Impact of sex and chronic resistance training on human patellar tendon dry mass, collagen content, and collagen cross-linking

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LeMoine JK, Lee JD, Trappe TA. Impact of sex and chronic resistance training on human patellar tendon dry mass, collagen content, and collagen cross-linking. Am J Physiol Regul Integr Comp Physiol 296: R119–R124, 2009. First published October 22, 2008; doi:10.1152/ajpregu.90607.2008.—Collagen content and cross-linking are believed to be major determinants of tendon structural integrity and function. Sex and chronic resistance training have been shown to alter tendon function and may also alter the key structural features of tendon. Patellar tendon biopsies were taken from untrained men (n = 8, 1 repetition maximum (RM) = 53 ± 3 kg), untrained women (n = 8, 1 RM = 29 ± 2 kg), and resistance-trained (10 ± 1 yr of training) men (n = 8, 1 RM = 71 ± 6 kg). Biopsies were analyzed for dry mass, collagen content, and collagen cross-linking (hydroxylysylpyridinoline). We hypothesized that these elements of tendon structure would be lower in women than men, whereas chronic resistance training would increase these parameters in men. Tendon dry mass was significantly lower in women than men (343 ± 5 vs. 376 ± 8 μg dry mass/mg tendon wet wt, P < 0.01) and was not influenced by chronic resistance training (P > 0.05). The lower tendon dry mass in women tended to reduce (P = 0.08) collagen content per tendon wet weight. Collagen content of the tendon dry mass was not influenced by sex or resistance training (P > 0.05). Similarly, cross-linking of collagen was unaltered (P > 0.05) by sex or training. Although sex alters the water content of patellar tendon tissue, any changes in tendon function with sex or chronic resistance training in men do not appear to be explained by alterations in collagen content or cross-linking of collagen within the dry mass component of the tendon.

human tendon; water content; hydroxyproline; hydroxylysylpyridinoline

TENDONS ARE THE FIBROELASTIC structures that connect muscle to bone and convey muscular force (5, 22, 23). The tensile strength of tendon tissue is estimated to be 50–100 N/mm² (5), a key feature as tendons are subjected daily to high forces. The composition of tendon tissue influences its strength and integrity, playing a key role in force transmission through the muscle-tendon complex (23). Animal and human cadaver studies indicate that tendon tissue is ~55–70% water (5, 23), with the remaining dry mass containing the extracellular matrix and tendon cells (5, 22). Collagen is the main protein component of tendon tissue, comprising ~65–75% of the dry mass (5, 22, 23). Collagen molecules are joined into fibrils and fibers by lysine-based cross-links [hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP)] (7, 22, 23, 30). Tendon dry mass, collagen content, and cross-links have been shown to impact tendon strength and mechanical properties (22, 23, 30, 52).

Tendon tissue is dynamic, highly metabolic, and quite responsive to exercise (23, 24, 29, 34). Tendons should therefore adapt in a positive manner to long-term loading, becoming more damage resistant and ensuring optimal muscular force transmission (23). In humans, acute exercise stimulates collagen synthesis (29, 39), and chronic exercise appears to increase tendon strength via structural adaptations at the whole tendon and the molecular level. With training, tendon cross-sectional area (CSA) is increased (24, 25, 35, 46), tendon mechanical properties are improved (27, 44), and there is a net positive balance of collagen (28). Although the impact of exercise training on collagen content and cross-linking in human tendon tissue is unknown, animal data show increased collagen content with chronic training (37, 54), accompanied by increased CSA and altered tendon mechanics (54). Taken together, these data indicate that the acute and chronic response of the tendon is to increase collagen content and/or the degree of collagen cross-linking, thereby improving tendon tissue integrity and force transmission.

Incidence of training-related tendon pathologies is significantly greater in women than men (10, 17, 21), and women <30 yr of age may be at the greatest risk for overuse injuries (31). The cause of this sex-related disparity is unknown, but female tendon tissue may have less collagen and cross-linking than male tendon tissue. Lower collagen content and less collagen cross-linking in women may also contribute to the potentially inferior tendon mechanics in women, namely, increased tendon strain and stress with decreased stiffness (26, 33, 53). Animal data show significantly less collagen per tendon weight in female than male tendons (36). In humans, tendon collagen synthesis is depressed in women compared with men at rest and after acute exercise (38). Additionally, the characteristic increase in tendon size that occurs in men with training does not occur in women (33, 53). These sex-related differences may be due to the biology of tendon cells, which have sex hormone receptors (17), as collagen protein synthesis after exercise is blunted by increased estradiol (33).

