher than in men [29], which may induce a greater elastic flexibility. In addition, women patellar tendon dry mass and collagen content per tendon weight has been reported to be lower compared to in men [30].

The tendon collagen content is determined by the balance between collagen protein synthesis rate and collagen protein breakdown rate (see Chap. 8). The tendon collagen turnover is slow and specifically recent data suggest that the tendon core of collagen is the same from the late teenage years until death [31], whereas the outer layer seems to responsive to anabolic stimuli such as training [32–34] and probably also hormonal stimuli [26, 27, 35, 36]. In young healthy women, a lower tendon collagen synthesis rate has been reported compared to age-matched men both at rest and in response to exercise at the same relative intensity [37]. Patellar tendon collagen synthesis rate was still enhanced over resting values 72 h after exercise in men whereas the synthesis rate was not different from resting values in women (Fig. 13.2). Unfortunately, the balance between synthesis and breakdown was not determined. Only limited validated methods exist in relating to determining tendon collagen breakdown rate *in vivo* in humans. However, these data similarly to cross-sectional including untrained and trained female runners [17] indicate that the response to mechanical loading may be reduced in women [23].

Influence of Estrogen on Tendon Structural and Biomechanical Properties

Introduction; Estrogen and Estrogen Signaling

The effect of sex hormones may differ between animals and human since the sex hormonal and

menstrual profile varies considerably between species, which questions the transferability of results from animal studies to humans. Based on this, the following is primarily based on human data.

In premenopausal women 17-β estradiol is the dominating type of estrogen. The estradiol level in women is in general several folds higher than in men until menopause [38, 39], but the concentrations of sex hormones fluctuate during the menstrual cycle. During the menses period in the early follicular phase (FP) the concentration of estradiol and progesterone are low, whereas the concentration is peaking just before ovulation (~day 14 of the regular cycle) and remains at a high level during the following luteal phase (LP) until next menses.

Estrogens primarily bind to the estrogen receptors (α & β) in the nucleus, but there are also bindings sites in the plasma membrane [40, 41]. Estrogen receptor distribution and the relative isoform (α- and β estrogen receptors) predominance varies within different tissue types and the expression is influenced by the estrogen level [42–45]. Activation of the two types of estrogen receptors seems to induce both similar and opposing effects [46, 47]. -Estrogen-binding to the receptor induce receptor activation, but also other stimuli can independently of estrogen-binding influence signaling pathways linked to estrogen receptor activation [48, 49].

Influence of Estrogen on Tendon and Ligaments

To get closer to an elucidation of the effect of estradiol on tendon and ligament it is relevant to compare results from women tested in the distinct menstrual phases or women with low compared to high endogenous concentrations of estradiol independent of menstrual phases.

In a systematic review from 2006, six of nine studies observed no significant effect of menstrual cycle on knee laxity [50]. Nevertheless, a meta-analysis of the data show that knee laxity was significantly greater around ovulation, when estrogen is peaking, medium in the LP and lowest in the FP [50]. The findings are supported by later data [51–53], but not all [18, 54]. The cyclic variation in knee laxity seems to be repeated in each menstrual cycle, but the magnitude and pattern of cyclic changes varying greatly among women [55]. An inverse relationship between circulating estradiol concentration and tendon stiffness has been observed in female handball players independent of cycle phase [28]. Similarly, cyclic changes in Genu recurvatum and general joint laxity have been reported, which underlines a systemic effect [55]. The greater knee laxity associated with high levels of estrogen seems influence landing biomechanics [56] and thereby the risk of injury. In support, exposure to high level of estrogen reduce maximal load to failure in rabbit anterior cruciate ligaments [57].

Until recently, a mechanistic explanation for the rapid changes in tendon and ligament laxity during the cycle was missing, since it is very unlikely that it is caused by any significant change in collagen content within days. Though, new *in vitro* findings have shown that short-term (24 h or 48 h) exposure to physiological concentration of estrogen decreases mechanical function of engineered ligaments by inhibiting the activity (61–77 %) of the crosslinking enzyme lysyl oxidase [58]. If the enzyme activity is similarly reduced *in vivo* when exposed to high level of estrogen it may explain the observations of greater knee laxity and a risk of sustaining an ACL injury around ovulation [58].

Inter-individual difference in the average level of estrogen between women may also influence the tendon and ligament structure and biomechanical properties over time. A positive correlation between circulating estrogen and human tendon collagen synthesis has been reported [27] (Fig. 13.3). Furthermore, in elderly hysterectomized women who were long-term users of estrogen replacement therapy (ERT), tendon collagen synthesis was higher and patellar tendon relative stiffness was lower compared to age matched postmenopausal women with very low levels of estrogen [27]. The latter observation may be explained by a general high tendon

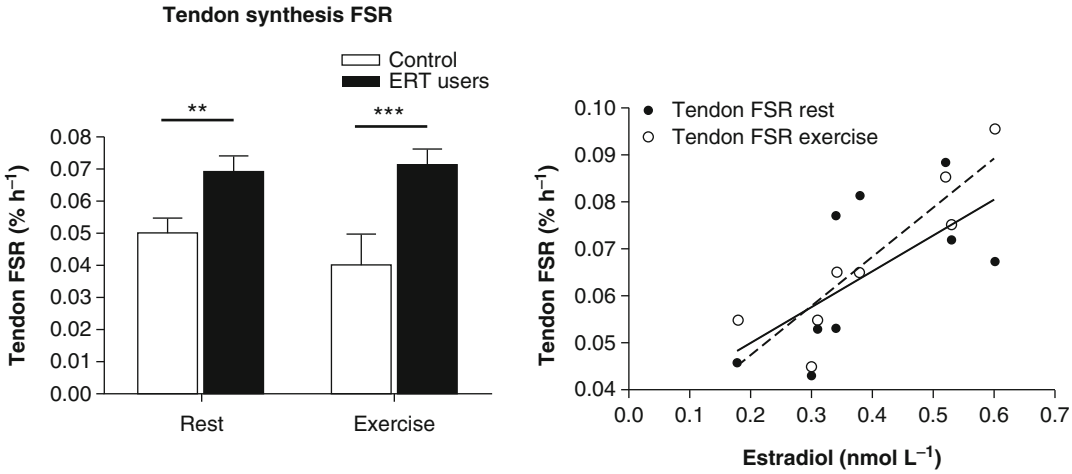

Fig. 13.3 *Top left:* patellar tendon collagen fractional synthesis rates (FSR) at rest and 24 h after exercise in postmenopausal women who used ERT and postmenopausal women who did not use ERT (control). $^{**}P < 0.01$ and $^{**}P < 0.001$, unpaired *t*-test, control vs. ERT users. *Top right*: relationship between tendon FSR and serum (s)-estradiol in ERT users at rest ($r^2 = 0.41$, $P = 0.06$) and postexercise ($r^2 = 0.80$, $P < 0.001$)

collagen turnover at high levels of estrogen since the collagen content was not changed. An enhanced tendon collagen turnover may indirectly reduce the possibilities for intra- and intermolecular cross-linking by enhancing the tendon collagen turnover on the top of a probably direct inhibiting effect on crosslinking enzymes [58]. In line with a lower tendon collagen synthesis in postmenopausal women not using ERT, estrogen deficiency in rats has been associated with down-regulation of fibroblast cell proliferation and density in the Achilles tendon [59].

It should be noted that estrogen may have divergent effect on the biomechanical properties of collagen rich tissues placed in anatomical distinct positions due to a disparity in loading profile and differences in relative distribution and numbers of estrogen receptors (α and β) [46, 60]. Knowledge in this field is very limited, but as an example, high versus low circulating estrogen in postmenopausal women has been found to be associated with reduced relative stiffness of the patellar tendon [27], which is rich in type I collagen as most tendon and ligaments (~60 %) [61]. Similarly, the rise in estradiol around ovulation [50] and during pregnancy [62] in premenopausal women is associated which enhanced knee joint laxity. In contrast, in arcus tendinous fasciae pelvis, which is rich in type III collagen (82 % of the total amount of collagen) the tensile strength of the tissue and the susceptivility to anterior vaginal wall prolapse are low in postmenopausel women charachterized by low estrogen level [63]. Similarly, hip joint flexibility was observed to be lower at low estrogen levels. The latter observation may be related to the content of type I collagen and the type I to (Type III + IV) ratio was observed to be lower in postmenopausal women compared to postmenopausal women on hormone replacement therapy (HRT) [63]. Similarly, low estrogen status may be detrimental for tendon and ligament healing [59]. In oophorectomized rats maximal stress upon the Achilles tendon, cell proliferation and density was reduced compared to sham-operated rats [59].

To sum up, effects of estrogen on tendon and ligaments are not fully elucidated and seem to depend on the age and the hormonal status of the women, the specific type of tendon and ligament in focus, and the degree of mechanical loading of the tissue. In women, presence of estrogen in contrast to low or lack of estrogen may be beneficial when tendon and ligaments are exposure to regular tissue loading [64] or recovering after an

injury as shown in animal studies [59, 65], since estrogen seem to have a stimulating effect on type I collagen synthesis in tendon [27]. Conversely, in active young female athletes physiological high concentration of estrogen may enhance the risk of injuries since a high level of estrogen seems to reduce the responsiveness to mechanical loading in collagen synthesis [49, 66] and is coupled to enhanced joint laxity [6]. The latter may be coupled to a greater risk of tendon and ligament injuries [5].

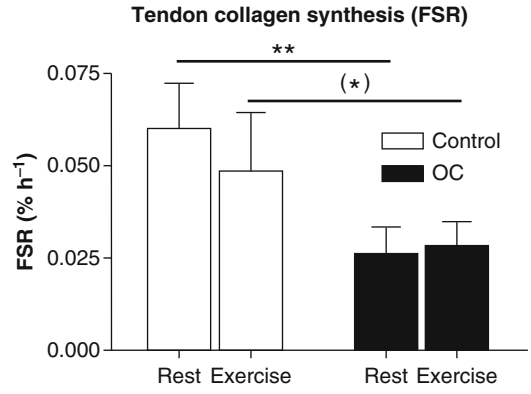

Fig. 13.4 Patellar tendon collagen fractional synthesis rates (FSR) at rest and 24 h after exercise in controls and oral contraceptive (OC) users. Values are means ± SE. Controls, women who had never used oral contraceptives who were tested in the follicular phase; OC, OC users. $^{**}P < 0.01$, (*) $P = 0.13$, unpaired t-test, control vs. OC

Influence of Oral Contraceptives on Tendon and Ligaments

Worldwide more than 100 million premenopausal women are using oral contraceptives (OC) [67]. Nevertheless, knowledge regarding the effect of OC on tendon and ligaments is sparse. Yet, use of OC in premenopausal women seems to influence tendon and ligaments differently than use of oral administration of ERT in elderly women [27, 36]. High endogenous estradiol is associated with high tendon collagen synthesis [27], whereas tendon collagen synthesis is lower in young OC users compared to control [36]. This may be related to OC has a marked inhibiting effect on the IGF-I level [26, 36]. Furthermore, in contrast to endogenous estradiol or ERT, cross-sectional data suggest that OC use reduces knee joint laxity in young athletes at ambient temperature [53, 68, 69], but not in all studies [28, 52, 70] and not after warming up the leg to 38° [53]. If tendon and ligament laxity is reduced in OC users compared to non-OC users, as in men, it might explain why OC users and men experience greater muscle damage and more delayed muscle soreness after heavy exercise than non-OC users [69, 71, 72], since a flexible tendon reduces the tensile loading on the connected muscle-tendon junctions and contractile muscle filaments during muscle contractions [73].

Use of OC inhibits endogenous secretion of estradiol and thereby the fluctuations in estrogen during the menstrual cycle [74]. In conjunction with this, the fluctuation in knee joint laxity during the menstrual cycle disappears [53], which diminished the enhanced risk of ACL injury in the ovulation phase [75]. Likewise, prospective data show no periodicity in non-contact ACL injury and ankle sprains in OC users in contrast to non-OC users. However, in the latter trial no overall significant difference in injury risk between groups was observed [76]. Actually, use of OC may have negative impact on tendon adaptation to regular training and thereby the risk of injury in athletes based on a lower tendon collagen synthesis rate (Fig. 13.4) and a reduced responsiveness to mechanical loading [35, 36].

Data on the effect of OC on tendon and ligament injury risk is limited. In a 12 month prospective study including Swedish female soccer players a lower rate of traumatic knee and ankle injuries was reported in the group of OC users (13 of 33 women) compared to in non-OC users (29 of 51 women) [77]. Furthermore, in a study including 4497 operatively treated ACL cases and 8858 age-matched controls with no ACL injury, OC use was linked to a lower relative ACL injury risk [78]. Nevertheless, in the latter study they did not control for sport participation which may differ between OC and non OC users. Furthermore, two studies have reported that OC does not seem to exert any protective effect on

ACL injury risk in skiers [7, 79]. Also, a higher risk of symptomatic Achilles tendinopathy has been reported in OC users [80]. Therefore, additional clinical studies are needed to clarify the biological and causal association between OC use and injury risk.

Influence of Androgen and Relaxin on Tendon Structural and Biomechanical Properties

Knowledge in relation to the effect of androgens on human tendon and ligament is limited and the exact role remains elusive. Androgen receptors have been localized in human ACL tissue [81], which seems to be a androgen-responsive tissue.

In women, testosterone, the most abundant androgen, fluctuates – although at low concentrations - during the menstrual cycle and is enhanced around the ovulation [81]. The level of testosterone is positively correlated with ACL stiffness, whereas estrogen and the estrogen to testosterone ratio are negatively correlated to ACL stiffness [81]. Since knee laxity is enhanced around ovulation [50] this suggests that estrogen seems to have the dominating influence on tendon and ligament biomechanical properties in women. Nevertheless, testosterone may have a potential counteracting role. In support of the later, animal data indicates that testosterone changes estrogen receptor α and β expression in upper site directions [82, 83] and reduces the stimulating effect of estrogen on mammary epithelial proliferation [83, 84].

In men, the level of testosterone is 7–8 times higher in men than women [85] and this is coupled to a higher tendon synthesis [37], a lower knee laxity [18], whereas tendon stiffness is higher [17]. It should be noted that the circulating concentration of estrogen is positively correlated to high tendon collagen synthesis rates in women [27]. Therefore, it can be hypothesized that testosterone has a more pronounced role on tendon and ligaments in men than estrogen in women, but this statement needs to be confirmed. An anabolic effect on testosterone on tendon collagen content and thereby tissue stiffness is supported by animal findings showing that testosterone increases collagen content in prostate [84, 86] and hip joint capsule [87], and reduces passive range of motion of the patellar tendon and ligament [88]. Furthermore, *in vitro* findings have shown that testosterone administration in high doses to human cultured tenocytes increased tenocytes number and changed the phenotype after short-term exposure [89].

Animal data suggest that testosterone may indirectly reduce tendon and ligament laxity by downregulating the expression of relaxin receptors [88] and thereby the effects of relaxin. Relaxin is linked to activation of collagenases, increased tendon laxity, enhanced risk of ACL injury, but also to improved ligament healing [90–92]. Interestingly, in contrast to testosterone, recent animal data suggest that progesterone and high doses of estrogen administration are coupled to enhanced knee laxity and enhanced expression of relaxin receptors [93]. Therefore, on the top of a likely direct effect of estrogen and androgens on tendon the sex hormones may indirectly influence tissue stiffness through changing the responsiveness to relaxin.

Testosterone may also have a positive influence on tendon and ligament adaptation to training, but the human data are primarily based on cross-sectional data comparing men and women [23]. Tendon synthesis rate in response to exercise is greater in men than women [23]. In addition, in elderly men a greater increase in tendon stiffness after 12 weeks of alpine skiing training has been observed compared to in postmenopausal women [94]. The latter should be seen in light of circulating estrogen levels is comparable between elderly men and women, whereas circulating testosterone is still enhanced in the men [11].

Similarly to estrogen, effect of testosterone on tendon and ligament injury risk is probably dependent of the physiological concentration and balance between unbeneficial effects of low, but also of high testosterone concentrations induced by administration of androgens.

Case reports indicate that high levels of testosterone or synthetic derivatives of testosterone may enhance the risk of tendon and ligament injuries [95–98]. Furthermore, analysis of human patellar tendon morphology and mechanical properties indicates that adaptation to strength training is influenced by long-term use of androgenic anabolic steroids [99]. This is supported by animal data which suggests that anabolic androgenic steroids reverse the beneficial effect of exercise on Achilles tendon adaptation, thus resulting in inferior maximal stress values [100]. The higher risk of tendon rupture in anabolic steroid users may be directly caused by a direct effect on tendon or may also be indirectly related to the enhanced muscle hypertrophy and gain in muscle strength which is not balanced by a similar degree of adaptation in the connected tendon [101, 102].

Conclusion

Sex differences in tendon biomechanical properties, tendon morphology and tendon collagen turnover suggests that sex hormones play an explanatory role in relation to injury risk. However, it is difficult to sort out the isolated effect of the sex hormones in cross-sectional studies comparing women and men or users and non-users of sex hormonal administration, since the groups may differ in many other ways e.g. training status. Nevertheless, both estrogen and testosterone in balanced physiological concentration seems to be important for tendon health and physical function, whereas very low or high concentrations of endogenous or exogenous administrated sex hormones may led to an enhanced risk of injuries and inadequate adaptation to mechanical loading.

The research up to now has primarily focused on the ACL, Achilles, and the patellar tendon. The future may elucidate whether sex hormones influence tendons and ligaments differently depending on their localization and function.

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