five were different at the time of ACL reconstruction surgery, six were different 6 months post-surgery, and eight were different 12 months-post surgery. L-methionine, Glyoxylic acid, and Malonic acid concentrations were significantly lower across all time points in ACL injured cases compared to uninjured matched controls. L-Lysine concentrations were significantly higher in ACL injured cases across all time points. Succinic acid concentrations were significantly higher in ACL injured cases at the time of injury and at 6 months and 12 months post-surgery. Arachidonic acid concentrations were significantly higher in ACL injured cases at the time of injury, the time of surgery, and 12 months post-surgery. The total percentage of explained variance by the first two principal components was 55.0% at the time of ACL injury, 58.0% at the time of ACL reconstruction surgery, 59.8% at 6 months post-surgery, and 56.6% 12 months post-surgery. As demonstrated in the figure, modest separation was observed between ACL injured cases and uninjured matched controls at the first two time points (time of injury and surgery) and notable separation was observed at the latter two time points (6 months and 12 months post-surgery). The proportion of cumulative explained variance with the first four principal components improved to 78.2% to 84.5% across all time points.

Conclusions: Plasma concentrations for several metabolites appear to differentiate between ACL injured cases and uninjured controls matched for sex, age, height, and weight. This differentiation between groups appears to improve at 6 and 12 months following ACL reconstruction. The metabolites identified may provide insight into the pathways associated with the initiation and progression of post-traumatic osteoarthritis following ACL injury; however, further research and longer term follow-up in this cohort is needed to confirm this.

Table. Summary of the 10 metabolites identified via Fisher-Ratio analyses. The signal area ratio (equal to the concentration ratio) is provided along with the p-value for the patient samples relative to the matched control group at each time point.

Metabolite	Time of ACL Injury Case/Control (p-value)	Time of ACL Surgery Case/Control (p-value)	6 Months Post Surgery Case/Control (p-value)	12 Months Post Surgery Case/Control (p-value)
L-methionine	0.41 (<0.001)	0.31 (< 0.001)	0.48 (<0.001)	0.57 (<0.001)
Glyoxylic acid	0.54 (<0.001)	0.54 (<0.001)	0.48 (<0.001)	0.54 (<0.001)
L-Lysine	1.33 (<0.001)	1.42 (<0.001)	1.13 (0.026)	1.27 (<0.001)
Malonic acid	0.64 (<0.001)	0.68 (<0.001)	0.61 (<0.001)	0.07 (<0.001)
Glycine	1.21 (0.020)	1.06 (0.260)	1.07 (0.240)	1.02 (0.237)
Lactic acid	0.91 (0.269)	0.88 (0.157)	0.90 (0.268)	0.77 (0.024)
Adenine	1.01 (0.442)	0.96 (0.331)	1.15 (0.034)	1.34 (<0.001)
Arachidonic acid	1.31 (0.014)	1.53 (0.002)	1.10 (0.170)	1.55 (<0.001)
Glutamic acid	1.50 (0.020)	1.17 (0.086)	0.99 (0.498)	1.12 (0.114)
Succinic acid	1.34 (0.006)	1.20 (0.467)	2.89 (<0.001)	2.34 (<0.001)

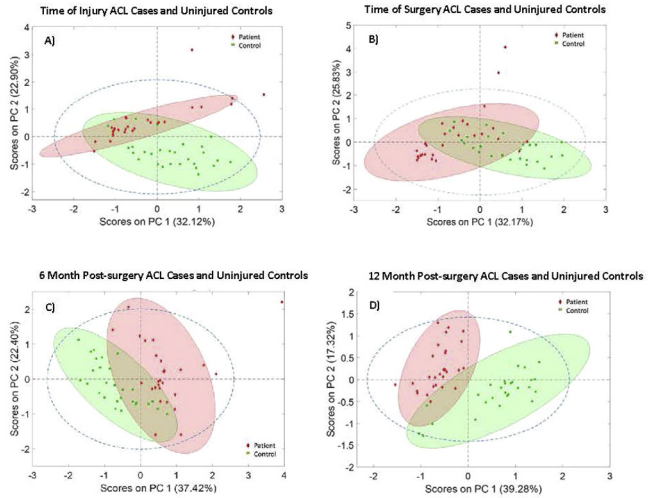

Figure. Principal Component Analysis plots and 95% confidence intervals for the two patient groups relative to the four time points of interest: A) time of injury, B) time of surgery, C) 6 months post-surgery, and D) 12 months post-surgery.

