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"Exploring the Paracrine Potential of Peri-Articular Muscle in Response to Knee Osteoarthritis Severity"

Skeletal muscles such as the *Articularis genu* (AG) undergo disuse-mediated myofiber type re-distribution, atrophy, and fibrosis attributable to knee osteoarthritis (KOA). Although peri-articular musculature can differentially secrete over 600 myokines in response to exercise or arthropathy, its paracrine influence on the symptomatic severity of KOA is largely underexplored. Myostatin (Mst) and irisin myokines have been of particular interest in cardiac research for their effective modulation of hypertrophic damage and endomysial fibrosis in cardiomyopathy, which are also hallmark structural features of the KOA musculature that can impair joint function. Measuring intra-articular concentrations of Mst and irisin in the synovial fluid (SF) of KOA patients can yield insight on the local inflammatory, hypertrophic, and fibrotic status of the quadriceps femoris, which can be histologically assessed by using the AG as a surrogate. We predict that Mst and irisin levels in the SF of KOA patients will align with clinical metrics of KOA symptomology and motility. Together with indicators of KOA severity such as synovitis histology validated with interleukin (IL)-6 levels in the SF for inflammation and collagen deposition measures validated with transforming growth factor (TGF) β-1 levels for fibrosis, measuring Mst and irisin will add to a panel of SF analytics after arthrocentesis to expand on KOA severity classification. *We predict that lower concentrations of irisin with higher concentrations of Mst, IL-6, and TGFβ1 in the SF will correlate with worse range of motion and patient-reported outcome scores from a validated questionnaire.* Refined assessment of the health status of peri-articular muscle before total knee arthroplasty would more precisely guide individualized strategies for peri-operative soft tissue conditioning such as the fine adjustment of frequencies during neuromuscular electrical stimulation. Ultimately, such measures and their association to symptomatic and structural KOA will help develop novel patient-centered approaches to maximize function and patient satisfaction.

To test our hypothesis, SF collected from 80 patients during total knee arthroplasty and cryopreserved in liquid nitrogen will be used to measure the proposed analytes by high-sensitivity, sandwich enzyme-linked immunosorbent assay (ELISA). SF will be thawed and divided into 100 µl aliquots for pre-treatment with hyaluronidase and centrifugation to remove matrix macromolecules and cellular debris. Cleared aliquots will be diluted 10-25 times in high-performance ELISA buffer. SF dilutions will be optimized per assay based on the sensitivity and detection range of each ELISA kit. Samples will be assayed in duplicate against Mst, irisin, IL-6, and TGFβ1, output measured in a Biorad microplate reader, and readings analyzed against corresponding standard curves. We will compare myokine concentrations between patients grouped by poor (≤85º), fair (90º-110º), and good (≥115º) ROM using one-way ANOVA with $\alpha=0.05$. Spearman's ρ will be used to correlate ELISA values with retrospective histological metrics for synovitis and fibrosis and pre-operative scores from the symptoms section of the Knee Severity and Osteoarthritis Outcome Scores (KOOS) survey corresponding to each patient.

This study will contribute to the overarching goal of better understanding the global and diverse effects of KOA on peri-articular muscle health and the potential development of a minimally invasive diagnostic tool to guide peri-operative soft tissue rehabilitation.