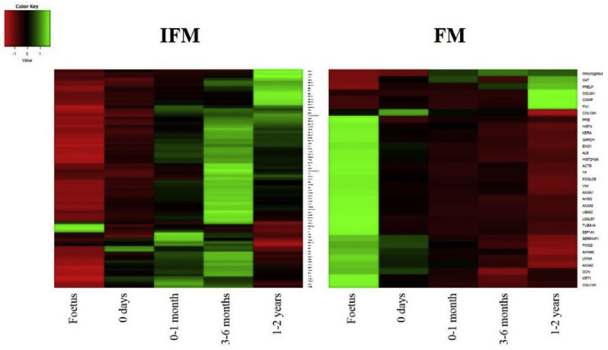
positional equine common digital extensor tendon (CDET) through development and characterised the proteome of the SDFT fascicular matrix (FM) and IFM through development.
Methods: SDFT and CDET were collected from foetuses and foals up to 2 years old, euthanised for reasons unrelated to this project. Samples were split into 5 age groups· foetus, 0 days (full term **but** did not stand after birth), 0–1 month, 3–6 months, and 1–2 years (n = 4, 0–1 month n = 3). Tendons with macroscopic evidence of injury were excluded.
For the biomechanical analysis, fascicles and IFM were dissected from the SDFT and CDET and tested using an electrodynamic testing machine. Fascicles and IFM were preconditioned (10 cycles) before being tested to failure. Force and displacement data were continuously recorded at 100 Hz for both fascicle and IFM tests[3].
To characterise the proteome of the SDFT FM and IFM, FM and IFM tissue was collected using laser-capture microdissection (LCM) and trypsin-digested for liquid chromatography mass spectrometry (LC-MS/MS). FM and IFM proteins were identified using Peaks®8.5 software (false discovery rate 1%, ≥2 unique peptides). Fold changes in protein abundance between the age groups for FM and IFM were calculated following label-free quantification (significance: fold change ≥2, p&lt0.05). Pathway analysis for the SDFT FM and IFM was carried out with the Ingenuity Pathway Analysis software (IPA).
Results: Biomechanical analysis of the viscoelastic properties of fascicles and IFM revealed a similar pattern through development for the fascicles and the IFM with similar values for both tendons until 1–2 years, where the CDET displayed higher values than the SDFT. Biomechanical analysis of the pull to failure properties for the IFM revealed a significant interaction between tendon and age (ANOVA, p&lt0.05) for force and extension at maximum stiffness, with the SDFT showing higher values than the CDET with development, especially in 1–2 years old tendons. Fascicles values were similar for both tendons through development.
Proteomic analysis of the SDFT FM and IFM returned 33 and 79 differentially abundant proteins, respectively, between age groups and heatmap analysis revealed distinct expression profiles for the FM and IFM through development. In the fascicles, proteins' abundance mainly peaked in the foetus and decreased with development whereas in the IFM most proteins' abundance increased with development and peaked at 3–6 months (Figure 1). Pathway analysis for the IFM predicted TGFB1 as an upstream regulator. TGFB1 was predicted to be inhibited in the foetus SDFT IFM and to become activated in the 3–6 months age group.
Conclusions: This is the first report of the SDFT IFM biomechanical properties and protein expression profile through development. These results highlight the importance of the IFM in the function of the energy-storing SDFT and support the post-natal specialisation of the SDFT IFM. Knowledge of protein expression in tendon IFM through development is providing the basis for identifying driving mechanisms for healthy tendons and targets to manipulate to optimise tendon health post injury.



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MECHANISMS OF ACTION OF CHONDROITIN SULFATE AND GLUCOSAMINE IN MUSCLE TISSUE: *IN VITRO* AND *IN VIVO* RESULTS. A NEW POTENTIAL TREATMENT FOR MUSCLE INJURIES?

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Purpose: In recent years, glycosaminoglycans (GAGs) have been widely evaluated as potential therapies for the treatment of musculoskeletal pathologies. Two of the most studied GAGs, the natural compounds chondroitin sulfate (CS) and glucosamine (GLU), have demonstrated beneficial effects for the treatment of osteoarthritis both in preclinical studies and in clinical trials. Both CS and GLU are considered symptomatic slow-acting drugs for OA and have demonstrated clinical efficacy and safety when administered to OA patients, showing a synergistic effect when combined. Therefore, the mechanisms of action of the combination CS+GLU have been largely studied in articular tissue. However, its potential therapeutic effects for muscle healing remain still unknown. In our previous work, we demonstrated that CS and GLU administration improves muscle healing and force recovery of the injured skeletal muscle in rats, thus suggesting an important role of these products as potential new therapies for the treatment of muscle injuries in sports medicine. The aim of the present study is to elucidate the mechanisms of action responsible for this interesting benefit.
Methods: Skeletal muscle biopsies were rinsed in a phosphate-buffered saline (PBS) containing 5 μg mL^{-1} de Amphotericine B and minced into fine pieces. The tissues were further digested in a solution containing 1.25 mg/mL Protease type XIV, for 1 h at 37ºC with intermittent shaking. The resulting tissue suspension were collected by centrifugation at 1500×*g* for 5 min and the digested muscle pellet was resuspended in warm, sterile PBS, triturated to liberate the human satellite cells. Differential centrifugations were used to enrich the cell fraction. The collected supernatants were filtered through a 100 μm cell strainer and then centrifuged at 1500×*g* for 5 min. The final cell-pellet was re-suspended in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and pre-plated for 2 h. Cell suspension was then transferred onto cell-culture dishes in Growth media (DMEM/M-199 medium (3:1) with 10% FBS, 10 μg/ml insulin, 2 mM glutamine, 25 ng/ml fibroblast growth factor, and 10 ng/ml epidermal growth factor) and cells were expanded in a growing monolayer. The effect of CS+GLU treatment in primary human skeletal muscle cells was evaluated in a cell proliferation assay. NF-κB intracellular levels were determined by Western Blot. TNF-α production was measured in culture medium supernatants by ELISA.
Results: An enhancement in cell proliferation was found in CS+GLU treatments at a concentration of 100 and 200 μg/ml, increasing 160-fold (p&lt0,01) and 204-fold (p&lt0,001), respectively, compared to untreated cells. In addition, myoblasts were then incubated with IL-6 (50 ng/ml) for 72 h in order to induce an inflammatory environment. The results showed an IL-6 induced-reduction on cell proliferation in all groups, although the data did not reach statistical significance. Therefore, an IL-6 inhibitory effect on cell proliferation in human muscle cannot be ensured. We also measured the effect of the combined treatment CS+GLU on NF-κB activation and TNF-α production in human skeletal muscle cells in primary culture. Despite of TNF-α levels were undetectable in cell supernatants, preliminary data showed a slight reduction on NF-κB signaling pathway. Global gene expression profiles, measured by microarrays and GeneChip Human Gene 1.0 ST Arrays (Affymetrix), will also be analyzed.
Conclusions: Musculoskeletal injures are the most common cause for severe, chronic pain and physical disability affecting hundreds of millions of people around the world and represent a major concern also in sports medicine. A preclinical study evaluated the impact of CS and GLU combination on muscle healing. The results showed that daily administration of both oral and intraperitoneal CS and GLU (combined) induced not only an increase in intramuscular CS deposition in the injured area but also improved muscle force and stimulated the growth of regenerating muscle fibers. The mechanisms of action involved in this potential therapeutic effect seem to be related with an increase in muscle cell proliferation, together with blocking NF-κB nuclear translocation and TNFα production. Although further investigation is required, these preclinical data suggests potentially positive effects of CS and GLU administration for the treatment of skeletal muscle injuries in sports medicine.