Eccentric rehabilitation exercise increases peritendinous type I collagen synthesis in humans with Achilles tendinosis

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It has been shown that 12 weeks of eccentric heavy resistance training can reduce pain in runners suffering from chronic Achilles tendinosis, but the mechanism behind the effectiveness of this treatment is unknown. The present study investigates the local effect of an eccentric training regime on elite soccer players suffering from chronic Achilles tendinosis on the turnover of the peritendinous connective tissue.

Twelve elite male soccer players, of whom six suffered from unilateral tendinosis and six were healthy controls, participated in this study. All participants performed 12 weeks of heavy-resistance eccentric training apart from their regular training and soccer activity. Before and after the training period the tissue concentration of indicators of collagen turnover was measured by the use of the microdialysis technique. After training, collagen synthesis was increased in the initially injured tendon (n = 6; carboxyterminal propeptide of type I collagen (PICP): pre 3.9 ± 2.5 µg/L, to post 19.7 ± 5.4 µg/L, P < 0.05). The collagen synthesis was unchanged in healthy tendons in response to training (n = 6; PICP: pre 8.3 ± 5.2 µg/L, to post 11.5 ± 5.0 µg/L, P > 0.05). Collagen degradation, measured as carboxyterminal telopeptide region of type I collagen (ICTP), was not affected by training neither in the injured nor in the healthy tendons. The clinical effect of the 12 weeks of eccentric training was determined by using a standardized loading procedure of the Achilles tendons showing a decrease in pain in all the chronic injured tendons (VAS before 44 ± 9, after 13 ± 9; P < 0.05), and all subjects were back playing soccer following the eccentric training regime.

The present study demonstrates that chronically injured Achilles tendons respond to 12 weeks of eccentric training by increasing collagen synthesis rate. In contrast, the collagen metabolism in healthy control tendons seems not to be affected by eccentric training. These findings could indicate a relation between collagen metabolism and recovery from injury in human tendons.

Overuse injuries of Achilles tendons, which results in chronic pain and a localized thickening of the tendon, are a major problem among middle-aged athletes (Clement et al., 1981; Kvist, 1994; Astrom & Rausing, 1995). Achilles tendon pain is often associated with a gradual onset, and structural changes of the tendon can be visualized on ultrasonography and magnetic resonance imaging (Neuhold et al., 1992; Fredberg et al., 2004). Previously it was believed that inflammation was the key contributor to these tendon overuse problems, but recent data do not support this explanation. For example, histological analysis of the localized thickening in painful Achilles tendons several days after a rupture or postmortem has shown areas with a high concentration of glycosaminoglycans and irregular fiber structure and arrangement but with a striking absence of inflammatory cells and inflammatory mediators (Movin et al., 1997; Alfredson et al., 1999). This has led to the assumption that chronic Achilles tendon disorder is a result of a degenerative process (Jozsa et al., 1990; Astrom & Rausing, 1995) hypothetically because of inadequate adaptation of the tendon to changes in loading pattern.

Treatment involving heavy load eccentric training has been shown to provide good clinical results in the treatment of Achilles tendinosis, with a decrease in pain and a higher percentage of return to the previous level of physical activity (Alfredson et al., 1998; Fahlstrom et al., 2003). However, data to support the pathophysiological background for these effects are few. A recent paper demonstrates that 12 weeks of eccentric training is associated with a normalization of the tendon structure and a decreased thickness (Ohberg et al., 2004). This illustrates the dynamic nature of the response to loading of the human tendon. In healthy tendons, it has been shown in both animal and humans that collagen turnover, and in animals cross-sectional area and tensile strength can be increased with physical training.
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(Tipton et al., 1975; Kiiskinen, 1977; Suominen et al., 1980; Michna & Hartmann, 1989; Simonsen et al., 1995; Rosager et al., 2002). Interestingly, it has been shown that physical training initially increases both synthesis and degradation of collagen (Langberg et al., 1999a, 2001) and only results in a net collagen synthesis after a long training period (Langberg et al., 2001). It is therefore hypothesized that overuse injury is a result of a mismatch between mechanical loading and adaptation of collagen, and that controlled eccentric rehabilitation exercises will increase collagen formation. In this study we investigated, using a microdialysis technique, the effect of a 12-week eccentric rehabilitation program on local collagen turnover in the area surrounding the Achilles tendon in high-level soccer players with chronic Achilles tendon disorders.

Methods

Subjects

Six elite male soccer players (highest division (Superliga) to third division) participated in the study, all with unilateral tendinosis (26 ± 1 years; BMI: 22 ± 1; duration of symptoms: 19 ± 7 months; positioning of the pain: 30–60 mm above the Achilles tendon insertion on calcaneus). The non-injured Achilles tendons on the non-injured leg functioned as a control. The results of the non-injured tendons were compared with data obtained on the tendons of six healthy elite male soccer players with no history of previous tendon problems (22 ± 1 years; BMI: 22 ± 1). None of the subjects were on any medication and they were all non-smokers. All subjects gave informed consent, and the study conformed to The Declaration of Helsinki and was approved by the Ethical Committee of Copenhagen (KF 01-157/98).

Experimental protocol

A standardized eccentric training program with two training sessions daily was performed for 12 weeks (Alfredson et al., 1998; Fahlstrom et al., 2003). The program is described in detail below. The concentration of markers of collagen type I synthesis and degradation in the interstitial tissue was determined before and after the 12 weeks of eccentric training. As training was performed on a daily basis, subjects were always studied 16h after the last training bout to exclude early recovery after acute exercise (Langberg et al., 1999a) and to ensure that the measurements reflected the basal condition during the training period. All experiments were started at 09.00 hours. During the measurements the subjects lay prone with the ankle joints in a relaxed neutral position (70–80°) at a room temperature of 25°C.

Eccentric training regime

All the subjects were instructed in the eccentric training by a physiotherapist, the exercises were demonstrated and a written manual describing the program was given. The subjects were told to perform the program two times daily for the following 12 weeks. Each training session consisted of three sets of heel raises each comprising of 15 eccentric repetitions on straight leg (full weight on the injured leg, forefoot on a step, going from maximum heel lift to maximal dorsal flexion in the ankle joint, and using the healthy leg to lift back up to maximal heel lift position) (mainly loading m. gastrocnemii) and 15 eccentric repetitions with bent knee (mainly loading m. soleus). To increase loading of the tendon, the subjects were told to wear a backpack containing 20% of bodyweight (BW). This load was increased when the subjects could perform the eccentric training without experiencing increased pain immediately after the training regime or the following morning. The subjects were informed that the eccentric training regime could result in increased pain during the first 3–4 weeks, but that it was important to continue the rehabilitation despite the increase in pain. This is in accordance with the pragmatic advice given at present (Alfredson et al., 2003; Fahlstrom et al., 2003). The subjects were allowed to continue participation in soccer training if the pain had not increased. During the whole period the subjects were told to note information on pain, training sessions, load increase and participation in soccer training. This was collected at intervals of 3 weeks throughout the entire training period.

Microdialysis

A microdialysis probe was inserted, under ultrasound guidance, into the peritendinous space just ventral to the Achilles tendons (both legs), with the active part of the fiber covering the painful area 30–60 mm above the Achilles tendon insertion on calcaneus (identical to the position in the healthy tendons) as previously described (Langberg et al., 1999a, 2001). The microdialysis catheters (molecular mass cutoff 3000 kDa) were constructed and sterilized as described earlier (Langberg et al., 1999a), and perfused with a Ringer acetate solution via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden) at a rate of 2 μL/min 3 nM [3H]-human type IV collagen (130 kDa; specific activity: 7.0 TBq/mg; NEN, Perkin Elmer, Life Sciences Inc., Boston, MA, USA) was added to the perfusate in order to mimic the in vivo recovery of the carboxyterminal propeptide of type I collagen (PICP) as a marker for collagen synthesis and the carboxyterminal telopeptide region of type I collagen (ICTP) as an indicator of collagen breakdown using the internal reference method (Scheller & Kolb, 1991). Human type IV collagen was used as an internal reference, as it is comparable in molecular size with PICP, and because no radioactive-labeled type I pro-collagen was commercially available.

After blood samples were taken (as described below) and the microdialysis catheters were positioned, the subjects rested for at least 90 min before starting the experiment to ensure that any reaction from the insertion trauma had minimized (Langberg et al., 1999b). Dialysate samples were collected every 30 min. The samples were immediately frozen to minus 80°C until analyzed within the following 1–2 weeks.

Blood samples

Blood samples were drawn before insertion of the microdialysis catheters from the antecubital vein of the arm. The blood samples, used for determination of collagen synthesis and degradation were centrifuged at 2000 × g for 10 min at 4°C, and the plasma was stored at −80°C for subsequent analysis.

Calculations

The interstitial concentrations (Ci) were calculated using the internal reference calibration method as previously described (Langberg et al., 1999a). Relative recovery (RR) of the internal reference was measured and used for calculation of Ci for each sample separately.
Collagen synthesis and degradation analysis

The concentration of PICP and ICTP, respectively, was measured in duplicate samples of plasma and dialysate by equilibration radioimmunoassays (RIA) (Orion Diagnostica, Espoo, Finland). All samples from each individual subject were analyzed in the same run. The intra-assay precision (coefficient of variation) is 2.7% at 214 µg/L for PICP and 4.9% at 6.1 µg/L for ICTP (Orion Diagnostica).

Evaluation of pain during loading

The amount of pain during standardized eccentric loading of the Achilles tendon performed with an additional load of 25% of BW in a backpack was measured on a 100 mm long pain scale (VAS), with 0 mm referring to no pain and 100 mm to severe pain. The eccentric loading was performed as three consecutive eccentric contractions of the calf muscle with straight knee from full plantar flexion to full dorsal flexion (identical to the exercise used during the training regime). The subjects were told to rate the pain immediately after the standardized loading. Determination was performed before the 12-week training period began as well as following the 12 weeks training period: in both cases before microdialysis evaluation was performed.

Statistics

All data are presented as mean ± SEM or range. Wilcoxon’s signed ranks tests were used (SPSS; standard version 7.5.3) to examine changes within groups, and Mann–Whitney U-test to examine changes between healthy and injured tendons. P ≤ 0.05 (two-tailed testing) was considered significant.

Results

All subjects declared by written training dairies that they had performed the 12-week training regime as described.

Collagen synthesis and degradation

There were no significant differences in the data obtained between the healthy tendons of the injured subjects when compared with the results from the healthy tendons of healthy subjects (P > 0.05). For this reason, the healthy tendons of the injured subjects were used as internal controls in the patients.

No differences between the injured and the healthy tendons could be detected for collagen synthesis before training (Fig. 1; P > 0.05). After training, an increased interstitial tissue concentration of PICP (collagen synthesis) was measured by microdialysis in the painful tendons (pre-training 3.9 ± 2.5 µg/L to post-training 19.7 ± 5.4 µg/L, P < 0.05; Fig. 1). In contrast PICP was unchanged in healthy tendons in response to training (pre-training 8.3 ± 5.2 µg/L to post-training 11.5 ± 5.0 µg/L, P > 0.05; Fig. 1). ICTP (collagen degradation) concentration was not affected by training either in the injured tendons or in the healthy tendons (P > 0.05; Fig. 2). The mean RR for collagen was 89 ± 1%, with no significant differences in RR between the healthy and injured tendons or between pre- and post-training.

Pain during loading (VAS)

In the group with healthy tendons, the standardized loading of the Achilles tendons did not result in pain, either before or following the training (Fig. 3). In contrast, all the injured Achilles tendons generated...
Before training

After 12 wks training

Fig. 3. Eccentric training and pain from loaded Achilles tendon. A 100 mm long pain scale (VAS) was used to monitor the pain in the Achilles tendons during eccentric loading with an additional load of 25% of body weight (0 mm referring to no pain and 100 mm to severe pain). The subjects were told to rate the pain immediately after the loading with an additional load of 25% of bodyweight. A 100 mm long pain scale (VAS) was used to monitor the pain in the Achilles tendons during eccentric loading.

The results showed that the level of matrix metalloproteinase (MMP) in the peritendinous tissue increases immediately after repeated loading of the respective tendon (Koskinen et al., 2004), and that both acute and prolonged loading of the human Achilles tendon can result in increased synthesis of the collagen type I (Langberg et al., 1999a, 2001). This emphasizes that exercised tendons undergo a constant turnover (breakdown and synthesis) making adaptation to changes in loading possible. In contrast, it has been found that the expression of MMP3 is decreased and the concentration of MMP inhibitors (TIMPs) increased in degenerated human Achilles tendons compared with healthy tendons (Ireland et al., 2001). Thus, it can be hypothesized that the homeostasis of the connective tissue is disturbed in overuse-injured tendons, and that the positive effect of eccentric rehabilitation of chronic overused tendons (Alfredson et al., 1998; Fahlstrom et al., 2003) derives from a stimulation the synthesis of collagen type I (Fig. 1) as part of a repair or regeneration process in the injured tissue. This would also explain why no increases could be found around the healthy tendons. If the synthesis of collagen in the healthy tendons already is sufficient to maintain the homeostasis of the tendon tissue, additional loading is unlikely to lead to any increase in collagen synthesis of the tissue.

The mechanism behind the development of chronic Achilles tendon injuries is highly debated in the literature, but there seems to be consensus that inflammation is not involved in the chronic stage of this condition (Movin et al., 1997; Alfredson et al., 1999). It has been hypothesized that in-growth of vessels and nerve endings can account for the pain and swelling found in the localized thickening in painful Achilles tendons (Ackermann et al., 2002, 2003; Alfredson et al., 2003; Fredberg et al., 2004). To support this notion, investigations of the painful area in chronic mid-portion Achilles tendinosis by using ultrasonography and color Doppler have shown neovascularization in the painful tendinosis area, where no such finding was present in pain-free control tendons (Alfredson et al., 2003; Fredberg et al., 2004). In addition, it has been shown by various methods that a decrease in flow within this hypervascularized area by either eccentric training (Fahlstrom et al., 2003), ultrasound-guided sclerosing of the neovessels (Ohberg & Alfredson, 2002) or steroids around (Fredberg et al., 2004) or within the tendon (Koenig et al., 2004) all resulted in decreased pain in patients with chronic Achilles tendinosis.

Whether the increased synthesis of collagen type I demonstrated in the present study is involved in the reduction in hypervascularization is not known, but one can speculate that an increased collagen type I production in the peritendinous area could minimize the in-growth and flow in the new vessels. In a
previous study, we have shown that loading of the Achilles tendon by itself can result in increases in MMPs in the peritendinous area (Koskinen et al., 2004). As the present data show an increase in collagen synthesis in the peritendinous area around the Achilles tendon, one could hypothesize that this increase in synthesis is a result of repair of microtears leading to a reduced hypervascularization and decrease in pain.

Along with the increase in collagen type I synthesis and probable healing of the tissue, all subjects were back playing soccer after the 12 weeks of eccentric training. The pain level during the standardized loading of the tendon was significantly reduced in the previously injured subjects (Fig. 3). These data are in line with findings of Alfredson et al. (1998) and Fahstrom et al. (2003).

In conclusion, the present study shows that 12 weeks of eccentric training stimulates collagen type I synthesis in chronically injured human Achilles tendon, and that this increased synthesis is accompanied by a significant reduction in pain in the tendon during loading.

Perspectives

It is well known that chronic painful Achilles tendons are difficult to treat. For professional soccer players, a chronic Achilles tendon often is the end of an active career. During the last decade, the use of eccentric calf muscles exercises has been shown to be effective in the treatment of chronic painful Achilles tendon problems, but the mechanism behind the good results is unknown. In the present study, we show that collagen synthesis of the Achilles tendon is stimulated by eccentric exercises of the calf muscle and that this increase is accompanied by a reduction in pain. This might indicate that the etiology of chronic painful Achilles tendons results from an inadequate adaptation of the tissue strength to the activity level of the patient, which subsequently results in an overuse of the tissue. Thus, the eccentric exercises act through a stimulation of the collagen synthesis, thereby removing the strength deficit and protecting the tissue against further overuse. Whether this is the only pathway through which eccentric exercise works is not known nor is it known which growth factors are involved in the process. These and similar questions can hopefully be answered in future research.

Key words: microdialysis, tendon, training, soccer.

References


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