# Gene Expression In Muscles Of Rats After 18 Weeks Of Exposure To High Force Versus Low Force Repetitive Movements

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## INTRODUCTION

Work related musculoskeletal disorders (WMSDs) account for over 600,000 injuries and illnesses. Occupation-related arm, wrist and hand injuries account for 23 % of all workplace injuries/ illnesses and require a median of 12 days away from work. In humans sensorimotor dysfunction is typical for this type of injury and is accompanied by inflammation, fibrosis and degeneration in tissues, but the pathogenic mechanism is incompletely understood.

We examined gene expression of fibrogenic proteins in muscles in a rat reach and grasp model of upper limb overuse with the hypothesis that fibrogenic processes would be altered with prolonged performance of a High-Force High-Repetition (HFHR) task. We used three reference genes to investigate the effects of task on the expression genes associated with musculoskeletal homeostasis.

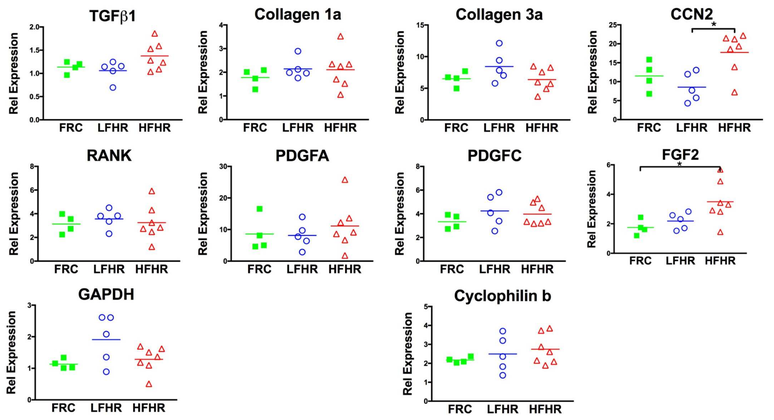
## METHODS




Studies were conducted on flexor digitorum muscles from adult female, Sprague-Dawley rats. Over a 6 week period rats learned to pull on a lever bar for 10 min/day, 5 days/wk, before then performing either a high force high repetition (HFHR; 53% max voluntary force) task or a low force high repetition (LFHR; 15% max voluntary force) task for 2 hrs/day and 3 days/wk, for 18 wks. Control rats were age-matched rats that were on the same food restricted diet as task rats, but that did not perform any task (termed food restricted controls, FRC).

## MUSCLE RESULTS

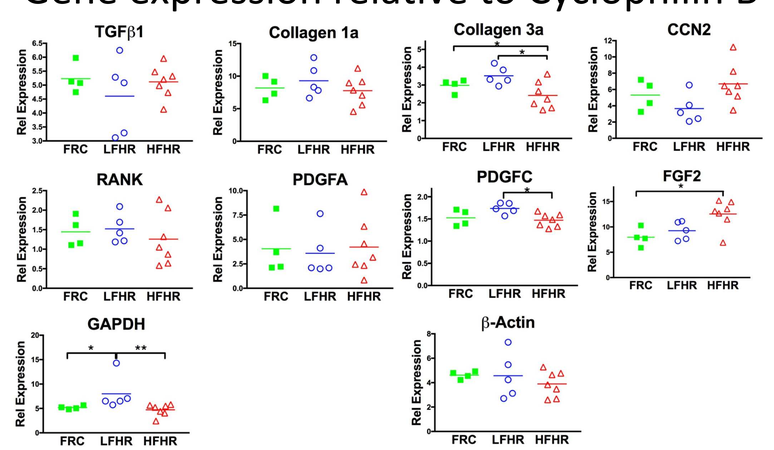
### Gene expression relative to β-Actin




### Gene expression relative to GAPDH




### Gene expression relative to Cyclophilin B




## RESULTS SUMMARY

1. Relative gene expression results differed according to the reference gene used.
2. FGF2 was higher in HFHR rat muscles ($p<0.05$), compared to FRC rat muscles for all three reference genes.
3. With β-actin as the reference gene, CCN2 was higher in HFHR rat muscles ($p<0.05$), compared to LFHR muscles, and FGF2 was highest in HFHR rat muscles ($p<0.05$), compared to FRC. This method also showed nonstatistical increases in TGFβ1 in HFHR rat muscles and Col3 in LFHR rat muscles.
4. With GAPDH as the reference gene, both CCN2 and TGFβ1 were higher in HFHR rat muscles than in LFHR rat muscles ($p<0.01$ each), and lowest in LFHR rat muscles than in FRC rat muscles ($p<0.05$ each). Similar to the other normalization methods, FGF2 was highest in HFHR rat muscles, compared to the other two groups ($p<0.01$ each).
5. With cyclopilin B as the reference gene, higher levels of collagen 3a and PDGFC were evident in muscles of LFHR rats, compared to the HFHR group ($p<0.05$ each).
6. TGFβ1 and CCN2 had a similar pattern of expression for all three reference genes with highest expression in the high force task group and lowest expression in the low force task group.
7. The data demonstrates that the expression of reference genes commonly used for analysis of gene expression can be regulated by the demand load in working muscles.

## CONCLUSIONS

1. Our data demonstrate that work tasks of higher demands induce changes in fibrotic gene expression.
2. These data make a strong argument for obtaining absolute values for muscle gene expression in this model.

## CLINICAL SIGNIFICANCE

The increase in CCN2 gene expression supports targeting it or its protein for reducing fibrogenic processes occurring with prolonged performance of repetitive high demand tasks. Since CCN2 is a downstream mediator of TGFβ1, targeting the CCN2 gene or protein in pathogenic fibrotic conditions would avoid the potentially more severe and widespread deleterious effects of targeting TGFβ.

While recent research has revealed the anti-fibrotic effects of FGF2, its higher expression in HFHR muscles may represent an attempted tissue repair effort in muscles performing prolonged high demand tasks. Therefore, FGF2 may be a good candidate as a biomarker for muscle fibrosis occurring with overuse.

## ACKNOWLEDGEMENTS

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