Altered cell metabolism in tissues of the knee joint in a rabbit model of quadriceps muscle weakness

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Background:
Quadriceps muscle weakness is a frequent associate of knee injuries in sports. More than 50% of athletes (e.g. soccer players) sustain another knee injury shortly after having ruptured their ACL, e.g. tendinopathy, muscle and ligamentous strains, stress fractures. The influence of quadriceps weakness on knee joint tissues and subsequently on knee joint homeostasis remains unclear. Therefore, the aim of the study was to measure the influence of chronic quadriceps muscle weakness on the molecular metabolism of tendons, ligaments, and menisci in a Botulinum A toxin (BTX-A) induced lapine muscle weakness model.

Methods:
Six one-year old New Zealand White rabbits were injected monthly with BTX-A (BOTOX, Allergan Inc, Toronto, ON) directly into the quadriceps muscle complex of one leg for six months. Five additional animals served as controls with injection of saline/dextrose. Muscle weakness was assessed measuring isometric knee extensor force, muscle wet weights, and histologic morphological analysis of the muscle tissue. For the final tests, a nerve cuff electrode was implanted to the femorals nerve under isoflurane/oxygen anaesthesia. Pelvis and femoral condyles were fixed to a stereotactic frame. The tibia was restrained to an instrumented bar, allowing measuring isometric knee extensor torque at angles of 90°, 100°, and 120° of knee joint flexion. For maximal stimulation a frequency of 100Hz was used. Then, the rabbits were sacrificed and muscle wet weights assessed, and embedded for histological morphology analysis (HE staining).

Molecular metabolism was assessed for patellar tendon, medial collateral ligament, medial meniscus, lateral collateral ligament, and lateral meniscus by assessing total RNA yield/tissue and measuring mRNA levels for Collagen I and III, MMP-1, MMP-3, MMP-13, TGF-β, Biglycan, IL-1, bFGF by RT-PCR. All tissues were subjected to RNA isolation using the TRIspin method and total RNA was quantified using the SYBR Green reagent. RT primers were used from a Qiagen Omniscript kit (Qiagen, Mississauga, ON), specifically validated primer sets for the rabbit were used for PCR of the specific molecules.

For analysis, Windaag software and customized Matlab programs were used. Statistical analysis were performed with student’s t-test and one way ANOVA. The level of significance was set at α=0.05.

Results:
BTX-A produced an average decrease in isometric knee extensor torque of 59-61% with the highest decrease at 80° of knee joint flexion, a decrease of contractile tissue of 36%, and a loss of quadriceps muscle wet weight of 47% (all results p<0.05), after 6 months. Loss of weights for the different muscle bellies changed between 31% for the small vastus lateralis, and 59% of the large vastus lateralis muscle.

Total mRNA levels decreased by 2 to 35% (all results p>0.05). RT-PCR showed tissue-specific lowered mRNA levels for both relevant anabolic and catabolic molecules indicating depressed tissue turn over. mRNA levels for Collagen I and Collagen III, two major anabolic factors, were significantly reduced in all 5 tissues. TGF-β was reduced significantly in the lateral meniscus and lateral collateral ligament as well as Biglycan in the medial meniscus and medial collateral ligament. bFGF levels didn’t change. (Figure 1)

For the catabolic factors, mRNA levels for MMP-1 and MMP-3 were significantly reduced in the patellar tendon, medial meniscus and medial collateral ligament, while MMP-13 was decreased for the lateral meniscus and lateral collateral ligament. IL-1 was reduced significantly in the medial and lateral collateral ligament and in the lateral meniscus. (Figure 2).

The patellar tendon that is linked more directly to the action of the quadriceps muscle didn’t show different alternations in comparing to the other tissues.

Discussion:
This study induced long-term muscle weakness of the quadriceps muscle in a rabbit model with BTX-A and analyzed the subsequent influence on mRNA levels for a number of relevant molecules in joint-associated tissues. Not only was significant muscle weakness documented, but also for the first time, histologic changes in skeletal muscle induced by repetitive application of BTX-A. Cell metabolism as assessed by specific mRNA levels associated with anabolism and catabolism was also significantly decreased indicating a new steady-state of joint function, giving insight in a new pathomechanism for further injury proneness in athletes. The histological changes show that repetitive BTX-A injection does not only lead to blockage of the pre-synaptic exocytosis of acetylcholine storing vesicles, but also to long-term muscle structural changes. However, no further force loss was documented in comparison to other data reported 4 weeks after a single injection of BTX-A. Several studies reported on changes in mRNA levels after exercise or increase in muscular strength in tendons. After an acute bout, down-regulation was seen (e.g. Coll I and III), in short-term recurrent or cyclic loading, an up-regulation of anabolic but not catabolic factors were found. In long-term chronic loading, both, anabolic and catabolic factors were up-regulated indicating that a new steady-state of knee joint function was reached. No study so far measured the influence of muscle weakness on tendons, menisci, and ligaments. Therefore our results with down-regulated anabolic and catabolic factors in a long-term chronic model seem to reflect a new adaptive image of hyperloading on connective tissues.

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Fig 1. mRNA levels of anabolic factors (relative changes (%)).

Fig 2. mRNA levels of catabolic factors (relative changes (%)).