We determined dry mass, collagen content, and collagen cross-linking using patellar tendon biopsy samples from three groups (21–35 yr of age): recreationally active men, recreationally active women, and chronically resistance-trained men (≥3 yr of consistent training). We hypothesized that these tendon structural characteristics would be lower in women than men and would be increased by chronic resistance training in men.
MATERIALS AND METHODS

Subjects

Three groups of healthy young adults (21–35 yr of age) were recruited to participate in the study: untrained men (n = 8), untrained women (n = 8), and chronically resistance-trained men (n = 8). Subject characteristics are presented in Table 1. Before enrollment into the study, subjects gave written consent for participation and completed a detailed health history questionnaire and interview regarding their health and exercise-training history. All individuals were apparently healthy and had no history of tendon pathologies. The study was approved by the Institutional Review Board of Ball State University.

All subjects in the untrained groups were recreationally to moderately active; i.e., the subjects did not perform aerobic or resistance exercise on a regular basis and did not participate in organized sports. At the time of the study, three of the eight resistance-trained men were engaged in competitive Olympic weightlifting, and one other subject had an extensive history of participation in the sport. Minimum requirements for resistance-trained men were as follows: they were engaged in resistance training, including lower-body training, ≥4 days/wk, ≥1 h/day, 10–15RM, and titrated to pH 8–13. HYP (model 56250, Sigma, St. Louis, MO) was quantified by HPLC and fluorometric detection (1100 Series, Agilent Technologies, Wilmington, DE) via the precolumn derivatization method previously described (19, 20) with modifications for human tendon. Briefly, tendon biopsy samples were placed in 6 M HCl (2 mg tendon wet wt/ml HCl) and hydrolyzed for 24 h at 100°C. A 250-μl aliquot of hydrolysate was removed and added to 750 μl of 6 M HCl; then the diluted hydrolysate was neutralized with 1 ml of 6 M NaOH and titrated to pH 8–13. HYP (model 56250, Sigma, St. Louis, MO) standards (1, 10, 50, 100, 150, 200, and 250 μM HYP) were prepared in water.

A 900-μl aliquot of each sample/standard was transferred to a 12 × 75 mm borosilicate tube, and 200 μl of borate buffer (0.7 M boric acid, pH 9.5), followed by 100 μl of OPA solution [50 μg of o-phthalaldehyde dissolved in 974 μl of acetonitrile (ACN) and 26 mg of β-mercaptoethanol], 100 μl of iodoacetamide reagent (140 mg/ml iodoacetamide in ACN), and 300 μl of 5 mM FMOC solution (9-fluorenylethylchlorormiformate in acetonitrile) were added to the sample/standard. Tubes were vortexed, and 60 s elapsed after the addition of each reagent. Ethyl ether (2 ml) was added to each tube, which was then tightly capped and vigorously shaken for 30 s to wash the contents. The organic layer was discarded, and the wash was repeated twice for a total of three washes. Derivatized samples and standards were injected onto the HPLC (1100 Series, Agilent Technologies) via an autosampler (5 μl) every 40 min with an intervening wash step. All samples and standards were run in triplicate. HYP separation was achieved via 4.6 mm column (Waters, Milford, MA) using an isocratic mobile phase [65% acetic acid–35% acetonitrile (3% glacial acetic acid and sodium acetate buffered to 4.3)] at a
flow rate of 1.0 ml/min. Peaks were monitored at 260 nm excitation/316 nm emission with a gain of 8 and integrated with chromatography software (ChemStation, Agilent Technologies). HP concentration was determined and used to calculate collagen content, as previously described (6, 20).

**HP and LP cross-link content.** Molecular cross-linking of collagen via lysine-based cross-links, purported to be the most prevalent cross-linking pathway in collagen (7, 30), was assessed by measurement of HP via HPLC and fluorometric detection (1100 Series, Agilent Technologies), as previously described (1, 2) with modifications for human tendon. HP and LP separation was achieved with an XTerra RP 18, 5-μm, 250 mm × 4.6 mm column (Waters) that had been equilibrated with 0.13% HFBA in 22% methanol (mobile phase A). Cross-links were then eluted with a 1.0 ml/min flow rate with mobile phase A followed by a secondary mobile phase using 0.1% HFBA in 75% ACN (mobile phase B). Fluorescence was monitored at 295 nm excitation/395 nm emission at a gain of 14, and peaks were integrated with chromatography software (ChemStation, Agilent Technologies). Chromatographs indicated that LP was present in only some human tendon samples at very low levels (not reliably reproducible); therefore, we do not report values for LP.

**Statistics**

For each variable (subject characteristics, tissue dry mass, collagen content, and collagen cross-links), a two-tailed t-test was used for group comparisons. Group comparisons were as follows: 1) men vs. women and 2) men vs. resistance-trained men. Significance was accepted at Ρ < 0.05. Values are means ± SE.

**RESULTS**

Men had a significantly greater amount of tendon dry mass than women (376 ± 8 vs. 343 ± 5 μg dry mass/mg tendon wet wt, Ρ < 0.01). The lower dry mass in women reduced (Ρ = 0.08) collagen content per wet weight tendon (339 ± 14 and 306 ± 11 μg collagen/mg tendon wet wt in men and women, respectively). Figure 1 compares the in vivo patellar tendon composition of men and women: tissue dry mass per tendon wet weight and collagen content per tendon wet weight.

When normalized to tendon dry mass, collagen content was comparable in men and women (903 ± 38 and 892 ± 29 μg collagen/mg tendon dry mass, respectively, Ρ > 0.05). HP cross-linking of collagen was similarly unaltered by sex (401 ± 47 and 418 ± 35 mmol HP/mol collagen in men and women, respectively, Ρ > 0.05). Figure 2 compares the dry tendon material composition of untrained men and women: collagen content per tendon dry mass and HP cross-linking to collagen.

Chronically resistance-trained men (10 ± 1 yr of resistance training) demonstrated no higher levels of either parameter than untrained men. Tendon dry mass was unaltered with chronic resistance training (376 ± 8 and 364 ± 20 μg tendon dry mass/mg tendon wet wt in untrained and resistance-trained men, respectively). Collagen content normalized to tendon wet weight was unchanged by training (339 ± 14 and 315 ± 10 μg collagen/mg tendon wet wt in untrained and resistance-trained men, respectively). Figure 3 compares the in vivo patellar tendon composition of untrained and chronically resistance-trained men.
between men and RT men. No differences (P > 0.05) were seen in these variables between men and RT men.

No differences (P > 0.05) were seen in collagen content of the tendon dry mass between these two groups (903 ± 38 and 881 ± 43 μg collagen/mg tendon dry mass in untrained and resistance-trained men, respectively). HP cross-linking of collagen was similarly unaltered by training (401 ± 47 and 424 ± 38 mmol HP/mol collagen in untrained and resistance-trained men, respectively, P > 0.05). Figure 4 compares the dry tendon material composition of untrained and resistance-trained men.

Tissue dry mass was lower in peritendon than tendon samples (361 ± 7 μg tendon dry mass/mg tendon wet wt vs. 166 ± 3 μg peritendon dry mass/mg peritendon wet wt). Collagen content per wet weight was therefore lower in peritendon than tendon tissue (320 ± 7 collagen/mg tendon wet wt vs. 123 ± 8 μg collagen/mg peritendon wet wt). Collagen content of the dry mass was only slightly higher in tendon than peritendon tissue (892 ± 20 μg collagen/mg tendon dry mass vs. 773 ± 90 μg collagen/mg peritendon dry mass). HP cross-linking was comparable in tendon and peritendon tissue (415 ± 23 and 412 ± 11 mmol HP/mol collagen in tendon and peritendon, respectively).

Coefficients of variation for triplicate injections were low for HYP (0.26 ± 0.06% and 0.83 ± 0.16% for standards and samples, respectively) and HP (0.54 ± 0.25% and 0.55 ± 0.11% for standards and samples, respectively). Coefficients of variation for assays of separate aliquots from the same tendon sample were also low (0.32% for HYP and 0.82% for HP).

DISCUSSION

The aims of this investigation were to determine the impact of sex and chronic resistance training on tissue dry mass, collagen content, and collagen cross-linking of human patellar tendon. The main findings were as follows: 1) sex decreased dry mass and tended to decrease collagen content per wet weight tendon but did not change the composition of dry tendon tissue, and 2) chronic resistance training (for 10 ± 1 yr) did not alter dry mass, collagen content, or collagen cross-linking.

As hypothesized, in vivo patellar tendon composition was altered by sex. Women had significantly less dry mass per tendon wet weight (37.6 ± 0.9% vs. 34.3 ± 0.5% dry mass) and a strong trend toward less collagen per tendon wet weight (33.9 ± 1.4% and 30.6 ± 1.1% collagen for men and women, respectively, P = 0.08). Women may synthesize less tendon material overall per tendon size, resulting in lower overall tendon dry mass and collagen content. Comparisons of tendon size and collagen synthesis in men and women support this theory. Tendon CSA relative to body size does not differ between sexes (33), but tendon collagen synthesis is significantly lower in women than men (38). Estrogen directly alters collagen kinetics (8, 15, 17), and inherently higher estrogen levels in women may therefore chronically depress collagen production, as tendon cells have estrogen receptors (8, 17). Thus, blunted collagen production via estrogen could explain the lower amount of dry mass in female tendons, as collagen comprises ~90% of dry mass.

