Title: Response to Diet-Induced Obesity Produces Time-dependent Induction and Progression of Metabolic Osteoarthritis in Rat Knees

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Abstract

Obesity, and corresponding chronic-low grade inflammation, is associated with the onset and progression of knee OA. The origin of this inflammation is poorly understood. Here, the effect of high fat, high sucrose (HFS) diet induced obesity (DIO) on local (synovial fluid) and systemic (serum) inflammation is evaluated after a 12-week obesity induction and a further 16-week adaptation period. For 12-weeks of obesity induction, n=40 DIO male Sprague-Dawley rats consumed a HFS diet while the control group (n=14) remained on chow. DIO rats were allocated to prone (DIO-P, top 33% based on weight change) or resistant (DIO-R, bottom 33%) groups at 12-weeks. Animals were euthanized at 12- and after an additional 16-weeks on diet (28-weeks). At sacrifice, body composition and knee joints were collected and assessed. Synovial fluid and sera were profiled using cytokine array analysis. At 12-weeks, DIO-P animals demonstrated increased Modified Mankin Scores compared to DIO-R and Chow ($p=0.026$), and DIO-R had higher Mankin scores compared to Chow ($p=0.049$). While numerous systemic and limited synovial fluid inflammatory markers were increased at 12-weeks in DIO animals compared to Chow, by 28-weeks there were limited systemic differences but marked increases in local synovial fluid inflammatory marker concentrations. Metabolic OA may manifest from an initial systemic inflammatory disturbance. 12-weeks of obesity induction leads to a unique inflammatory profile and induction of metabolic OA which is altered after a further 16-weeks of obesity and HFS diet intake, suggesting that obesity is a dynamic, progressive process.

Keywords: Metabolic Osteoarthritis, Synovial Fluid, Rat, Systemic Inflammation, Knee Joint

Introduction

Increasing rates of obesity have been attributed to the consumption of a "western-type" high fat, high sugar diet[1]. Obesity is associated with osteoarthritis (OA)-related changes both clinically and in animal models[2]. Likely, metabolic OA may be one subtype of OA with a distinct inflammatory signature, and a unique OA trajectory, when compared with other subtypes (e.g. post-traumatic, genetic.)[2–5].

Previously, we and others have shown that long-term exposure to high fat or western-type diets result in OA related changes in animal models of diet-induced obesity[6–8]. Furthermore, these OA-related changes may be explained by the inflammatory signatures and metabolic dysfunction resulting from the increased body fat of these animals[3,6,7,9]. Moreover, high fat high sucrose diet-induced obesity significantly alters the synovial fluid cytokine, adipokine, and growth factor concentrations in rats after 28-weeks, and many changes are associated with OA-like structural changes[3,10]. However, comparative inflammatory marker profiles from serum and synovial fluid over time, in the context of a high fat, high sucrose diet-induced metabolic OA, are unknown, but are critical to understanding this disease trajectory.

Similar to humans, outbred Sprague Dawley rats demonstrate obesity prone and obesity resistant phenotypes after exposure to a high energy obesity-inducing diet[11]. A diet-induced obesity protocol is typically conducted over a 10-12 week induction period, allowing animals to reach "heterostasis," at which time obese animals demonstrate increased mass, and presumably increased metabolic disturbance, when compared with chow-fed controls[11]. These two phenotypes (prone and resistant to obesity while on a high fat, high sucrose diet) can be used to evaluate intrinsic changes related to obesity in two groups of different mass but presumably similar metabolic disturbance.

Although high fat diets, resultant obesity, and OA-related changes have been explored using a variety of animal models, much of this work was done over time periods beyond the obesity induction period[6,7,9,10,12–14]. As such, we wanted to explore OA-related changes in the knees of high fat, high sucrose (HFS) diet induced obese rats at the end of their obesity induction period; 12-weeks on the diet. To illustrate changes over time, and to provide mechanistic insight, we present findings from animals on the HFS diet for 28-weeks, representing a 16-week adaptive period after the onset of obesity. Other outcomes related to knee joint histology and gut microbiota specific to this 28-week group can be found elsewhere[10].

