Introduction: Severe irreversible tissue damage occurs early in the osteoarthritic (OA) disease process, prior to the onset of radiographically observable changes. It is generally agreed that degradation of the molecular components of articular cartilage is integral to its destruction. Since the degradation by-products of OA are first released from the cartilage matrix into the synovial fluid (SF), joint fluid analysis should provide much information about the metabolic status of a specific joint. As one of the most powerful methods in analytical chemistry, high resolution nuclear magnetic resonance spectroscopy (NMRS), is particularly well suited to the assessment and quantification of wide variety of low molecular weight metabolites present in body fluids such as SF. In principle, quantitative $^1$H-NMRS data can be recorded from all proton-containing metabolites of interest in one single-pulse experiment. It is likely that a number of competing metabolic processes occurring in the joint will change with OA onset. The purpose of this study was to investigate the nature of these changes wherein we used NMRS to compare the metabolic profiles of normal human synovial fluid with that from four progressive stages of osteoarthritis: mild, moderate, marked and severe.

Methods: Synovial fluid was collected from patients undergoing arthroscopic debridement for knee osteoarthritis. The specimens were kept in low temperature storage until processing. The degree of OA severity was documented at arthroscopy. Twenty-five samples were analyzed; 5 samples judged to be free of osteoarthritis, and 5 samples each from the mild, moderate, marked and severe OA categories. Samples were made in a 60:40 ratio of SF:D$_2$O in 5 mm NMR tubes for a total controlled volume of 600 µl. Data were acquired on a Varian 12T system (500.011 MHz-$^1$H) using the CPMG pulse sequence D-90$_0$,-t-(180$^\circ$,-t),-acquire (d=3 sec, t= 1ms, 2n= 48 ms, acquisition time = 1.33 sec, 256 scans, 16,384 data points, spectral width 6000 Hz) and using gated presaturation at the water resonance frequency to suppress the intense water signal.

Chemical shifts were referenced to a known concentration external-internal standard of TSP contained inside a co-axial capillary tube at the center of each NMR sample. Peak areas obtained using a Marquardt-Levenberg line fitting routine/integration were normalized with respect to the peak area from the TSP. Changes in metabolic profiles of the SF samples were assessed for the following metabolites: glucose, choline, creatine/creatinine, citrate, pyruvate, N-acetyl-glycoproteins, alanine, lactate, glutamine/glutamate, CH$_3$- and CH$_2$-lipids, valine, iso-leucine and leucine. Changes in metabolite levels were tested for significance using one way ANOVA with SIGMASTAT, a computer software statistical package.

Results: The levels of glucose, lactate, pyruvate, citrate, creatine/creatinine, hydroxymyobutrate; the amino acids lysine, leucine, glutamine and glutamate; and the mobile components of (-CH$_2$)$_n$ chain, CH3-terminal end lipoproteins, choline headgroup components of high density lipoproteins and mobile components of hyaluronic acid (N-acetyl-glycoproteins) were all found to be significantly different in progressive OA human synovial fluid with respect to normal synovial fluid (p<0.05), as depicted in Figure 1. Moreover, a linear increase in concentration across the five levels was observed for some metabolites (glycerol, pyruvate, glucose and creatine/creatinine), while for others, a sharp initial increase in concentration with OA onset was followed by incremental increases with progressive OA, leading to an eventual decrease in severe OA (lipoproteins, hyaluronic acid, glutamine and glutamate).

Discussion: Our results indicate that not only does the metabolic profile of synovial fluid from patients in progressive stages of OA differ markedly from that of normal synovial fluid, but, in addition, an actual biochemical pattern of joint metabolism begins to emerge. The steady and pronounced increase in glucose levels may be the result of increased metabolic demand with progressive OA. Similarly, the rise in lactate levels indicates an increasingly hypoxic and acidic intra-articular environment, further exacerbating cartilage degradation. The initial sharp increase in lipoprotein levels in early OA may reflect increased synovial permeability to these macromolecules, caused by inflammatory processes; the subsequent decrease in concentration is probably the result of increased fatty acid utilization for energy with progressive OA.

Increases in the N-acetyl-glycoprotein signal intensity suggests an elevation in the concentration of mobile components/side chains of hyaluronic acid, indicating degradation of this substance into smaller polymeric units with progressive OA. Interestingly, the levels of the branched amino acids, valine and isoleucine, are both gradually diminished suggesting their depletion as alternate substrates for glycolytic processes with OA progression.

In summary, this study shows that a pattern of metabolic changes occurs with increasing OA severity suggesting increased metabolic demand leading to the use and eventual near-depletion of alternate sources of energy in end-stage osteoarthritis. Such information about the biochemical processes underlying degenerative changes will be valuable in better understanding osteoarthritic pathogenesis and progression.

Acknowledgments: Physician Services, Inc.

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