Women may bind more water per given amount of tendon tissue, decreasing dry mass and collagen per wet weight tendon. Estrogen and progesterone administration significantly increased plasma volume in women (49), and estrogen also increased water retention during dehydration (48). These hormones may therefore affect tendon tissue, again because of the presence of estrogen receptors on tendon cells (8, 17). Greater water infiltration of tendon tissue would increase interfibrillar spacing and decrease the overall amount of collagen in tendon tissue. The concept that increased tissue water content weakens women’s tendons is supported by findings from human cadavers that air-dried plantaris tendons were 50% stronger than damp tendons (52).
Contrary to our hypotheses, collagen content per dry mass and HP cross-linking of collagen were equal in women and men. On the basis of the similarity of these structural components of tendon dry mass, collagen quality may be inferior in women. Type I collagen dominates in tendon tissue (22) and has high tensile strength and low elasticity (4, 22). Type III collagen is also present in tendon tissue; it is structurally similar to type I collagen but forms thinner, weaker fibers (4, 40). The exact proportions of type I and III collagen in healthy human tendon tissue are unknown, as are potential sex differences. It has been shown, however, that type III collagen mRNA is elevated in women compared with men (50), whereas type I collagen is decreased at tendon rupture sites (13) and type III collagen is increased in degenerated tendons (13, 45). Thus the apparent weakness of female tendons (3, 10, 21, 26) may be due to a greater amount of type III collagen in women than men.

Contrary to our hypotheses, in vivo tendon composition and the nonwater, biosynthesized material of the tendon appear unaltered by chronic resistance training in men. In light of the mechanical improvements in human tendon with exercise training (24, 25, 27, 44), the lack of change in dry mass, collagen, or cross-linking in resistance-trained men was quite surprising. Previous data from humans (24, 25, 32, 46) and animals (41, 54) demonstrate increased tendon CSA with training of sufficient duration. Increased tendon CSA with training is associated with improved force transmission and tendon strength (24, 54). Given that the members of the resistance-trained group had trained consistently for an average of 10 yr, with training programs of intensity and duration at or above those that have been shown to induce tendon alterations, it is likely that tendon CSA was larger in these resistance-trained men than in the untrained men. However, it appears that the tendon material is altered in proportion to the change in tendon size, maintaining equal proportions of dry mass, collagen content, and collagen cross-linking in trained and untrained men.

To our knowledge, this investigation provides the first report of tendon tissue dry mass, collagen content, and collagen cross-linking from in vivo healthy human patellar tendon samples. Previous data from human cadaver and animal models (5, 9, 14, 23, 47) indicate that tendon tissue is ~30–45% dry mass. In healthy, untrained men, we show that 37.6 ± 0.9% of the tendon is composed of dry mass. Similarly, the collagen content of nonpathological human tendon has been studied only in cadavers (5, 12, 51). The present study demonstrated that collagen comprises a greater amount of the tendon’s wet weight: 33.9 ± 1.4% (combined mean of all 3 groups) vs. ~20% in previous investigations (12). Additionally, collagen was shown to comprise 90.3 ± 3.8% (combined mean of all 3 groups) of the patellar tendon dry mass vs. ~60–75% in previous cadaver studies (5, 51). These differences are likely due to tissue sampling and collection methods (in vivo tendon biopsy vs. cadaver tissue) or differences in subject groups, as earlier data are mostly derived from older individuals (5, 51).

Perspectives and Significance

Knowledge of tissue dry mass, collagen content, and collagen cross-linking of in vivo healthy human tendon is key in understanding tendon structure and integrity, given that collagen comprises the vast majority of the nonwater tendon tissue. The present study examined the impact of two separate conditions that have been shown in the literature to alter tendon strength and collagen kinetics: sex and chronic exercise training. In the patellar tendon of women, lower overall tissue dry mass and potentially lower collagen content may indicate structural discrepancies that predispose women to tendon injury. Chronic resistance training did not alter the tendon on the level of its dry mass, collagen content, or collagen cross-linking. The lack of change in these parameters with chronic resistance training suggests that resistance training in women may not ameliorate their deficiency in tendon dry mass and collagen content. Future investigations should examine other material/molecular elements of the patellar tendon, such as other cross-link species and the proteoglycan structures, which may be altered by sex or exercise.

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