Glycerol induced paraspinal muscle degeneration leads to hyper-kyphotic spinal deformity in wild-type mice

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INTRODUCTION: Degenerative spinal disorders, including kyphotic deformity, are associated with a range of degenerative characteristics of the paraspinal musculature[1,2,3]. It has therefore been hypothesized that paraspinal muscular dysfunction is a causative factor for degenerative spinal deformity; however, experimental studies demonstrating causative relationships are lacking. To better unravel the cause-and-effect association between paraspinal muscle degeneration and spine degeneration and deformity, we developed and characterized a novel model of paraspinal muscle degeneration using repeated intramuscular glycerol injections in C57BL/6 wild type mice. It was hypothesized that the induced paraspinal muscle degeneration would lead directly to kyphotic spinal deformity and mild degeneration of the IVDs.

METHODS: Male and female mice received either glycerol or saline injections bilaterally along the length of the paraspinal muscles at four timepoints, each separated by 2 weeks; sacrifice occurred two weeks following the final injection. Immediately after sacrifice, micro-CT was performed to measure spinal deformity; paraspinal muscle biopsies were taken to measure active, passive and structural properties; and lumbar spines were fixed for analysis of intervertebral disc (IVD) degeneration. All data were analyzed by two-way analysis of variance (ANOVA), with factors of group (glycerol and saline) and sex (male and female). For significant interactions, Sidak multiple comparisons were used to compare individual group differences between males and females within the two-way ANOVA. Significance was set to $\alpha = 0.05$. All data are reported as means ± 95% CI. All experiments were approved by the University of Guelph Animal Utilization Protocol (#4533) in accordance with all relevant guidelines and regulations; reporting of methods here follow ARRIVE guidelines.

RESULTS: Glycerol-injected mice demonstrated clear signs of paraspinal muscle degeneration and dysfunction: significantly ($p < 0.01$) greater collagen content (Figure 1A), lower density, lower absolute active force, greater passive stiffness (Figure 1B) compared to saline-injected mice. Further, glycerol-injected mice exhibited spinal deformity: significantly ($p < 0.01$) greater kyphotic angle than saline-injected mice (Figure 1C). Glycerol-injected mice also demonstrated a significantly ($p < 0.01$) greater IVD degenerative score (although mild) at the upper-most lumbar level compared to saline-injected mice.

DISCUSSION: The pathophysiology driving degenerative spinal disorders and spinal deformity is complex and has been suggested to involve the paraspinal muscles[14]. However, direct evidence in support of this hypothesis is incomplete. Previous studies have attempted to demonstrate that paraspinal muscle pathophysiology can precede, and directly lead to, negative changes to the spine[5]. While presenting compelling hypotheses, these studies have been unable to unravel the sequence of events that lead to kyphotic deformity. To our knowledge this is the first study to investigate whether impaired paraspinal muscle function can precede and thus lead to spinal deformity. While the current results demonstrate a clear link between paraspinal muscle myopathy and spinal deformity, this should not be directly extrapolated to humans, who have clear functional and morphological differences when compared to rodents.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings provide direct evidence that combined morphological (fibrosis) and functional (actively weaker and passively stiffer) alterations to the paraspinal muscles can lead to negative changes and deformity within the thoracolumbar spine. This confirms the long-standing hypothesis that paraspinal muscular dysfunction is a causative factor for degenerative spinal deformity and should be considered in the treatment strategies for spinal deformities.

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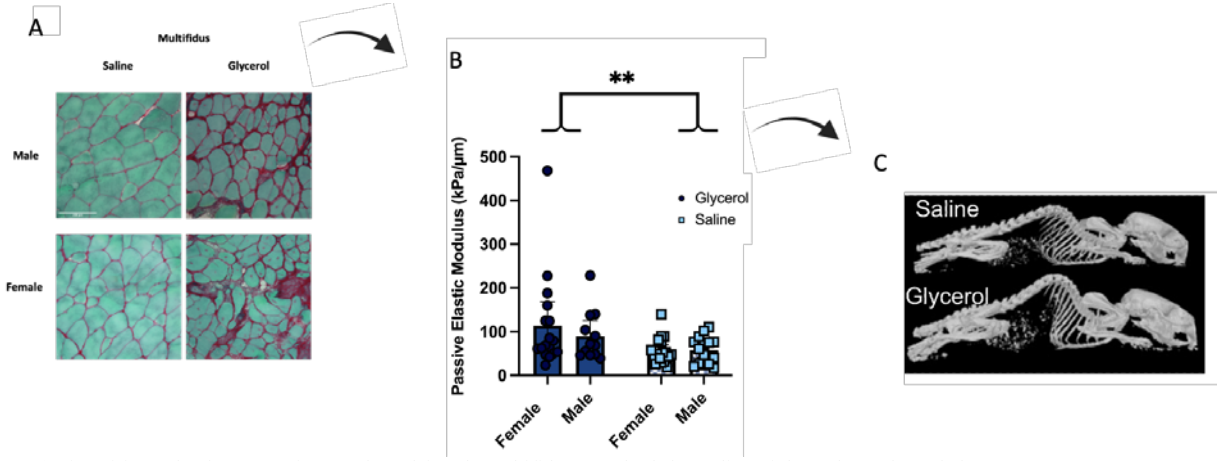

Figures 1 (A) Picrosirius red + fast green from male and female multifidus muscle (left = saline, right = glycerol) Scale bars = 100 μm. (B) increased passive stiffness of muscle fibre bundles from the ES (light squares = saline, dark circles = glycerol; n = 62 total fibre bundles). **p = 0.0092, significant effect of group. (C) 3-D model of representative female mouse skeletons, demonstrating greater thoracolumbar kyphosis in the glycerol mouse.