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HIGHLIGHTED TOPIC | Role of Inflammation in Skeletal Muscle, Connective Tissue, and Exertional Injuries: To Block or Not to Block?

What is the impact of inflammation on the critical interplay between mechanical signaling and biochemical changes in tendon matrix?

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Kjaer M, Bayer ML, Eliasson P, Heinemeier KM. What is the impact of inflammation on the critical interplay between mechanical signaling and biochemical changes in tendon matrix? J Appl Physiol 115: 879 – 883, 2013. First published April 25, 2013; doi:10.1152/japplphysiol.00120.2013.—Mechanical loading can influence tendon collagen homeostasis in animal models, while the dynamics of the human adult tendon core tissue are more debatable. Currently available data indicate that human tendon adaptation to loading may happen primarily in the outer tendon region. A role of inflammation in this peritendinous adaptation is supported by a rise in inflammatory mediators in the peritendinous area after physiological mechanical loading in humans. This plays a role in the exercise-induced rise in tendon blood flow and peritendinous collagen synthesis. Although inflammatory activity can activate proteolytic pathways in tendon, mechanical loading can protect against matrix degradation. Acute tendon injury displays an early inflammatory response that seems to be lowered when mechanical loading is applied during regeneration of tendon. Chronically overloaded tendons (tendinopathy) do neither at rest nor after acute exercise display any enhanced inflammatory activity, and thus the basis for using anti-inflammatory medication to treat tendon overuse seems limited.

collagen; exercise; matrix; growth factors; tendinopathy

THE QUICK ANSWER TO THE QUESTION in the title is that inflammation does have an impact on mechanical signaling and the subsequent biochemical adaptations in tendon tissue (18, 27). The detailed response to this question is somewhat more complicated, as the available data reveal a marked difference in the role of inflammatory pathways for tendon adaptation, dependent on the experimental model and the initial condition in the studied tissue. Factors of importance to consider are human vs. animal studies, in vitro vs. in vivo systems, loading pattern and intensity, peritendinous vs. tendinous tissue, and whether studies are performed in healthy or injured/overloaded tendon.

BACKGROUND AND QUESTIONS

Tendons transfer muscle contractile force to bone and thereby ensure bodily movement and are exposed to significant amounts of loading during repetitive physical activity, either in relation to occupational tasks or to leisure activity like sports. The adaptive response of tendon to loading and overloading is by far not fully understood. In rat tendons, it has been clearly demonstrated that mechanical loading can lead to increased collagen expression (15, 33), and in humans there are data from microdialysis and stable isotope studies to support that tendon collagen protein synthesis rises in response to loading, at least in the outer parts of the tendon (22, 24, 30). Somewhat in contrast, acute exercise does not upregulate mRNA expression of collagen, or growth factors that stimulate collagen synthesis, in human patella tendon (14, 40). Furthermore, a very recent study reveals that the core of the human Achilles tendon has almost no turnover in adult life (16), and earlier studies on human biceps tendon and on horse tendons provide further support for a low rate of tissue turnover in tendons (6, 42). So the question still remains, whether human adult tendons in fact demonstrate any significant dynamics with regard to the primary collagen structure of the tendon when subjected to exercise. The fact that tendon hypertrophy is seen in response to long-term loading (10), along with the data from stable isotope and microdialysis studies (22, 24, 30), suggests that human tendon tissue is relatively adaptable. These observations, combined with the finding of negligible tendon core turnover (16), could indicate that a large degree of tendon adaptation happens in the outer region of the tendon (for further elaboration of this discussion, we refer to Ref. 16). The
question of this review is whether inflammation plays a role in the adaptation of tendon tissue to loading.

**ROLE OF INFLAMMATORY MEDIATORS IN ADAPTATION OF HEALTHY TENDON TO LOADING**

Tendon loading, either acutely or chronically, can influence tendon blood flow, peritendinous metabolism, and peritendinous concentration of prostaglandins (e.g., PGE$_2$) (23, 24) and cytokines (21). Furthermore, along with these changes, also collagen synthesis (22, 24, 30), proteolytic enzyme activity [matrix metalloproteinases (MMPs)], (19) and collagen degradation (22) are found to increase. To what extent inflammatory activity, or a change therein, is important for the mediation of exercise-induced changes in circulation, metabolism, or matrix changes in or around the tendon tissue is still not clear. The idea that inflammatory mediators should play a role in the adaptation of tendon to mechanical loading has been indicated initially within the vasculature.