The purpose of these studies is to understand differences in rate of OA progression related to body fat, and the associated local and systemic inflammatory environments related to the accumulation of adiposity at 12- (post-obesity induction) and after 28-weeks (adaptive period). We **hypothesize** that after 12-weeks of obesity induction differential rates of OA progression will be observed based on the response to the diet, and that the time-course of inflammatory marker profiles of obese animals will indicate a systemic to local disease trajectory of metabolic OA. Furthermore, by quantifying serum and synovial fluid inflammatory marker profiles, we may gain novel insight into the dynamic inflammatory environment due to diet-induced obesity, and therefore contribute critical information regarding the mechanisms contributing to metabolic OA development and progression.

Methods

Animals. Fifty four male, 8-12 week old male Sprague-Dawley rats were purchased from a specific pathogen free facility (Charles River Laboratories) and maintained at the University of Calgary with standard monitoring thereafter. Animals were individually housed on a 12h dark/light cycle. A total sample size of 54 rats was based on detecting a minimal meaningful

difference in Modified Mankin Score of 27 to provide α=0.05 and power of 80%[10]. At the start of the study, animals were allocated into either the high fat/high sucrose diet-induced obesity group (DIO, 40% of total energy as fat, 45% of total energy as sucrose, n=40, **custom Diet #102412, Dyets, Inc,**)[3], or the standard chow low fat diet group (Chow 12% fat, 3.7% sucrose, n=14, **Lab Diet 5001**) for a 12-week *ad libitum* feeding intervention [15–17]. All experiments were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee. Animal mass was recorded weekly throughout the obesity induction period, and after 12-weeks, DIO animals were stratified into tertiles according to changes in body mass, resulting in an Obesity Prone group (DIO-P, top 33% of animals by change in body mass, n=13), an Obesity Resistant group (DIO-R, bottom 33% of animals by change in body mass, n=13), and a middle tertile group, which was not further considered here. After the 12-week obesity induction period (20-24 weeks old), half of the animals from each group (DIO-P, n=6, DIO=R, n=6, Chow n=7) were euthanized. The remaining animals were followed for an additional 16 weeks, or 28 weeks in total (36-40 weeks old). Primary outcomes included body composition and Modified Mankin knee joint scoring. Secondary outcomes included cytokine, adipokine, and growth factor measurements in serum and synovial fluid.

Body Composition. Animals were euthanized by barbiturate overdose (Euthanyl®, MTC Animal Health Inc., Cambridge, Ontario, Canada). Immediately after sacrifice, body composition was measured using Dual Energy X-ray Absorptiometry and calculated by software for small animal analysis (Hologic QDR 4500; Hologic, Bedford, MA).

Knee Joint Histology and Modified Mankin Scoring: Joints were harvested, decalcified, and cut into serial sagittal 8µm thick sections according to previously described methods[3,10]. Alternate slides were stained sequentially with haematoxylin, fast green and

safranin-O stains (Fisher Scientific) using an auto stainer (Leica ST 5010). Slides were dehydrated, coverslipped, and allowed to dry at room temperature for several days before being scored and imaged at 10x and 25x (Zeiss Axiocam® Icc 5 camera, Zen 2011 Zeiss imaging system, Carl Zeiss Inc., Toronto, Ontario, Canada).

A Modified Mankin Score was used to describe the volumetric damage in each joint. Five areas were evaluated: the medial and lateral tibial plateau, the medial and lateral femoral condyle, and the patella. These five sites were assigned a score based on the standard 14-point Mankin scale[18]. Subchondral bone and synovium were then assessed using a 5 and 4 point criteria, respectively that was adapted from the rat-specific OARSI metric[19]. Meniscal damage was scored on a scale of 0-5. The final Modified Mankin score was obtained by adding the five site-specific Mankin scores, the two corresponding OARSI scores, and the meniscal damage score[3,10,18–20]. The Modified Mankin Score thus has a total range from 0-98 across all sites. The inter-rater reliability between the two independent blinded assessors used in this study was $r>0.9$.

Cytokine, Growth Factor and Adipokine Measurements. Animals were sacrificed following a 12h fast, and blood was collected immediately via cardiac puncture. Serum was stored at -80°C until analysis. Synovial fluid was collected shortly after sacrifice using the Whatman chromatography paper method[21]. Samples were weighed, diluted 1:30, centrifuged at 13,500 rpm, and stored at -20°C overnight. Samples were aliquoted 24 hours later and stored at -80°C until analysis. Twenty-seven serum and synovial fluid cytokines and adipokines were quantified using a Rat 27 Multiplex Discovery Assay with Luminex®xMAP technology (Eotaxin, EGF, Fractalkine, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, IP-10/CXCL10, GRO/KC, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1, leptin, LIX, MIP-1α, MIP-2, RANTES, VEGF; Eve Technologies, Calgary, AB). Synovial fluid from the left

and right limbs of each animal was pooled for quantification. Urea was evaluated in duplicate in serum and synovial fluid using ELISA (Sigma Aldrich Urea Kit, Eve Technologies, Calgary, AB) as previously reported[3,10,22].

Statistical Analysis. Blood analytes, synovial fluid analytes, and right knee joint Modified Mankin Scores were evaluated for all animals within each time point. Levene's test for equality of variance was conducted on all outcomes. If positive, or in the case of ordinal Modified Mankin Scores, non-parametric Kruskal-Wallis tests were used to determine differences by obesity response (DIO-P, DIO-R, Chow) and between dietary groups (DIO, Chow). If equal variances were calculated, one-way ANOVAs were performed to assess differences between the groups. Bonferroni corrections were used in all cases to control for multiple testing error. If statistical differences were not observed between DIO-R and DIO-P, data were reported as the mean of all DIO animals compared to the mean of chow animals. Pearson (continuous data) Spearman (Modified Mankin Scores) correlations were used to identify associations between outcomes. Body fat and Modified Mankin Score were dichotomized based on the median within the 12-week groups to enable the calculation of a preliminary odds ratio to assess the relationship between a higher body fat and Modified Mankin score. All statistical tests were conducted in IBM SPSS Statistics 20 (α=0.05).

Results

Body Mass, Body Fat, and Modified Mankin Scores. DIO-P animals had increased body mass compared to DIO-R and Chow at both 12- and 28-week time-points (*Fig. 1A).* Further, DIO-P animals had increased body fat *(Fig. 1B)* compared with both the DIO-R and chow animals at both 12- and 28-week time points. Although DIO-R animals consistently

demonstrated increased body fat percentage compared to Chow animals, average body mass was similar between these groups at both 12 and 28-weeks. Body fat and body mass were positively associated with Modified Mankin Score at 12-weeks (r=0.75 and r=0.70 respectively, p=0.001), but only body fat remained positively associated at 28-weeks (r=0.60, p=0.001). All animals demonstrated increases in body mass, body fat, and Modified Mankin Score between the induction and adaptation periods ($p<0.05$).

At 12-weeks, DIO-P animals exhibited significantly higher Modified Mankin Scores compared to both DIO-R (p=0.05) and Chow animals (p=0.001 *Fig. 1C),* and DIO-R animals had significantly higher Modified Mankin Scores when compared to Chow (p=0.049). Further, at 12-weeks, DIO-P animals had significantly higher medial tibial plateau (p=0.047) and lateral femoral condyle (p=0.008) damage compared to Chow animals (*Table 1).* DIO-P animals had increased lateral femoral condyle (p=0.015), bone (p=0.015), and meniscal damage (p=0.026) compared to DIO-R animals. Synovium scores were higher in DIO-P animals compared to chow (p=0.05), and all DIO animals had higher synovium scores when pooled and compared to chow (p=0.023).

At 28-weeks, DIO-P and DIO-R animals had similar Modified Mankin Scores that were significantly higher than Chow values (p=0.002). The relationship between body fat and Modified Mankin scores and regional damage by group at 28-weeks was reported elsewhere[10]. Of the Chow animals at 28-weeks, three animals demonstrated elevated Modified Mankin Scores, and meniscal subscores of 3-4, while the remaining Chow animals had meniscal subscores of 0-1*(Figure 2B).* Chow animals with elevated Modified Mankin Scores had similar mass (mean: 838.7 [776.5-908.5g] vs. 875.3 [867.0-890.0g]) with similar body fat (mean: 31.5 [21.8-37.4%] % vs. 34 [28.7-42.5%]) compared to the remainder of the 28-week Chow group.

