FLUID INDUCED SHEAR STRESS INCREASES HUMAN OSTEOARTHritic CHONDROCYTE PRO-INFLAMMATORY MEDIATOR RELEASE IN VITRO

**Methods:**
Isolation of Articular Chondrocytes: Articular chondrocytes were isolated from cartilage obtained as autopsy specimens from patients undergoing primary total knee arthroplasty. Briefly, cartilage was removed from the joint surfaces, diced and placed in 0.25% trypsin for 30 min. The cartilage was washed with Dulbecco’s phosphate buffered saline (PBS) and placed in 20 ml of DMEM/F12 containing gentamicin and a mixture of bacterial collagenases, Type II and IV (Worthington), at a final concentration of 1.0 mg/ml each. The cartilage samples were incubated for a 36 hour period at 37°C in 5% CO2 until assayed.

Cytokine Determination: Monospecific antibodies to IL-6 and MCP-1 (R&D Systems) were purchased and levels of cytokine expression were measured via ELISA.

Statistical analysis: Statistical analysis was carried out using Friedman's method for randomized blocks followed by Wilcoxon's signed ranks tests. Values of p<0.05 were considered significant. Bonferroni's correction for multiple comparison was applied.

**Results:**
FISS increased human osteoarthritic chondrocyte release of IL-6 by 141% and 229% following application of FISS for 6 and 12 hours, respectively, relative to control cultures. (Table I). FISS increased human osteoarthritic chondrocyte release of MCP-1 by 139% and 198% following application of FISS for 6 and 12 hours, respectively, relative to control cultures.

<table>
<thead>
<tr>
<th>Sample Tested</th>
<th>IL-6 (Mean±SE)</th>
<th>MCP-1 (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98±26</td>
<td>238±48</td>
</tr>
<tr>
<td>Shear 2 Hours</td>
<td>326±85*</td>
<td>644±16</td>
</tr>
<tr>
<td>Shear 6 Hours</td>
<td>326±85*</td>
<td>644±16</td>
</tr>
<tr>
<td>Shear 24 Hours</td>
<td>326±85*</td>
<td>644±16</td>
</tr>
</tbody>
</table>

*Denotes p<0.05 vs. control culture

**Discussion:**
It is generally recognized that mechanical loading of normal joints contributes to the maintenance of the articular cartilage extracellular matrix. However, the precise relationship between mechanical loading and chondrocyte metabolism remains undefined. Areas of high shear stress may lead to cartilage degeneration and loss of joint function. This study shows that FISS regulates the expression of the pro-inflammatory mediators, IL-6 and MCP-1. The effects of increased production of pro-inflammatory cytokines may influence degredation of extracelluar matrix and cartilage homeostasis.

**Acknowledgement:** This work was supported by VA RR&D Merit Review A857-RC

**References:**

**Introduction:**
Cartilage metabolism depends in part on a cellular response to mechanical forces, including shear stress and hydrostatic pressure, that occur during normal joint loading. Mathematical models of tissue loading histories by Carter et al. propose that cartilage undergoes a tissue differentiation process that includes degeneration and ossification that is accelerated by shear stresses, but remains inhibited by intermittently applied hydrostatic pressure (1,2). Previous in vitro studies confirm that chondrocytes in culture continue to respond to a variety of loading conditions (3,4). A second major contributing factor to cartilage metabolism involves the state of the joint environment. A number of pro-inflammatory mediators are associated with inflammation including prostaglandin E2 (PGE2), nitric oxide, interleukin-1beta and interleukin-6 (IL-6). Analysis of chondrocytes retrieved from osteoarthritic joints has demonstrated that they secrete the pro-inflammatory cytokine, IL-6, which is associated with bone remodeling (5,6). Villager et al. demonstrated that interleukin-1 stimulates chondrocyte release of the C-C chemokine monocyte chemoattractant protein-1 (MCP-1) (7). Previous studies have associated MCP-1 with monocyte attraction to sites of inflammation (8). One potential mechanism influencing cartilage degeneration is the secretion of the pro-inflammatory mediators IL-6 and MCP-1, that create an environment in which bone remodeling occurs and loss of cartilage form and function is experienced. The purpose of this study was to quantify the effects of fluid induced shear stress (FISS) on cultured articular chondrocyte metabolism. This study tested the hypothesis that FISS would increase secretion of the pro-inflammatory mediators, IL-6 and MCP-1, in human osteoarthritic chondrocytes in vitro.

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