**Tendon blood flow.** Tendon blood flow of healthy human tendon has been investigated by the use of radio-labeled xenon washout and with laser Doppler flow, and it has been demonstrated that acute exercise will elevate the blood flow, both around and within the tendon up to 8- to 10-fold in an intensity-dependent manner (20). This regulation of blood flow is partly exerted by release of vasoactive substances, and, when prostaglandin release was inhibited by nonsteroidal anti-inflammatory drug (NSAID) administration, the exercise-induced rise in tendon blood flow was reduced by ~30% (20). Furthermore, it was demonstrated that this effect was specific for cyclooxygenase-2 (COX-2)-mediated pathways, in accordance with this pathway being inducible by exercise (20). The findings from that study indicated that COX-2-specific mechanisms are responsible for the exercise-induced increase in synthesis of the prostaglandins that mediate blood flow regulation, and that an increase in tissue prostaglandin plays a significant role for blood flow in peritendinous connective tissue during physical loading in vivo. In human patella tendon, it has been shown that both COX-1 and COX-2 were expressed at relatively higher levels in tendon than in skeletal muscle, although the level of expression was not changed in tendon tissue in response to an acute bout of mechanical loading (43). In support of a connection between tendon loading and release of inflammatory mediators, tendons from mice subjected to treadmill running showed increased concentrations of PGE$_2$ (48), and also in vitro studies on human fibroblasts show a rise in production of inflammatory mediators like PGE$_2$ and leukotriene B4 (26) in a stretching magnitude-dependent pattern (46, 47).

**Regulation of matrix synthesis by inflammatory mediators.** To gain further knowledge of the role of prostaglandins in tendon adaptation, NSAIDs have been used to manipulate prostaglandin release. Thus it has been demonstrated that NSAID was able to diminish the PGE$_2$ response to mechanical loading of human fibroblasts isolated either from patellar tendon or from hand tendon that were stretched in vitro (1, 26). In humans, we investigated the effect of NSAID on the local peritendinous concentration of PGE$_2$ along the surface of human patella tendon and demonstrated that, when anti-inflammatory medication was provided for 3 days before the experiment, local PGE$_2$ levels were lower at 72 h after exercise compared with the nonblocked situation (8). Along with this blockade of inflammatory mediators, the exercise-induced increase in peritendinous collagen synthesis was reduced (8). These findings suggest that intact activation of inflammatory pathways is important for the physiological rise in the peritendinous collagen synthesis that is normally seen with mechanical loading of tendon tissue. In rat tendon cells, anti-inflammatory medication did not affect expression of collagen, but did lead to upregulation of several collagenases (44), while in rat mammary, extracellular matrix anti-inflammatory medication reduced extracellular matrix protein content of tenascin C and laminin (32). It is important to note that, in the two latter studies, no systematic mechanical loading was applied, so although the results do provide some information regarding role of inflammatory pathways for matrix changes, it can only, to a limited degree, provide conclusive evidence in regards to the interaction between inflammation and mechanical loading.

The effect of NSAIDs on training-related adaptation of tendon tissue has been investigated in humans. In elderly individuals, consumption of NSAID had no influence on tendon adaptation to training with regard to tendon cross-sectional area (7), and also the training-induced decrease in tendon deformation and strain was unaffected by NSAID intake. Thus, based on these results, NSAID intake does not seem to have a great impact on adaptation of tendon tissue to training.

Also interleukin-6 (IL-6) has been suggested to play a role in tendon adaptation to loading. Prolonged running exercise results in a rise in the peritendinous tissue concentration of IL-6 (21), and similar exercise has been shown to induce peritendinous collagen synthesis (24). Based on this, the exercise-induced rise in IL-6 was suggested to be the “inflammation-mediator” of mechanical stimulation of collagen synthesis (2). It was shown that human recombinant IL-6-infused peritendinously in humans, to mimic the rise in tissue concentration seen with exercise, could elevate collagen synthesis in the peritendinous tissue to a similar degree as exercise did (2). This suggests that IL-6 is an important stimulator of collagen synthesis and can act independently of any mechanical tendon loading (2). In support of these human data, Legerlotz et al. (25) found that stretched fascicles from bovine extensor tendons increased the expression of IL-6 mRNA following loading at 30% failure strain in vitro. This occurred concomitant with an increase in collagen type I gene expression.

**Mechanical loading, inflammatory mediators, and proteolytic activity.** In humans, MMP activity has been shown to increase in the Achilles peritendinous tissue in response to acute running exercise (19). In addition, in vitro studies on rabbit Achilles tendon cells demonstrated that the combination of mechanical stretch and the inflammatory cytokine, IL-1β, synergistically increased the expression and activity of proteolytic enzymes (4). Also, human patellar tendon fibroblasts responded to IL-1β with increased MMP-1 expression, and this induction was either repressed or additionally induced by stretching, depending on the magnitude of stretching (4 vs. 8%) (47). This illustrates that mechanical loading, together with inflammatory cytokines, can alter matrix proteolytic enzymatic activity. IL-1β and mechanical stretch can both, as individual treatments, induce COX-2 expression in tendon fibroblasts, and combining these two treatments can induce a more pronounced response (35). However, low doses of IL-1β can also repress expression of some genes induced by mechanical stretch [e.g.,

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transforming growth factor (TGF)-β1, MMP-27, a disintegrin and metalloproteinase with thrombospondin motif-5) (35). Thus it appears that there are some interactions between inflammatory cytokines, loading, and the potential for matrix degradation. In further support of this, an accumulation of inflammatory cells into rat tendon injected with carrageenan, a vegetal polysaccharide devoid of endogenous proteolytic activity, resulted in increased proteolytic (MMP) activity and a decreased content of MMP inhibitors. However, this did not influence tendon hydroxyproline content or mechanical properties (28). This illustrates that an inflammatory response with increased MMP activity does not necessarily lead to tendon degradation. Interestingly, this study showed that systematic mechanical load was necessary to protect tendon collagen bundles cultured in the presence of inflammatory cells (and thus high MMP activity) from degradation and loss of mechanical integrity (28). This suggests that mechanical loading is a primary mechanism for reducing susceptibility of collagen fibrils in tendon tissue to enzymatic degradation. This protective effect of tensile strain is supported by findings made in collagen matrices (37). Here collagen fibrils under tensile stress were shown to be less susceptible to enzymatic degradation than fibrils under lower tensile load (37). These findings underline the importance of mechanical tension for homeostasis of collagen-rich tissues.

SUMMARY

In summary, it appears that inflammatory mediators do play a role in the adaptation of healthy human tendon tissue to mechanical loading, at least in the outer region of the tendon tissue. Thus both inflammatory markers and collagen turnover are modulated in the peritendinous tissue with loading, and removal of the inflammatory response to physiological tissue loading seems detrimental for the collagen response. It is more questionable that inflammation should play a role in the tendon core, as the collagen turnover appears to be very limited in this region.

INFLAMMATORY MEDIATORS IN TENDON INJURY

Tendinopathy. Chronically overused tendons (tendinopathy) display characteristics of degenerative changes in the tissue, but in general lack signs of inflammatory responses (17, 36). Thus the tendinopathic tendon demonstrates changes in cell shape and density, presented as a rounding of the normally elongated tendon fibroblasts and showing areas of increased cell density, as well as areas with very low cell numbers. Furthermore, the normally well-aligned collagen fibers loosen up the organization, and a thickening of the tendon, due to accumulation of proteoglycans and thus water, is seen (17, 36). In addition, a chronic upregulation of the expression of several structural proteins (collagens), proteolytic enzymes (MMPs), and growth factors is observed in tendinopathy (e.g., VEGF, TGF-β1, and IGF-1) (17, 18, 36). However, there is no sign of any upregulation in the expression of inflammatory mediators like TNF-α, IL-1, or PGE2 (17, 34). Somewhat in contradiction to the lack of inflammatory upregulation in tendinopathic tendon is the demonstration of a, at least short-term, clinical effect of anti-inflammatory treatment with glucocorticoids (9). An explanation for this paradox could be that, in the resting situation (where tendon surgical material is normally obtained), no inflammatory signs are present, whereas in another situation, like after heavy mechanical loading (e.g., running), an inflammatory response could potentially be present. Although speculative, this would fit with the clinical observation that exercise does worsen tendon symptoms and that tendon swelling is often observed after acute exercise. To address this issue, patients with chronic tendinopathy of their Achilles tendons were investigated with biopsy sampling from both the painful and the healthy part of their tendon after an acute bout of exercise, in both a situation with placebo and one with previous treatment with anti-inflammatory medication (NSAIDs) (34). After acute exercise, the painful part of the tendon showed increases in expression of collagen (I and III) and TGF-β1, while levels of IL-1, IL-6, IL-10, TNF-α, and COX-2 were either downregulated or unchanged, compared with the healthy region of the tendon. Thus there were no indications of an inflammatory response to exercise in the affected area. The NSAID treatment only diminished the IL-10 response, whereas all other expression responses were unaffected by anti-inflammatory medical treatment (34). These findings point opposite of the original hypothesis (above) and demonstrate that inflammatory signaling is not exaggerated in tendinopathic compared with healthy regions of the tendon after physical activity. In line with this, no convincing evidence exists for the substantial historical use of anti-inflammatory drugs (e.g., NSAIDs), in treatment of painful or overloaded tendon (3, 9, 13). Meanwhile, it cannot be excluded that tendinopathic conditions only demonstrate inflammatory characteristics early in the disease, and several authors have suggested a two-phase response, with an early inflammatory dominated response followed by a later degenerative response (29), whereas others maintain the idea that tendinopathy is a degenerative phenomenon without inflammation (41), but neither of these suggestions has any solid human data to back it up. In vitro, it has been demonstrated that tendon cells subjected to PGE2 differentiate into nonfibroblasts, and, given the release of PGE2 seen in response to tendon loading, it suggested that intense repetitive loading could, due to high PGE2 levels, negatively affect tendon cells and thus lead to development of tendinopathy (48). This could be one of the explanations for the development of tendinopa-
thy. It might, however, also be that local unloading of tendon cells, due to microruptures of collagen fibers, is an initial step in pathological changes seen in tendon injuries (5). In this scenario, it would be the absence of tensile stimuli that would trigger catabolic alterations of tendon tissue (5). Currently, however, the mechanism for development of tendon overuse injury/tendinopathy is not clear, and further studies in the area are necessary.