For all groups and all animals, 12-week median body fat was 33% and median Modified Mankin Score was 16, whereas at 28-weeks, the median body fat was 40% and the median Modified Mankin Score was 40 (*Fig. 2A*, *Fig. 2B*). At 12-weeks, all DIO-P animals were in the top 50th percentile of body fat and Mankin Score, half of the DIO-R animals were on each side of the body fat and Modified Mankin Score median, and all chow animals were in the bottom 50th percentile of body fat and Modified Mankin Score. At 12-weeks, the odds of being in the upper half of the Modified Mankin Score given a body fat percentage of ≥ 33% was 18 (1.79-1590, p=0.003).

At 28-weeks, all DIO-P animals were in the top 50% percentile of body fat and Modified Mankin Score. One DIO-R animal was in the bottom 50% of body fat, and two were in the bottom 50th percentile of the Modified Mankin Score. While all Chow animals were in the bottom 50% percentile of body fat, 8 were also in the bottom 50th percentile of the Modified Mankin Score. The 3 Chow animals specified above with uncharacteristically high meniscal damage were in the top 50th percentile of the Modified Mankin Score. At 28-weeks, the odds of being in the upper half of the Modified Mankin Score given a body fat percentage of ≥ 40% was 11 (1.6-76.8, p=0.008).

Serum Inflammatory Profiles. There were no detectable differences in the serum inflammatory profiles of DIO-P and DIO-R animals after 12-weeks on the HFS diet. Of the 27 analytes measured, 12 were increased in the serum of all DIO animals compared to Chow (*Table 2)*, seven of which demonstrated a positive significant association with the Modified Mankin Scores. Three markers, leptin, MIP-2, MIP-1α, were also elevated at the 28-week time point. No differences were detected in serum inflammatory marker concentrations between DIO-P and DIO-R animals.

Synovial Fluid Inflammatory Profiles. Two markers, leptin and IL-1β, were elevated in the synovial fluid of DIO animals at the 12-week time point, and leptin remained elevated at the 28-week time point (*Table 3).* At 12-weeks, DIO-P animals demonstrated increased synovial fluid IL-5 and IL-6 compared to DIO-R. Moreover, both synovial fluid leptin and IL-1β were significantly associated with the Modified Mankin Scores.

Synovium Alterations and Synovial Fluid Inflammatory Markers. Nine animals had a synovium histological subscore of 2 or greater (n=8 DIO, n=1chow), indicating proliferation of synovial tissue and infiltration of inflammatory cells[19]. These animals also demonstrated a trend toward increased synovial fluid leptin and IP-10 concentrations (p=0.33, p=0.27, respectively) compared to animals with synovial subscores less than 2. Furthermore, leptin and IP-10 levels in 12-week animals with synovial subscores greater than 2 were similar to previously reported DIO SF concentration levels observed at 28-weeks in this model (SF leptin p=0.482; SF IP-10 p=0.519)[3].

Discussion

Time-course changes associated with OA damage and inflammatory profiles in metabolic OA are unknown. Therefore, the purpose of this study was to characterize the OA-related changes observed in the knees of DIO-P and DIO-R male rats after a 12-week obesity induction period and a subsequent 16-week exposure period, compared to Chow diet control animals. Our findings suggest that immediately following 12-weeks of obesity induction may be a critical time-point to observe differential rates of progression of OA between DIO-P and DIO-R animals. Furthermore, data from the 12-week animals suggest a preliminary body fat threshold of 33% for increased odds of OA progression. At the 12-week induction phase, we observed increases in the majority of serum systemic inflammatory markers, with few increased local biomarkers in the

synovial fluid. Taken with previous reports of few differences detected systemically and vast differences detected locally at 28-weeks with this diet[10], these data suggest that the role of a metabolic influence on progression of joint damage is a dynamic, evolving process that may be systemically initiated. Although the present study is limited by evaluating only two time points, it does provide an indication of a systemic to local trajectory for metabolic OA, which then becomes focused at the level of the local knee environment as OA progresses.

When evaluating serum inflammatory profiles between groups at these two time points, it appears that a distinct subset of factors are increased and strongly correlated with the Modified Mankin Scores after the obesity induction phase at 12-weeks. Further, three of these factors (leptin, MIP-1α, MIP-2) were increased in DIO animals compared to Chow at both 12 and 28 weeks[3,10]. It is possible that the state of dysregulation of systemic factors, and not the specific dysregulated factors indicated here, may be critical in this process, as we evaluated a comprehensive, but not an exhaustive set of biomarkers. Thus, such changes in inflammatory markers facilitate our understanding of the associated disease process. However, after the post-induction phase and by 28-weeks on the HFS diet, we speculate that the extensive systemic changes observed after obesity induction either become attenuated, or a systemic heterostatic altered set point is achieved. The body may create an altered set point for low-level chronic inflammation as a strategy to cope with the initial change in the inflammatory environment. This altered set point could facilitate regulation of the positive feedback mechanism by which low-level chronic inflammation operates, to protect vulnerable tissues and organs. Due to the dynamic nature of obesity and metabolic disturbance, this potential attenuation or heterostasis could be further clarified by future evaluation of systemic inflammatory marker profiles at

several time points during and after the obesity induction period, as well as during the post-induction adaptation phase.

The similar degree of OA damage between DIO-P and DIO-R animals by 28-weeks may be due to an eventual overriding of the protective mechanisms against metabolic dysregulation from continued HFS diet intake in the DIO-R animals. However, changes in the rate of progression are evident between DIO-P and DIO-R rats after a 12-week obesity induction period and may be, in part, due to differences in mass between these two groups of animals. There is a strong body of evidence that indicates inflammation related to body fat may drive this process, so mechanical outcomes were not considered here[3,6,9,10,12,14]. However, it is likely that mechanical factors may also contribute to the OA disease process, both from the perspective of gross joint loading and local mechanobiological alterations. Therefore, future efforts should consider the interface of mechanical and biological outcomes to better understand the observed difference in rate of progression in DIO-P and DIO-R animals.

The 12-week time-point provides novel insight into potential mediators of the increased rate of progression observed in DIO-P animals. Concordant with increased DIO-P joint damage, we observed increased synovial fluid protein concentrations of IL-5 and IL-6 in DIO-P compared to DIO-R animals at the 12-week time-point. IL-6 is reported to modulate leptin secretion from adipocytes *in vitro*[23], so increased synovial fluid IL-6 in DIO-P may lead to increased leptin expression thereafter, contributing to OA induction[24] and increased Modified Mankin Scores in all DIO animals compared to Chow-fed animals. Leptin, in turn, has been widely implicated in OA onset and progression across both animal and human models of OA[24–26]. However, the complex interaction between these molecules in the context of metabolic OA warrants further

investigation, and these data provide a platform for future mechanistic work evaluating the potential influences of these factors over time.

After the post-induction period and an additional 16-weeks on the diet, there are marked differences in the synovial fluid of DIO animals that were not observed at 12-weeks. It is difficult to determine if these differences are due to obesity-related inflammation or the local OA-like disease process, as degenerative processes can also contribute to biological alterations[27]. Emerging data indicate the importance of the infrapatellar fat pad in both joint homeostasis and OA-related changes within the joint[28–30]. Further, the synovium is critical in these processes[4]. Here, we observed evidence of synovial involvement, as DIO animals demonstrated increased synovial histological alterations compared to control animals. Moreover, we observed trends toward increases in specific SF constituents (leptin and IP-10) among animals with higher synovium histological scores, findings that may indicate an early role of the synovium in metabolic OA development[10]. However, Leptin and IP-10 can also be produced by the infrapatellar fat pad, which was not evaluated here[29]. As the levels for SF leptin and IP-10 at 12-weeks are consistent with 28-week levels in DIO animals, future work should consider evaluating synovial and infrapatellar fat pad involvement in the early progression of metabolic OA. As the cell types resident in the infrapatellar fat pad and the synovium are different, and therefore likely the inflammation that results from these tissues is also different, subsequent contributions from these tissues to the intra-articular environment may also be different. Ongoing work aims to clarify the relative contribution of obesity-related inflammation and/or the local OA disease process by quantifying gene expression in the fat pad and the synovium with HFS diet-induced obesity[13]. Such data will assist in guiding our understanding of the relative contribution of specific inflammatory mediators to the synovial fluid.

Both synovial fluid and serum leptin concentrations were positively associated with Modified Mankin Scores at both time points[3,10]. Leptin levels appear to be consistent as the disease process became more established by 28-weeks on the diet. However, leptin may act synergistically with other pro-inflammatory cytokines to initiate this process, or activate other mediators downstream[24]. Specifically, leptin may induce IL-1β, which has been shown to affect matrix metalloproteinase activity and may induce NO activity, therefore leading to cartilage degradation[31–33]. Furthermore, leptin and IL-1β could act in synergy, as this synergism is reported to induce NOS type II, nitrate, PGE2, IL-6, and IL-8 in chondrocytes[34–36]. However, the precise mechanism by which leptin may lead to cartilage degradation is still unknown, and the relationship between leptin and IL-1β in this model requires both earlier time points, and quantification between 12 and 28-weeks, to address this issue directly. The relationship between leptin and IL-1β may be critical to clarifying this potential cornerstone of metabolic OA.

Joint damage was also evident in a subset of Chow animals at 28-weeks. These data suggest that even in a Chow group with no intervention, a subset of animals approach increased damage at an accelerated rate. Although this was only detected in 3 of 11 animals that were neither heavier nor fatter than the remainder of the 28-week Chow animals, increased meniscal damage suggested induction of OA in this subset of animals via unknown trauma affecting meniscal integrity. As Sprague-Dawley rats are outbred and thus genetically heterogeneous, it is possible that the OA exhibited in these three Chow animals could be due to other factors (e.g. genetic, mechanical factors) not evaluated here. Considering that damage in these animals was found at the level of the menisci, which was not the focus of the damage in the obese animals, this may also speak to other mechanisms operating in this subset of Chow animals. Furthermore, there were no detectable differences in the array data in the serum or synovial fluid of these

animals compared to the remaining control animals. Therefore, future work is required to better understand and describe the factors leading to meniscal damage and ultimately elevated OA scores in this subset of non-obese animals.

There are a number of limitations to this work. Based on our *a priori* power calculation, a minimal number of animals/group was used this in this study. Future studies may include fewer groups with larger N/group based on the present information. However, given the minimal number of animals used, the preliminary odds ratios presented here demonstrate wide 95% CIs. Future analyses using time-course data may enable a multivariable logistic regression in which the influence of inflammatory marker concentrations (i.e. leptin, IP-10) and/or gene transcription data on the relationship between higher Modified Mankin Scores and body fat percentage could be explored.

The influence of obesity on the joint is dynamic as progressive damage occurs, and therefore the role of a metabolic/mechanical influence on progression of joint damage will change over time. Future work should incorporate more time points, and specifically, earlier time points, to clarify tissue responsiveness and identify early critical changes in this OA-like disease process with HFS diet. Likely, a new understanding of the processes that occur early in the obesity induction period may provide mechanistic insight into how these inflammatory factors contribute to the consequences observed during the post-obesity induction phase.

Conclusion

Different rates of OA progression were captured after a 12-week obesity induction period and an additional 16 week post-induction adaptive period. We identified dysregulation of pro-inflammatory markers in serum and synovial fluid that may be involved in accelerated OA progression (IL-6, Leptin, IL-1β, IP-10), and warrant further investigation. Understanding the

source and triggers of these markers within the joint (infrapatellar fat pad, synovium, etc) and the responsiveness of joint tissues to low-level inflammation, may facilitate the development of targeted treatments for metabolic OA. We conclude that inducing metabolic syndrome by exposure to a high fat high sucrose diet leads to dynamic, progressive response patterns of OA-like changes in the rat knee. Future studies will focus on elaborating the details of induction and transition points, as well as engaging collaborators to evaluate response patterns in humans.

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Figure Legends:

Figure 1A: Cross-sectional body mass is increased for DIO-P animals at both time-points compared to resistant and chow animals ($p<0.05$). Resistant and chow animals have similar body mass at both time-points measured. Data are shown as raw values within each group at each time point, and * indicates $p<0.05$ between prone compared to resistant and chow.

Figure 1B: Graded increases in body fat were observed at both time-points. At 12- and 28-weeks, prone animals had increased body fat compared to both resistant ($p=0.026$) and chow animals ($p=0.001$), and resistant animals had more body fat compared to chow ($p=0.005$). Data are presented as raw values within each group at each time-point. * indicates $p<0.05$ for prone compared to resistant and chow, # indicates $p<0.05$ for resistant compared to chow.

Figure 1C: Cross-sectional Modified Mankin Scores mirrored body fat at both time points. At 12- and 28-weeks, prone animals had increased Modified Mankin Scores compared to both resistant (p=) and chow animals (p=), and resistant animals had more body fat compared to chow (p=). At 28-weeks, prone and resistant animals exhibit similar scores, greater than the chow control fed group. Three chow animals with high meniscal sub-scores are shown in black. Data are presented as raw values within each group at each time-point. * indicates $p<0.05$ for prone compared to resistant and chow, # indicates $p<0.05$ for resistant compared to chow.

Table 1: Modified Mankin Scores by region post 12-week obesity induction period indicate different rates of progression between prone and resistant animals. Data are shown as median and (minimum-maximum). # indicates $p<0.005$ for prone compared to resistant, ^ indicates $p<0.05$ for prone compared to chow, and * indicates $p<0.05$ for resistant compared to chow.

Figure 2A: Relationship between body fat and Modified Mankin Scores for 12-week animals. Horizontal (grey) line indicates median of Modified Mankin Scores (16) and cutoff value for fiftieth percentile of Modified Mankin Scores; vertical (black) line indicates median of Body Fat Percentage (33%) and cutoff value for fiftieth percentile based on body fat. These cutoffs indicate an increased odds of 18 (1.79-1590, p=0.003) for an animals with 33% body fat to have a Modified Mankin Score of 16 after the obesity induction period.

Figure 2B: Relationship between body fat and Modified Mankin Scores for 28-week animals. Horizontal (grey) line indicates median of Modified Mankin Scores (40) and cutoff value for fiftieth percentile of Modified Mankin Scores; vertical (black) line indicates median of Body Fat Percentage (40%) and cutoff value for fiftieth percentile based on body fat. After the adaptive period (28-weeks), animals with 40% body fat are have an increased odds of 11 (1.6-76.8, p=0.008) of having a Modified Mankin Scores greater than 40%. Black circle animals had scores of 3-4 in meniscal damage; all other control animals scored 0-1.

Table 2: All serum analytes were similar between obesity prone (DIO-P) and obesity resistant (DIO-R) animals. When all DIO animals were considered together, serum analytes leptin, MIP-2, and MIP-1α were consistently increased in DIO animals compared to chow at both time-points. Moreover, these analytes had a positive significant association with Modified Mankin Scores at 12-weeks. The next five analytes are increased in obese (DIO) serum at 12-weeks, have a positive significant association with Modified Mankin Scores, but demonstrate similar levels in DIO and Chow serum at 28-weeks. The remaining analytes are increased in DIO serum compared to Chow at 12-weeks, but do not display a significant positive association with Modified Mankin Scores. * indicates $p<0.05$ between obese and chow animals at 12-weeks, ‡ indicates increased at 28-week[10].

Table 3: Synovial fluid analytes increased in obesity prone (DIO-P), obesity resistant (DIO-R), and all DIO animals compared to chow control-diet animals. * indicates $p<0.05$; ‡ indicates increased in DIO at 28-weeks[10].

Figure 1:

Table 1

Figure 2A:

Figure 2B:

Table 2: Serum Analytes by Group after 12-week HFS obesity induction ‡ indicates increased at 28-weeks[10]

Table 3: