The effects of cyclical loading on tendon fibroblast metabolism in vitro

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Introduction:
It is well established that tendon fibroblasts (tenocytes) will synthesize or degrade collagen in response to their mechanical environment. An imbalance in this metabolic process due to an improper mechanical loading may result in either tissue hypertrophy or atrophy. Although it has been recognized that tensional loading in vivo influences these changes the relationship between the rate of cyclical loading and tenocyte metabolism which results in changes to matrix turnover have not been established. Using fibronectin as a marker of matrix synthesis activity and MMP-1 as a marker for tendon degradation, a study was conducted to determine the effects of continuous cyclical loading on tenocyte metabolism by measuring MMP-1 and fibronectin production.

Methods:
Tenocytes were obtained from tendon biopsied from a single donor following protocols developed previously. Equal numbers of cells were cultured onto 0.05% collagen type I coated silicone flasks and subjected to 0.5Hz, 1Hz or 2Hz cyclical loading at 6% strain for 48 hours. Non-stretched or static silicone flasks seeded with tenocytes were used as controls. Observations and sample collections were performed at 0, 6, 24 and 48 hours of the experiment beginning 72 hours after cells were seeded. These included cell culture medium sampling to determine total MMP-1 and fibronectin, and capturing cell images on silicone surfaces for image analysis. Each media sample was tested in duplicate using commercially available ELISA kits following protocols provided by the respective manufacturers. Image analysis using Image J analysis software provided the information necessary to estimate cell numbers on silicone surfaces. Data were analyzed using Microsoft Excel® and SPSS statistical package software (ver. 13.0). All experiments were conducted with the approval of the local ethics committee review board and the Royal Liverpool NHS trust.

Results:
Twelve (n=12) samples were examined from silicone flasks seeded with tenocytes subjected to various amounts of tensile loading. The amount of MMP-1 and fibronectin concentrations at different time points relative to that at 6 hours are summarized in figures 1A and 1B.

In all groups, MMP-1 and fibronectin concentrations at 24 and 48 hours were increased as compared to that measured at 6 hours. Cells at 1Hz loading produced the highest amount of MMP-1 and fibronectin, which was significantly higher compared to other cell cultures. Cell proliferation was highest with 2Hz of cyclical loading however, these number rapidly reduced at 48 hours. Observations of cells at this time point revealed that large numbers of these cells detached from the surface. The rates of cell proliferation for all other cell cultures were fairly similar although cell cultures in static flasks appear to lag at 48 hours. This was however not statistically significant (Mann-Whitney U test: p>0.05).

Figure 1A & 1B: Fibronectin and MMP-1 % increase from concentration levels at 6 hours.

By measuring the amount of MMP-1/fibronectin production in relation to the proportion of cells growth (not shown here) and later calculating the ratio of MMP-1 to fibronectin concentrations, the proportion MMP-1 to fibronectin was found to be lowest in cultures subjected to 1Hz cyclical loads and highest in cultures subjected 2Hz. In comparison to that of static cultures, this value was significantly lower (0.62 vs. 0.90) denoting a nett increase in synthetic activity compared to degradation. Although cultures subjected to 2Hz demonstrated the highest ratio of MMP-1/fibronectin (0.97), this value was not statistically different to that of static cultures (Mann-Whitney U test: p>0.05).

Conclusion: In complementing previous in vivo studies, collagen synthesis and MMPs were measured when tendons were either immobilized or subjected to dynamic exercises. These studies have noted that: (1) when tendons are reused, reduction in collagen synthesis can lead to tendon weakening or atrophy, and (2) when tendons are overexerted (or overused), collagen catabolism takes place following an increase in MMPs concentrations. Conclusion: In complementing previous in vivo findings, this study demonstrates that a balance in MMP-1 and fibronectin production is important to ensure that tendon quality is preserved. This balance can be maintained by ensuring cyclical tensile loading is applied but at moderate rates.

References: