INTRODUCTION
Our research in the past few years has focussed on understanding how tendon overuse injuries develop. For this purpose, we modified the rabbit Achilles tendon overuse model originally presented by Rais (1961). Our modifications allowed us to control and measure the loading inputs to the tendon, load the tendon for one or multiple bouts, and evaluate the biological response of the tendon to this loading. We report here our findings of changes in the tendon following one day or 11 weeks of repetitive loading.

METHODS
The experimental model has been described in depth previously (Archambault et al. 1998), but will be reviewed here in brief. The rabbit was placed supine in a sling with the left leg placed in the loading apparatus (Figure 1). The foot was attached to a footplate interfaced with a stepping motor via a strain-gauge instrumented torque cell. The right leg served as the contralateral control, not subjected to the loading protocol. For the loading protocol, the motor moved the ankle joint through a range of 55° at 1.25 Hz. Surface electrodes (acute protocol) or a tibial nerve cuff (chronic protocol) were used to stimulate the triceps surae muscles for 132 ms, 50 Hz at 3-4 times motor threshold. Stimulation was superimposed to the motion of the ankle joint so that the muscles were active during both the eccentric and concentric phases of loading about full ankle dorsiflexion.

For the chronic protocol, loading was done with 4 adult female NZW rabbits for 2 hours, 3 days per week for 11 weeks. For the acute protocol, loading was done for 6 hours, then the animals were sacrificed at 12, 24 and 48 hours post-loading.

Figure 1. Apparatus used to load the rabbit Achilles tendon.

For each session of loading, the damage index was calculated based on a model of tendon fatigue behaviour (Schechtman & Bader 1997). A damage index is the ratio of the number of cycles done in a session to the number of cycles needed for failure at a given load. Force on the tendon during the loading protocol was calculated from the moments measured at the ankle joint. A maximal tensile load value of 1010 N for the rabbit Achilles tendon was used in the calculations (Nakagawa et al., 1996).

For the chronic protocol, tendons were evaluated for histological changes, water content and DNA content. For the acute protocol, tendons were evaluated for the expression and production of collagenase (MMP-1) and stomelysin (MMP-3).
RESULTS
As a result of the chronic loading, no changes were observed in the experimental and contralateral tendons at dissection or under the microscope. Matrix organization and cellularity were similar in all sections of the tendons (Figure 2). Water content and DNA content in the tissues did not change in response to the loading protocol. The average damage index for a 2 hour session of loading was 0.002, with peak moments of 30-40% of the isometric maximum.

Figure 2. Micrograph of Achilles tendon of a contralateral (left photo) and experimental (right photo) leg.

The 6 hour acute loading protocol resulted in an average damage index of 0.005 (n=3 rabbits). Peak tendon forces were similar to those obtained during the chronic protocol. At sacrifice, no visible changes were observed in the tendons. The loading bout did not induce the expression or production of MMP-1 or MMP-3 in the Achilles tendon or paratenon at 12, 24, or 48 hours post-loading (Figure 3).

Figure 3. Gene expression of collagenase and stromelysin in Normal, Contralateral and Experimental tendons.

DISCUSSION
With the chosen loading parameters, acute and chronic repetitive loading did not induce an overuse injury in the Achilles tendon of mature rabbits. This is in contrast to the report of an injury response in the Achilles tendon of immature rabbits with chronic loading by Backman and co-workers (1990). Rais (1961) had also reported that a 6 hour loading session produced inflammatory changes in the tendon. Both these studies used a movement frequency that we felt was unphysiological and used immature animals. These two factors may be responsible for the observed differences in injury response.

Our results suggest that the Achilles tendon of healthy adult rabbits is not easily injured during normal activity. It is possible that the muscles cannot produce enough force to cause substantial damage to the tendon, as indicated by our calculation of the damage index. This would suggest that repetitive loading within the range of normal activity is not sufficient to damage a tendon, nor induce enzymes such as collagenase or stromelysin that could result in matrix degradation.

We now hypothesize that tendon overuse injuries occur as a result of a combination of factors, not only repeated mechanical loading. In vitro data with rabbit tendon cells has shown that the application of a mechanical stimulus in combination with an inflammatory cytokine (IL-1β) resulted in greater production of stromelysin than if either stimulus is presented alone (Archambault et al., submitted). Future experiments will test this combined etiology hypothesis in vivo.

REFERENCES
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