Tendon rupture. After severe damage or rupture to tendon, healing is, especially in the early phase, associated with inflammatory activity (39). This initial inflammatory period is followed by a later period of proliferation and remodeling, where inflammation is less pronounced and scar formation and fibrosis occur (39). This supports the view that tendon healing has transient changes in degree of inflammation. The inflammatory phase seems to be influenced by the degree of mechanical loading during the healing process, at least in animal models. Thus, when rat tendons were mechanically loaded, inflammation-associated genes were altered, both in response to a single loading episode or with more continuous loading (11, 12). The induced expression of inflammatory mediators like TNF-α and IL-1β during early tendon healing was lowered when tendons were loaded (12). Interestingly, this was associated with an improved matrix synthesis, and thicker and stronger tendons, and is suggestive, but not proven causal, for a beneficial role of mechanical loading and inflammatory signaling upon tendon healing. In addition, enzymes involved in production of nitric oxide (NO) were also induced by one loading episode during healing (11), and NO has previously been shown to improve tendon healing (31). In line with this, inhibition of inflammatory mediators like PGE₂ (45) and NO (31), during tendon healing after acute injury, appears to inhibit the healing progression. However, inhibition of other inflammatory mediators, like TNF-α (38) and IL-1β (unpublished data), does not appear to influence tendon healing.

CONCLUSION

In conclusion, it has, in human studies on healthy tendons, been found that both inflammatory markers and collagen turnover increase in the peritendinous tissue with loading. There is also some evidence provided that certain inflammatory cytokines can mediate increased collagen synthesis in peritendinous connective tissue, and that removal of the inflammatory response to physiological tissue loading is detrimental for this collagen response. In contrast to the peritendinous tissue, the actual core tendon tissue of adult humans seems much less dynamic (6, 16), and inflammatory pathways presumably play a negligible role there, apart from regulating the exercise-mediated blood flow (Fig. 1).

Chronically overloaded tendons (tendinopathy) do neither at rest nor after acute exercise display enhanced inflammatory activity, although some suggest that inflammatory mediators, such as PGE₂, may play a role in the initial phase of the disease. After acute tendon injury (rupture), an early inflammatory response is seen, and studies on animals indicate that mechanical loading applied during healing of the tendon may modulate this response in a beneficial manner.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.K. conception and design of research; M.K., M.L.B., and K.M.H. performed experiments; M.K. analyzed data; M.K. interpreted results of experiments; M.K. prepared figures; M.K., P.E., and K.M.H. drafted manuscript; M.K., M.L.B., P.E., and K.M.H. edited and revised manuscript; M.K., M.L.B., P.E., and K.M.H. approved final version of manuscript.

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