412
A SYSTEMATIC REVIEW OF THE SECRETOME OF SKELETAL MUSCLE CELLS

A. Florin [1], C. Lambert [1], C. Sanchez [1], A. Mobasheri [2], Y. Henrotin [1]. [1] *Bone and Cartilage Res. Unit, Arthropôle Liège, Univ. of Liège, CHU Sart-Tilman, Belgium, Liège, Belgium;* [2] *Dept. of Regenerative Med., State Res. Inst. Ctr. for Innovative Med., Vilnius, Lithuania., Vilnius, Lithuania*

Purpose: Proteomic studies of the secretome of skeletal muscle cells will help to understand the processes that govern the synthesis and organization of skeletal muscle. In this review, we have summarized recent mass-spectrometry based proteomics discoveries of skeletal muscle cells in response to disease, exercise or metabolic stress.

Methods: A literature search was performed in the Pubmed/Medline and Scopus electronic databases, focusing on articles published between July 2004 and May 2018. Only papers published in English and reporting the analysis of the secretome of isolated skeletal muscle myoblasts or of skeletal muscle explants of all species by mass spectrometry were included.

Results: A total of 17 papers were identified and analyzed. Published research included comparative analysis of differentially expressed proteins between healthy and unhealthy (Duchenne muscular dystrophy and insulin-resistant cells) muscle cells and comparison of the secretome of skeletal muscle cells during myogenesis and after insulin stimulation or exercising. The proteins were separated into several categories (extracellular matrix, growth factors and cytokines, enzymes, enzymatic inhibitors, cytoskeletal and miscellaneous proteins) and their differential secretion was compared and important differences were highlighted. In total, 288 proteins were listed in this systematic review as being present in the secretome of muscle cells. Among them, 11 proteins were differentially regulated by physical exercise (all upregulated), 28 during myogenesis (3 up- and 25 downregulated), 27 by insulin stimulation (14 up- and 13 downregulated) and finally 164 proteins secreted by insulin-resistant muscle cells (20 up- and 144 downregulated).

Conclusions: This systematic review of the secretome of skeletal muscle cell in health and diseases provides a comprehensive overview of the most regulated myokines in pathological conditions. These myokines may be therapeutic targets or biochemical markers of muscle diseases.

413
EXPLORATORY PROTEOMICS OF HUMAN SYNOVIAL FLUID IN KNEE OSTEOARTHRITIS: TOWARDS BIOMARKER IDENTIFICATION

N. Ali [1], J. Tjörnstand [2], P. Neuman [2], E. Folkesson [1], V. Hughes [1], P. Onnerfjord [3], M. Englund [1]. [1] *Clinical Epidemiology Unit, Orthopedics, Dept. of Clinical Sci., Lund Univ., Lund, Sweden;* [2] *Dept. of Orthopedics, Skåne University Hospital, Sweden;* [3] *Molecular Skeletal Biology, Section for Rheumatology, Dept. of Clinical Sci., Lund Univ., Lund, Sweden*

Purpose: There is a lack of valid and robust biomarkers in the field of OA diagnosis, prognosis, and treatment evaluation. Thus, our aim was to perform mass spectrometry (MS) of human synovial fluid using a global discovery approach, to identify biomarker candidates associated with meniscus degradation and/or knee OA.

Methods: Synovial fluid was sampled from 3 different subject groups: *i)* end-stage medial compartment knee OA patients undergoing arthroplasty (n=11, 3 men, 8 women; age range 55-80 years), *ii)* knee arthroscopy patients who had knee symptoms but no or only minor knee trauma, typically having a degenerative meniscal tear (n=7, 3 men, 5 women; age range 50-64 years), and *iii)* deceased human donors (within 48 hours post mortem) without known chronic knee disease (n=13, 8 men, 5 women; age range 19-79 years). These 3 groups were roughly assumed to represent *i)* end-stage knee OA, *ii)* early knee OA, and *iii)* healthy controls, respectively. For all 3 groups, we required no visual signs of blood contamination to be present in the synovial fluid. For the cadaver knee tissues (controls), we further required the meniscus and the weight-bearing femoral surface of cartilage/bone to be macroscopically intact. All synovial fluids were centrifuged and freshly frozen and stored at -80°C. For the analysis, 50 µL of synovial fluid from each sample was mixed with MS-safe proteinase inhibitor cocktail and hyaluronidase, and further depleted of the 7 most abundant proteins with the multiple affinity removal system (MARS Hu7 spin cartridge). After reduction and alkylation, the samples were precipitated and the pellets were further digested with sequencing grade trypsin (Promega. Following digestion, samples were cleaned with a 30kDa filter and desalted. The samples were further spiked with iRT peptides before analysis by MS. The samples were analyzed with an EASY-nLC 1000 coupled to an Orbitrap Fusion mass spectrometer. The samples were analyzed with data-independent acquisition settings. The raw MS data were further analyzed with Spectronaut™ software for protein identification and quant data extraction. The quant data extracted using Spectronaut™ were further imported into the R