An Obesity Induction Rat Model to Study The Metabolic Subtype of Osteoarthritis in Rats

University of Calgary, Calgary, AB, Canada.

Disclosures:

Introduction: Fat cells have the capacity to initiate inflammatory cascades.[1] Both, high fat feeding and resultant obesity are associated with inflammation, and inflammation is thought to exacerbate OA.[1] Traditionally understood as a consequence of loading or trauma, OA is also observed in non-loadbearing joints, such as the wrist, and recent literature suggests that inflammation from high fat feeding is enough to cause OA, suggesting that obesity is an independent risk factor for the onset of OA.[2-3] Sprague-Dawley rats respond well to diet-induced obesity (DIO) when compared with other rodents, and can be easily characterized as obesity prone when given free access to a high fat diet.[4] Recently, it has been suggested that OA progresses at a higher rate with a high fat diet independent of an increase in mass.[5] Leptin is an adipokine responsible for satiety signaling, that has been implicated in an increased rate of OA progression in obese individuals and cartilage damage.[6] Griffin and colleagues demonstrated that obese leptin-deficient animals do not develop OA despite their increase in mass while leptin-intact animals did.[7] However, the relationship between the rate of OA progression in a mechanically perturbed joint and the presence of a high fat diet and obesity has not been studied but represents a clinically realistic scenario. Therefore, the purpose of this study was to evaluate the effect of a high fat, high sucrose diet, and resultant obesity, on the local inflammation and rate of OA progression in ACL transected and intact contralateral rat knees. In order to achieve this aim, OA progression in obese animals was compared to lean surgical controls. We hypothesized that high fat diet animals (1) show more severe OA progression in the ACL transected and contralateral knee when compared to lean control animals and (2) have greater synovial fluid leptin concentrations in the ACL transected and contralateral intact knee.

Methods: Seventeen male Sprague-Dawley rats (8-12 weeks old) were randomly separated into two groups. The two groups consisted of DIO (n=12) group and control (n=5) group animals, where DIO animals were fed a fat high sucrose food (40% fat, Diet #102412, Dyets, Inc) and control animals received lean chow (LFD, 13.5% fat, LabDiet 5001). After the baseline data collection, animals were randomized to unilateral ACL-X or surgical sham groups. Animals were followed for 16 weeks post-surgery and sacrificed. At sacrifice, synovial fluid (SF) blood were collected and evaluated using a Rat Cytokine Array/Chemokine Array 27-Plex Panel (EMD Millipore) detected by Luminex xMAP. Following sacrifice, both knee joints were harvested, cleaned, and whole joints were fixed for histological analysis, embedded into paraffin blocks, and cut into 8um sections. OA damage was determined by the Osteoarthritis Society International (OARSI) scoring system. SF leptin concentrations were compared between limbs using Wilcoxon-paired tests and between groups using Kruskal-Wallis tests. Experimental and Contralateral limb Mankin Scores were compared using Wilcoxon Signed-Rank Test. All statistical tests were performed at α=0.05.

Results: The DIO animals had a 51.5±13% increase in body mass after the obesity induction, compared with a 28±7% increase for the LFD animals (p=0.001). Blood Leptin concentration was higher in DIO animals (3680±3985 pg/mL) compared to that obtained in LFD animals (15735±6173 pg/mL, p<0.001). SF Leptin concentration at sacrifice was higher in all DIO animals, regardless of surgery or limb, when compared to LFD animals (Figure 1). OARSI scores for experimental limbs were the same (DIO median=14, LFD median=12), but contralateral scores were higher (more severe damage) for the DIO compared to the LFD animals (p=0.025, DIO median=11, LFD median=8). Non-surgical LFD, age-matched control animals had OARSI Scores of 3 in both knees.

Discussion: To date, obesity induction success is measured by increased change in gross body mass over time compared to controls.[4] DIO animals demonstrated almost twice the percent change in body mass than LFD animals, confirming a successful obesity induction. DIO animals also demonstrated twice the concentration of blood leptin when compared to LFD controls. Although body composition was not measured, we can speculate that the DIO animals have greater fat mass when compared to LFD due to the increase in systemic Leptin concentration, which has been shown to be strongly correlated with fat mass, and is positively associated with OA damage.[8] To our knowledge, SF has not been profiled in a preclinical model comparing DIO to LFD in the presence of post-traumatic OA (ACL-X). Additionally, despite no differences in experimental limb OA damage, DIO animals demonstrated higher OARSI scores in their contralateral limbs, and higher SF leptin in both knees compared to LFD animals. Therefore, these results suggest that obesity and HFD seem to contribute to an increase rate of OA progression in this model, and potentially a unique OA trajectory when compared to a post-traumatic OA model.

Significance: These data suggest that obesity may affect Osteoarthritis through different mechanisms than ACL-X surgery alone. HFD and obesity are independent contributors to the rate of OA progression when evaluating non-operative limbs. This study suggests that obesity induction is a method of bilateral OA progression, distinct from the progression cross-sectionally...
demonstrated in LFD ACL-X animals.

Acknowledgments: University of Calgary Eyes High, Alberta Innovates Health Solutions, Alberta Osteoarthritis Team, Alberta Graduate Education Council, Killam Foundation, the Canada Research Chair Programme and the Natural Sciences and Engineering Research Council.

References:

**Figure 1:** Synovial Fluid (SF) Leptin concentration from experimental (EXP) and contralateral (CON) limbs of low fat diet (LFD) and diet induced obesity (DIO) rats at time of sacrifice. There were no differences between limbs for either group, but the DIO animals demonstrated elevated SF Leptin levels bilaterally when compared the LFD group.

ORS 2014 Annual Meeting
Poster No: 1217