INTRODUCTION:
Osteoarthritic (OA) chondrocytes exhibit a refractory response to soluble factors that are otherwise stimulatory of extracellular matrix synthesis. In particular, the role of insulin-like growth factor I on OA chondrocyte metabolism is reduced in spite of evidence of the receptor. A number of mechanisms have been implicated in the refractory nature of the IGF-I in these cells. Early studies showed that insulin can compete for the IGF-I receptor in chondrocytes. In some cell types, different forms of insulin-receptor can be active in the regulation of glucose uptake and cell metabolism. With respect to cartilage, insulin has proven effective as a stimulus for chondrocyte metabolism depending on the level and the type of delivery.

In addition to responsiveness to growth factor stimulation, articular chondrocytes exhibit a range of response to different types of mechanical stimulation. In vitro, addition of hydrostatic pressure induces increased matrix protein expression in normal chondrocytes depending on the level, frequency and duration of loading. With loading regimens up to twelve hours, intermittent hydrostatic pressure decreases the expression of matrix metalloproteinases and proinflammatory cytokines.

In osteoarthritis, onset of cartilage damage manifests as focal lesions that will progress without appropriate intervention. The challenge is to understand the types of factors, either mechanical or biological, that may serve as adjunctive therapies to be used in concert with surgical approaches. The objective of this study was to examine the effect insulin (INS) and intermittent hydrostatic pressure (IHP) on matrix gene expression in articular chondrocytes from osteoarthritic cartilage. This research addresses the hypothesis that extracellular matrix repair in osteoarthritic chondrocytes and repair are critically dependent on specific mechanical loading regimens acting through cell specific mechanoreception.

METHODS:
Articular chondrocytes were harvested from autopsy samples of cartilage obtained following total knee arthroplasty under an approved human subject protocol. The cartilage samples were randomly acquired with respect to sex and age. The chondrocytes were plated in primary culture at a density of 1 x 10^6 cells/60 mm^2 culture plate and maintained in serum containing medium. Prior to testing, the culture were changed to serum-free medium overnight. The medium was changed again to serum-free medium and the cultures were exposed to IHP with and without addition of insulin (10 micrograms/ml). Insulin was added to serum-free tissue culture medium (DMEM/F12) following solubilization in mild base and neutralization. IHP was applied by servo-hydraulic instrumentation using a loading regimen of 4 hours loading per day for 4 days. Each patient sample was distributed into individual culture plates as triplicates for testing under four conditions, (1) no INS, no IHP, (2) INS, no IHP, (3) No INS, IHP, and (4) INS with IHP. Following the loading, total RNA and protein were isolated. Gene expression was quantified by mRNA signal levels using real-time PCR. The data were expressed as quantity of target RNAs (ribosomal 18S RNA and mRNA for aggrecan, type II collagen, actin, matrix metalloproteinase-2 as a function of a reference RNA standard curve for each product. Each of the triplicate cultures was analyzed in RT-PCR as triplicate samples and the RT-PCR data were averaged to obtain a mean signal level per culture plate. The results for the triplicate samples for each of the four conditions were then subjected to statistical analysis using the General Linear Methods with Tukey’s correction for multiple comparison with a significance level of p<0.05 (two-tailed).

RESULTS:
In this study, the reactivity of each patient to INS and IHP was unique. In Patient A, significant changes were observed in ribosomal RNA levels with respect to OA chondrocyte cultures having addition of INS with IHP compared to cultures without INS or IHP (p<0.05). Similarly, in this patient, aggrecan mRNA and Col2A mRNA exhibited significant increases with addition of INS with IHP compared to cultures without INS or IHP (p<0.05). In addition, addition of INS alone was sufficient to increase Col2A expression when compared to culture exposed to IHP alone. Actin levels were also increased in this patient in response to insulin.

The effect of INS addition together with IHP was sufficient to increase the aggrecan mRNA level significantly in 4 of the six patients when compared to addition of INS or to culture without INS or IHP. In the case of the type II collagen gene expression, five of six patients exhibited a significant increase (p<0.05) in Col2A mRNA levels when compared to addition of INS alone or IHP alone. None of the six patients showed a significant increase in MMP-2 expression with addition of INS, IHP or the combination with INS with IHP. With respect to the dose responsiveness of the OA chondrocytes to insulin, INS at concentrations of 1 and 10 micrograms/ml exhibited comparable stimulatory effects on Col2A when compared to INS at a concentration of 0.1 micrograms/ml or chondrocytes maintained without insulin.

DISCUSSION:
This study demonstrated that addition of insulin to OA chondrocytes was sufficient in some patients to induce a general metabolic shift up that was evidence by an increase in ribosomal RNA or actin. In contrast to these metabolic markers, MMP-2 was generally not altered by the addition of insulin. In some patients, the addition of intermittent hydrostatic pressure resulted in changes in either aggrecan or type II collagen. However, the majority of the patients were responsive to the addition of both insulin and hydrostatic pressure. The relative change in the matrix protein expression was patient specific. The data show that, similar to DNA array analysis, the responsiveness of OA chondrocytes to changes in culture conditions, either mechanical loading or addition of a growth factor such as insulin, will depend in large part on the background metabolic state of the cells. At present, a single factor by which generalized chondrocyte metabolism can be normalized is unclear. With a single intervention, such as mechanical loading alone, this has been possible. However, the data presented here suggest that responsiveness of cellular metabolism to different stimuli will carry with it unique sets of gene changes.

Figure 1

Heterogeneity of Gene Expression in OA Chondrocytes

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