Increased expression of IL6 family members in tendon pathology

INTRODUCTION:
Tendinopathy, characterized by chronic tendon pain, swelling and impaired performance, is a common syndrome in both recreational and elite athletes as well as in the general population. Although its aetiology remains poorly understood, tendinopathy is considered to be a primarily degenerative condition. However, inflammation may still play a role in the early initiation of the disease, as some studies indicate that acute overloading may induce an inflammatory response in tendon tissue. The aim of this study was to investigate gene expression of IL6 and other members of the IL6 family in painful and ruptured human tendon compared to healthy controls. Expression of COX2 and other genes known to be upregulated in tendinopathy (COL1A1 and VEGF) were also investigated. We analysed samples from normal, painful and ruptured Achilles tendon (AT) as well as normal and painful posterior tibialis tendon (PTT), to test whether similar patterns of gene expression are observed in similar pathologies of different tendon types.

METHODS:
Specimens: AT samples were obtained from cadavers (normal) or from patients undergoing surgical procedures to treat chronic painful tendinopathy or ruptured tendon. PTT samples were obtained from patients undergoing surgery for other reasons (normal) and from patients with PTT dysfunction (painful). All procedures had local ethics committee approval and appropriate informed consent was obtained.

Gene Expression Analysis: Total RNA was isolated from frozen tissue samples by a modified Tri-Spin protocol. mRNA levels of 8 genes of interest were quantified using quantitative real-time PCR: COL1A1, VEGF, COX2, OSM, LIF, CNTF, IL6 and IL6R. 18S was used to check the quality of the RNA. TOPI and EIF4A2 were used as housekeeping genes. To detect differences in gene expression between groups a Mann-Whitney U test was performed on the 2 values. Significance was established at p<0.05.

RESULTS:
COL1A1 and CNTF expression were significantly elevated 14fold and 2.9fold respectively in painful PTT compared to normal PTT (Fig.1B). In painful AT COL1A1 expression significantly increased 9fold compared to control. COX2 and IL6 expression significantly increased 2fold and 6.3fold respectively in painful compared to normal AT; IL6R expression decreased 3.6fold. In the ruptured AT, VEGF, COX2, OSM, LIF and IL6 expression were significantly higher compared to normal (4.1, 11, 39, 20 and 180fold) and painful AT (7.2, 5.6, 31, 14 and 28fold). IL6R expression in the ruptured was 2.7fold less than in normal AT (Fig.1A).

DISCUSSION:
The expression of some IL6 family members was upregulated in pathological tendon. However, contrary to our expectation painful AT and PTT showed different expression patterns, indicating a substantial difference between those two tendinopathies. The increase in expression of IL6 and COX2 found in the painful AT does not occur in the painful PTT. This could be a result of differences in gross mechanical loading and lead to an altered cell environment. The mechanical properties of the AT have been shown to be altered by tendinopathy, leading to lower tendon stiffness and higher strain during isometric plantar-flexion compared to healthy AT. Previous studies have demonstrated that the concentration of IL6 and COX2 rises with increased mechanical strain. Hence, the higher strains perceived by cells in tendinopathic AT may be the cause of the upregulation of IL6 and COX2 seen in the painful AT in our study. In contrast, the PTT appears to lengthen permanently with tendinopathy (personal observation of operating surgeon), resulting in a loss of support of the foot arch, which is likely to reduce mechanical strain at the cell level.

The expression of the IL6 family members OSM and LIF was increased in ruptured compared to normal and painful AT. Both OSM and LIF have been shown to stimulate release and suppress synthesis of proteoglycans in articular cartilage. Similarly, CNTF, which was upregulated in painful PTT, has also been shown to inhibit proteoglycan synthesis, although without affecting proteoglycan release. One might assume that OSM, LIF and CNTF might function in a similar way in tendon, thereby decreasing proteoglycan content. However, this seems surprising since there is an increase in glycosaminoglycan and mRNA expression of the proteoglycans aggrecan and biglycan has been shown to increase in AT tendinopathy. Collagen metabolism has also been shown to be influenced by IL6 and OSM. Collagen degradation is stimulated in tendon tissue treated with OSM in combination with IL1α and in cartilage tissue treated with the combination of IL1α and either IL6 in the presence of its soluble receptor or OSM. However, IL6 can also stimulate collagen synthesis, as IL6 injections led to an increased concentration of a procollagen marker in the peritendinous space around the human Achilles tendon.

SIGNIFICANCE:
We have shown that IL6 family members are highly expressed in tendon, and that they are upregulated with tendon pathology. However, the role and function of the IL6 family members in tendons needs to be further clarified. It remains to be established whether the difference in gene expression between pathological AT and PTT is due to differences in loading, tendon structure or composition or reflects a difference in the type, progression or healing of tendinopathy. Given those differences, it is possible that the success of different treatment options may depend on the anatomical site of the tendinopathy.

REFERENCES: