Using diet-induced obesity to understand a metabolic subtype of osteoarthritis in rats

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S U M M A R Y

Osteoarthritis (OA) in obese individuals is often attributed to joint loading. However, a subtype of OA, Metabolic OA, may be due to obesity-related intrinsic factors but remains to be evaluated experimentally against a known OA progression model.

Objective: To evaluate if obesity contributes to OA onset using a high fat/high sucrose diet-induced obesity (DIO) model with anterior cruciate ligament-transsected rats (ACL-X).

Methods: Sprague Dawley rats (n = 33) consumed high fat/high sucrose or chow diets for 12 weeks, were randomized to one of three groups: a unilateral ACL-X group, sham surgery group, or naïve non-surgical group. These animals were followed for an additional 16 weeks. At sacrifice, body composition, knee joint Modified Mankin scores, and 27 serum and synovial fluid cytokines and adipokines were measured.

Results: Experimental limbs of obese ACL-X, obese Sham, and lean ACL-X animals had similar Modified Mankin scores that were greater than those obtained from lean Sham and naïve animals. Obese contralateral limbs had similar OA damage as ACL-X and Sham limbs of obese and ACL-X limbs of lean animals. Obese contralateral limbs had similar OA damage as ACL-X and Sham limbs of obese and ACL-X limbs of lean animals. Obese contralateral limb Modified Mankin scores had a strong correlation (r = 0.75, P < 0.001) with body fat percentage. Serum leptin and synovial fluid IP10/CXCL10 best described Modified Mankin scores in contralateral limbs of obese animals.

Conclusions: Mechanical factors produced OA damage in experimental limbs, as expected. Interestingly, OA damage in obese contralateral limbs was similar to mechanically perturbed limbs, suggesting that obesity may induce OA in a non-mechanical manner.

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Traditionally, Osteoarthritis (OA) has been thought to be due to the mechanical wear and tear of joints1,2. Obesity has been associated with OA due to increased mass and therefore increased joint load3,4. However, obese individuals were found to develop OA in hands 5–8 times more frequently than non-obese people5. Since loading of joints in the hand is independent of body mass, this result suggests that factors other than loading caused by body mass may cause OA in obese individuals. Recently, a subtype of OA, called metabolic OA, has been identified as a disorder that displays a unique OA trajectory that is potentially independent from the biomechanical contribution to joint load6.

Due to the multi-factorial nature of OA, it is challenging to identify early onset of the disease. One of the animal models developed to induce OA reliably is a Post-Traumatic Arthritis (PTA) model7. The probability of PTA after a ligament injury is greater than 50%8. One of the best validated and widely accepted models of PTA is anterior cruciate ligament transection (ACL-X), which creates instability in knee joints7.

In order to understand metabolic OA, diet-induced obesity (DIO) models must be compared to validated models of OA. There is an evolving body of work aimed at understanding the effects of DIO on OA. In a genetic obesity murine model, obesity was associated with elevated OA progression, loss of muscle function, and increased blood serum levels of specific cytokines10. In another study, DIO was superimposed on an intra-articular fracture in mice. Only fractured knee joints from animals in the obese group had more...
severe OA than the contralateral control limbs. The obesity group also demonstrated increased levels of selected serum cytokines, and decreased adiponectin concentrations\textsuperscript{11}. Additional studies also found that DIO affects the rate of progression of OA, but not onset\textsuperscript{12,13}.

Obese patients often present with knee OA without a history of intra-articular knee injury\textsuperscript{9}. Furthermore, diets used in previous animal studies may not be the best model of a typical human western-type diet, because the high percentage of fat (>50% kcal from fat) could be considered extreme\textsuperscript{14}. Rather, it is thought that the obesity epidemic in North America is driven by processed foods containing high fats and simple carbohydrates\textsuperscript{15}. Additionally, in rodent obesity OA models, synovial fluid inflammatory profiles have not been determined for comparison between local and systemic inflammatory environments.

Therefore, the purpose of this study was to develop a preclinical metabolic model of OA using a high fat/high sucrose (DIO) diet and ACL-X in an attempt to understand local and systemic inflammatory mechanisms that potentially link obesity to joint damage. Experimental and contralateral limbs of diet-induced obese and lean animals were evaluated at 16 weeks post-surgery in the presence of ACL-X in male Sprague Dawley rats. We hypothesized that animals exposed to DIO have a higher incidence of OA onset in contralateral control limbs, and in the ACL-X limbs compared to lean animals.

**Methods**

**Animals**

Thirty-three male, 8–12-week old Sprague Dawley rats were housed individually on a 12 h dark/light cycle. Animals were randomized to either the high fat/high sucrose diet-induced obesity group (DIO; 40% of total energy as fat, 45% of total energy as sucrose, n = 21, custom Diet #102412, Dyets, Inc), or the standard chow low fat diet group (LFD; 12% fat, n = 12, Lab Diet 5001) for a 28-week ad libitum feeding intervention\textsuperscript{11,16,17}. The high fat/high sucrose diet consisted of (g/100 g): casein (20.0), sucrose (49.9), soybean oil (10.0), lard (10.0), Alphacel (5.0), AIN-93M mineral mix (3.5), AIN-93 vitamin mix (1.0), nucleotides (0.3), and choline bitartrate (0.25). The energy density of the high fat/high sucrose diet is 4.6 kcal/g and 3.34 kcal/g for chow. All experiments were approved by the University of Calgary’s Animal Care Committee.

**Surgery**

After a 12-week obesity induction period, the two groups were randomized into an ACL-X with DIO (n = 10), a LFD (n = 4), a sham surgery group with DIO (n = 5), a LFD (n = 3), a DIO naive control group (n = 5), and a LFD naive control group (n = 5). ACL-X and sham surgeries were performed unilaterally in a randomly assigned hind limb. The ACL was cut mid-substance using a surgical hook\textsuperscript{18}. An anterior drawer test was conducted to confirm ACL transection, and ACL transection was confirmed at sacrifice. Sham surgery was conducted by entering the joint via a lateral incision, spraying the joint with saline, and closing the joint capsule. Contralateral limbs served as non-operated controls. Six DIO and Five LFD age-matched animals were analyzed as non-operated controls.

**Body composition**

At 16-weeks post-surgery (36–40 weeks old), animals were sacrificed by barbiturate overdose (Euthanyl\textsuperscript{16}, MTC Animal Health Inc., Cambridge, Ontario, Canada). Immediately after sacrifice, body composition was measured using Dual Energy X-ray Absorptiometry with software for small animal analysis (Hologic QDR 4500; Hologic, Bedford, MA). Body fat percentage was calculated as the value of body fat divided by total body mass.

**Preparation of knee joints**

Joints were harvested by cutting the femur and tibia/fibula 2 cm above and below the joint line. Excess muscle was trimmed away and joints were fixed in a 10% neutral buffered formalin solution (Fisher Scientific Company, Ottawa, Ontario, Canada) for 10 days at room temperature. Joints were then decalcified for 2 weeks at room temperature, using Cal-Ex II solution (10% formic acid in formaldehyde, Fisher Scientific). The solution was changed daily and the end of decalcification was determined by chemical testing with a 5% ammonium oxalate solution (Fisher Scientific) until no precipitate was observed for 5 days. The intact joints were processed in an automatic paraffin processor (Leica TP 1020, Leica Microsystems Inc., Concord, Ontario, Canada). Samples were dehydrated in a graded series of alcohols, cleared in xylene, and infiltrated with Paraplast\textsuperscript{8} Plus wax (Fisher Scientific). Whole knee joints were embedded in paraffin wax and stored at room temperature until sectioning.

Serial, sagittal plane sections of 8 μm thickness were obtained using a Leica RM 2165 microtome. Sections were mounted onto Super Frost plus slides (Fisher Scientific) and allowed to dry at 40°C for 4 days. Sampling was done approximately every 80 μm. Alternate slides were stained sequentially with haematoxylin, fast green and safranin-O stains (Fisher Scientific) using an auto stainer (Leica ST 5010). Sections were then dehydrated in a graded series of alcohols, cleared in xylene, and mounted with cytoseal 60 mounting media (Richard Allan) using an auto cover slipper (Leica CV 5030). Slides were dried at room temperature for several days before being evaluated using a light microscope (Zeiss Axiosstar plus, Carl Zeiss Inc., Toronto, Ontario, Canada). Images were digitized using a Zeiss AxioCam\textsuperscript{8} Icc 5 camera and analyzed using the Zen 2011 Zeiss imaging system. Sections were examined under 10× and 25× objectives and scored for OA degeneration using a Modified Mankin scoring system\textsuperscript{19}.

**Osteoarthritis scoring**

Joints were scored by two independent, blinded observers. A Modified Mankin Score was developed, where five areas were scored on the standard 14-point Mankin scale\textsuperscript{20}: the medial and lateral tibial plateau, the medial and lateral femoral condyle, and the patella. Subchondral bone and synovium were then assessed using a five and four point criteria, adapted from the rat-specific OARSI metric\textsuperscript{21}. The final Modified Mankin score was determined by adding the five site-specific Mankin scores to the two corresponding OARSI scores\textsuperscript{20,21}.

**Cytokine, growth factor, and adipokine measurements**

Animals were fasted for 12 h prior to sacrifice and blood collected immediately following sacrifice via cardiac puncture\textsuperscript{22}. Synovial fluid was collected shortly after sacrifice using the Whatman chromatography paper method\textsuperscript{23}. Samples were diluted 1:60, spun at 13,500 revolutions per minute, and stored at −20°C overnight. Samples were aliquoted 24 h later and stored at −80°C until analysis\textsuperscript{24}.

Twenty-seven serum and synovial fluid cytokines and adipokines were quantified using a Rat 27 Multiplex Discovery Assay with Luminox\textsuperscript{8}xMAP technology (Eotaxin, EGF, Fractalkine, IL-1β, 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, vascular endothelial growth factor (VEGF), Eve Technologies, Calgary, AB). Urea was evaluated in duplicate in serum and synovial fluid using an ELISA (Eve Technologies, Calgary, AB)\textsuperscript{14,25} to adjust for volumetric differences in synovial fluid concentration. Blood glucose was assessed using a Trinder assay (Sigma, Oakville, ON, Canada).

**Longitudinal kinetics**

Twelve weeks post obesity induction, 1 week post-surgery, 8 weeks post-surgery, and 16-weeks post-surgery, ground reaction forces were measured during locomotion using a 1-m custom runway with two embedded side-by-side \(7.5 \times 30\) cm, 3-dimensional force plates (Bertec, Columbus, OH, 500 Hz). A high-speed camera (200 Hz) confirmed speed and ground contact. Animals were acclimatized to the runway prior to measurements. Successful trials included a uniform speed and a single hind limb contact on each plate. A minimum of three successful trials was needed for inclusion into the analysis.

**Statistical analysis**

Wilcoxon rank sum tests were used to evaluate paired between-limb outcomes within each group. Kruskal–Wallis tests were used to evaluate between group outcomes relative to naïve age-matched controls. Friedman’s test was used to evaluate longitudinal repeated measures kinetic data. Linear regression was used to evaluate each analyte with body fat to predict Modified Mankin Scores. Spearman correlations were used to associate Modified Mankin scores with cytokines by diet (IBM SPSS Statistics 20, \(\alpha = 0.05\)).

**Results**

**Body composition and joint damage**

DIO diet group animals had a higher percent body fat, absolute amount of body fat, and body mass than animals in the LFD group \((P < 0.001, \text{Fig. 1})\). DIO animals had a \(68.3 \pm 7.0\%\) increase in body mass, whereas LFD animals had a \(31.4 \pm 3.0\%\) in body mass over the study period.

There were no differences between limbs in either the number of lesions or the Modified Mankin Scores between DIO ACL-X and DIO Sham groups (Fig. 2). Modified Mankin scores were similar in DIO ACL-X, DIO Sham, DIO naïve, and LFD ACL-X group animals, but their values were higher than those measured in LFD Sham and LFD naïve control limbs \((P < 0.01)\). The number of cartilage lesions was similar in the experimental limbs of DIO ACL-X, DIO Sham, and LFD ACL-X group animals. Modified Mankin scores and the number of lesions were higher in the contralateral knees of DIO ACL-X than in LFD contralateral limbs, independent of surgery \((P < 0.01, \text{Fig. 3})\).

**Synovial fluid, cytokines, and adipokines**

No detectable statistical differences were found between ACL-X and Sham operated experimental or contralateral limbs in either dietary group for any of the 27 outcome measures. Therefore, synovial fluid analyte data were pooled into the following groups: DIO experimental, DIO contralateral, LFD experimental, and LFD contralateral. Four analytes were increased in DIO compared to LFD animals (Table I), but there were no differences in analytes by surgery. Synovial fluid leptin, IL-1α and IP10/CXCL10 were higher in DIO compared to LFD animals \((P < 0.05)\), while VEGF trended to be higher in DIO animals, but was not significant \((P > 0.05)\). Values measured for the remaining analytes are provided in Supplementary Table 1.

**Serum cytokines and adipokines**

DIO animals had higher blood glucose (7.5, 6.5–8.5 mmol/L) than LFD animals (6.0, 7.0–5.1 mmol/L, \(P = 0.046\)). Three synovial fluid analytes were higher in DIO animals compared to LFD animals, and two of those were also higher in the serum of the DIO animals: leptin \((P = 0.001)\) and IP10/CXCL10 \((P = 0.008, \text{Table I})\). Serum VEGF was also higher in DIO animals compared to LFD animals \((P = 0.047)\).

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**Fig. 1.** Relative body fat (a, %), body mass (b, g), absolute body fat (b, g), and demonstrated by group: Obese anterior cruciate ligament transected (DIO ACL-X, \(n = 10\)), Obese Sham (DIO Sham, \(n = 5\)), Obese Naïve non-operated control (DIO Naïve, \(n = 6\)), Lean chow ACL-X (LFD ACL-X, \(n = 4\)), Lean chow sham (LFD Sham, \(n = 3\)), Lean chow non-operated naïve (\(n = 5\)). Regardless of surgery (ACL-X or sham), DIO animals had more body fat and body mass when compared to lean (LFD) animals at sacrifice (16–weeks post-surgery, 28–weeks post obesity intervention, \(P < 0.001\)). Data are shown as means and 95% confidence intervals (CI) of the mean, and * indicates significant differences between DIO and LFD for body fat outcomes, and # indicates significant differences between DIO and LFD body mass \((P < 0.05)\).
Associations with joint damage, body fat, cytokines, and adipokines

Modified Mankin scores and body fat percentage were positively related in the experimental \((r = 0.60, P = 0.004)\) and contralateral limbs \((r = 0.75, P < 0.001)\) of all DIO animals. Body mass was not significantly related to Modified Mankin scores in the contralateral \((r = 0.40, P = 0.07)\) or the experimental limbs \((P = 0.215)\). Body mass and percent body fat were not related in either limb of LFD animals.

Increases in contralateral limb IP10/CXCL10 were associated with increases in percent body fat \((r = 0.39, P = 0.043)\), and Modified Mankin scores \((r = 0.65, P < 0.001)\) in DIO animals. Also, synovial fluid IP10/CXCL10 was positively related with synovial fluid leptin \((r = 0.95, P < 0.001)\), synovial fluid IL-1\(\alpha\) \((r = 0.85, P < 0.001)\), and synovial fluid VEGF \((r = 0.96, P < 0.001)\).

Serum leptin and serum IP10/CXCL10 were positively correlated with percent body fat \((r = 0.47, P = 0.005\) and \(r = 0.28, P = 0.044\), respectively). Serum leptin was also positively associated with Modified Mankin scores in the contralateral limbs of DIO animals \((r = 0.40, P = 0.001)\), synovial fluid leptin from both limbs \((r = 0.47, P = 0.013)\), and synovial fluid IP10/CXCL10 \((r = 0.51, P = 0.004)\), IL-1\(\alpha\) \((r = 0.48, P = 0.006)\), and VEGF \((r = 0.40, P = 0.015)\) from both limbs. There was no relationship between any of the analytes and the percent body fat or Modified Mankin scores in LFD group animals.

![Fig. 2.](image)

(A) Modified Mankin composite score was measured by adding the 14-point Mankin score from 5-sites (medial tibial plateau, medial femoral condyle, lateral tibial plateau, lateral femoral condyle, and patella) and the 4- and 5- point Rat OARSI score for synovium and bone, respectively. Data are demonstrated by group: Obese anterior cruciate ligament transected (DIO ACL-X, \(n = 10\)), Obese Sham (DIO Sham, \(n = 5\)), Obese Naïve non-operated control (DIO Naïve, \(n = 6\)), Lean chow ACL-X (LFD ACL-X, \(n = 4\)), Lean chow sham (LFD Sham, \(n = 3\)), Lean chow non-operated naïve (\(n = 5\)). Experimental operative limbs are shown in black and contralateral non-operative limbs are shown in grey. Data are shown as means and 95% CI. P-values were calculated between each group and the lean naïve control group, and significant differences are indicated by \(\dagger\) \((P < 0.05)\). When compared to LFD naïve non-operative age and sex matched control limbs, all DIO experimental limbs, DIO ACL-X and DIO SHAM, had similar damage to LFD Anterior Cruciate Ligament Transected (ACL-X) experimental limbs, but greater damage than the LFD SHAM experimental limbs and LFD naïve control limbs \((P < 0.01)\). Although non-surgical, DIO ACL-X and SHAM contralateral limbs had similar damage to DIO ACL-X, DIO SHAM, and LFD ACL-X experimental limbs, which was greater than LFD ACL-X contralateral limbs, both LFD SHAM limbs, and LFD naïve control limbs \((P < 0.01)\). (B) Number of lesions by limb by group. P-values were calculated between each group and the lean naïve control group, and significant differences are indicated by \(\dagger\) \((P < 0.05)\). Experimental operative limbs are shown in black and contralateral non-operative limbs are shown in grey. The number of cartilage lesions was similar in the experimental limbs of DIO ACL-X, DIO Sham, and LFD ACL-X group animals. The number of lesions were higher in the contralateral knees of DIO ACL-X and DIO Sham group animals than in LFD ACL-X and LFD Sham group animals \((P < 0.01)\). Data are shown as means and 95% CIs of the mean.

![Fig. 3.](image)

Fig. 3. Sagittal plane histological slides cut at 8 \(\mu\)m. Representative contralateral non-operative limbs from LFD ACL-X group (top, Modified Mankin score of 11) and DIO ACL-X group (bottom, Modified Mankin score of 29).
Table I

Summary of cytokine, adipokine, and growth factor protein levels that were increased in either synovial fluid and serum of obese animals. Data are presented for all surgical groups pooled by diet: Obese (DIO, n = 15) and Lean (LFD, n = 7). Synovial fluid biomarkers differed by diet (P < 0.05), but not by surgery or between contralateral and experimental limbs. Therefore, synovial fluid protein levels are presented as obese (DIO) and lean chow (LFD) groups by experimental and contralateral non-surgical limb. P-values were calculated between DIO and LFD experimental limbs, DIO and LFD non-surgical contralateral limbs, and DIO and LFD serum protein levels. Data are presented as means and 95% CIs of the mean, significant P-values between DIO and LFD animals are bolded. Synovial fluid leptin, IL-1α and IP10/CXCL10 were higher in DIO high fat high sucrose animals compared to LFD group animals (P < 0.05). Synovial fluid VEGF trended to be higher in DIO animals, but was not significant (P > 0.05). Of the three synovial fluid analytes that were higher in DIO animals compared to LFD animals, two were also higher in the serum of the DIO animals: leptin (P < 0.001) and IP10/CXCL10 (P < 0.005). Additionally, serum VEGF was higher in DIO animals compared to LFD animals (P = 0.047). Data are shown as means and 95% CIs of the mean.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>DIO Exp (ng/mL) Mean (CI)</th>
<th>n = 15</th>
<th>LFD Exp (ng/mL) Mean (CI)</th>
<th>n = 7</th>
<th>P-value</th>
<th>DIO Con (ng/mL) Mean (CI)</th>
<th>n = 15</th>
<th>LFD Con (ng/mL) Mean (CI)</th>
<th>n = 7</th>
<th>P-value</th>
<th>DIO Serum (ng/mL) Mean (CI)</th>
<th>n = 15</th>
<th>LFD Serum (ng/mL) Mean (CI)</th>
<th>n = 7</th>
<th>P-value</th>
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<tbody>
<tr>
<td>IL-1α</td>
<td>76.4 (18.1–134.7)</td>
<td>11.8 (4.5–18.9)</td>
<td>0.022</td>
<td>25.6 (12.2–38.9)</td>
<td>8.8 (4.5–13.1)</td>
<td>0.001</td>
<td>0.2 (0.1–0.2)</td>
<td>0.02 (0.0–0.3)</td>
<td>0.0 (0.1–0.2)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>IP-10</td>
<td>25.1 (0.3–49.7)</td>
<td>4.9 (0.0–9.8)</td>
<td>0.044</td>
<td>6.7 (3.0–10.4)</td>
<td>116.1 (15.4–220.7)</td>
<td>&lt;0.001</td>
<td>37.1 (27.8–45.5)</td>
<td>13.7 (7.4–20.0)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leptin</td>
<td>3250.4 (556.8–7057.6)</td>
<td>200.0 (72.0–328.0)</td>
<td>0.002</td>
<td>1290.1 (331.9–2248.4)</td>
<td>141.0 (79.0–202.2)</td>
<td>&lt;0.001</td>
<td>18.5 (14.8–24.2)</td>
<td>2.4 (1.9–3.0)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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<tr>
<td>VEGF</td>
<td>58.2 (0–136.7)</td>
<td>18.2 (0–51.2)</td>
<td>0.18</td>
<td>22.2 (0.4–46.1)</td>
<td>4.5 (2.4–6.6)</td>
<td>0.06</td>
<td>0.08 (0.07–0.09)</td>
<td>0.06 (0.04–0.09)</td>
<td>0.047</td>
<td></td>
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</table>

Linear regression modeling

Linear regression modeling was used to evaluate the combined predictive value of significantly associated outcomes with Modified Mankin Scores. Contralateral limb Synovial fluid IP10/CXCL10 and percent body fat were significant predictors of joint damage in the contralateral limbs of DIO and LFD animals (r² = 0.70, P < 0.001, Table II). When body fat percentage and leptin are evaluated in contralateral limbs of all animals, a smaller degree of variation in the Modified Mankin scores is explained (r² = 0.54, P < 0.001). Contralateral limb IL-1α and VEGF were not associated with percent body fat or Modified Mankin scores. Serum leptin and synovial fluid IP10 (r² = 0.75, P < 0.001) explained more of the variance in Modified Mankin scores in contralateral limbs than serum IP10/CXCL10 and synovial fluid IP10/CXCL10 (r² = 0.63, P < 0.001), serum leptin and synovial fluid leptin (r² = 0.55, P < 0.001), or serum leptin alone (r² = 0.37, P = 0.001).

Longitudinal kinetic analysis

Only 3 DIO ACL-X, 3 LFD ACL-X, 3 DIO SHAM, and 3 LFD Sham animals met all inclusion criteria. LFD ACL-X animals unloaded the experimental limb most, 1 week post-surgery, whereas LFD Sham animals did not demonstrate decreased experimental limb loading [Fig. 4(a)]. DIO experimental limbs, regardless of surgery, were unloaded most at week 8 [Fig. 4(b)] and continued to be unloaded at 16 weeks compared with DIO contralateral limbs. All contralateral limb normalized and absolute peak vertical ground reaction forces measured, regardless of diet or surgery, were statistically similar across all time-points (P > 0.05).

Discussion

Obesity is strongly associated with OA. A subtype of OA, metabolic OA, has been suggested to occur independently of the mechanical contribution of obesity to joint load6. We evaluated this potential subtype in a rat model by superimposing DIO on ACL-X surgery.

DIO has been observed to increase the severity of OA in intra-articular fractured limbs of mice over a 20 and 24 week period11,13. However, in a rabbit model of varus bowing, there was no difference in the experimental limbs of DIO compared with LFD group animals over 38 weeks12. Our results show a similar degree of knee joint damage in DIO ACL-X and LFD ACL-X animals suggesting that DIO does not affect animals over the period studied here.

Among all mechanical models of OA, ACL-X is considered the most severe26. Therefore, it is possible that mechanical drivers from ACL-X may over-ride the OA processes originating from metabolic factors. Some of the experimental limbs reached end-stage OA (Mankin score ≥7/14) in one or more of the evaluated regions; therefore differences in OA may have occurred prior to sacrifice19. Future work should evaluate shorter time periods to determine the combined effects of DIO and ACL-X on the rate of OA progression.

The similarity in knee joint damage in DIO Sham and DIO ACL-X was not expected, since previous studies found that ACL-X consistently produced greater damage than sham interventions11,12,21. DIO animals may exhibit an immune response that is activated upon surgery given the low-level of systemic inflammation11. Generally, sham surgery serves as a surgical control and is not associated with OA damage, which is consistent with our findings in LFD sham animals25. Therefore, there appears to be an obesity-driven response causing increased damage in DIO Sham compared to LFD Sham knees.

We hypothesized that obesity affects OA onset. All DIO non-surgical contralateral limbs had the same Modified Mankin scores as the DIO surgical limbs, regardless of ACL-X or sham surgery. For DIO animals, body fat explained the majority of the variance in Modified Mankin scores from both limbs, whereas body mass and Modified Mankin Scores did not have a significant statistical association in either limb. These data suggest that OA damage is not simply caused by over-loading of joints from increased body mass.

Table II

Summary of outputs from linear regression modeling estimating Modified Mankin Scores across all surgical animals: obese (DIO, n = 15) and lean (n = 7). Where synovial fluid analytes are included, non-surgical contralateral limb values were used. B-coefficients and Predicted Modified Mankin Scores for each model are presented as mean and 95% CI of the mean. R-squared (r²), F-statistics, standard error of the estimate, and P-value of the model are also provided.

<table>
<thead>
<tr>
<th>Factors included</th>
<th>r²</th>
<th>β</th>
<th>F-statistic</th>
<th>Standard error of estimate</th>
<th>Predicted Modi</th>
<th>Mean</th>
<th>CI lower limit</th>
<th>CI upper limit</th>
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<tr>
<td>Serum systemic marker</td>
<td>0.75</td>
<td>4.66</td>
<td>32.3</td>
<td>5.35</td>
<td>&lt;0.001</td>
<td>19.6</td>
<td>15.5</td>
<td>24.3</td>
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<tr>
<td>Synovial fluid marker</td>
<td>0.73</td>
<td>2.14</td>
<td>18.5</td>
<td>6.53</td>
<td>&lt;0.001</td>
<td>19.6</td>
<td>15.5</td>
<td>24.3</td>
</tr>
<tr>
<td>Body fat</td>
<td>0.53</td>
<td>6.67</td>
<td>13.1</td>
<td>6.32</td>
<td>&lt;0.001</td>
<td>18.5</td>
<td>14.7</td>
<td>22.5</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.55</td>
<td>7.82</td>
<td>13.5</td>
<td>8.44</td>
<td>&lt;0.001</td>
<td>19.6</td>
<td>15.5</td>
<td>24.3</td>
</tr>
</tbody>
</table>
The interplay of mechanical and biological effects of obesity on animal joints has not been widely explored and remain to be clarified\(^\text{38}\).

The intervention diet used in this study incorporated higher fat energy and higher simple carbohydrate energy compared to the standard diet. Previous animal work evaluating obesity and OA utilized either monogenic models of obesity or high fat diets\(^\text{10,11}\). However, the liver has been shown to be differentially affected following a high fat or high sucrose diets\(^\text{39}\), and specific macro-nutrients may affect leptin resistance differently independent of body fat\(^\text{40}\). We suggest that although the resultant obesity may be similar, the intrinsic changes due to high fat/high sucrose diet may affect OA differently compared with an exclusively high fat diet.

Specifically, advanced glycation end-products, harmful compounds thought to contribute to degenerative disease, are associated with increased blood glucose, and have been implicated in the pathogenesis of oxidative-based diseases such as type 2 diabetes mellitus\(^\text{32}\). Through hyperglycemic pathways, oxidative stress and advanced glycation end-products have also been suggested to contribute to cartilage degradation\(^\text{41}\). Here, DIO animals had higher blood glucose concentrations compared to LFD animals. By inducing obesity through a high fat/high sucrose diet, we speculate that this study may have replicated a more clinically relevant metabolic dysregulation\(^\text{42}\). However, to our knowledge, the relative contribution of high fat and high sucrose to advanced glycation end products remains unknown.

Neither body fat percentage nor body mass correlated with the Modified Mankin scores in LFD animals regardless of surgery, possibly because of the homogeneity of body fat percentage and body mass in this group. However, despite being smaller and leaner, LFD animals sustained similar damage due to ACL-X surgery as DIO ACL-X, DIO Sham, and DIO contralateral limbs. OA in the experimental limb of LFD ACL-X animals may be largely caused by changes in joint loading conditions, whereas DIO Sham and contralateral limb damage may be driven primarily by metabolic factors.

To clarify the role of intrinsic factors related to obesity, 27 serum and synovial fluid inflammatory markers, cytokines, and hormones were measured. Despite similar Modified Mankin scores, the local (synovial fluid) and systemic (serum) inflammatory cytokine concentrations differed between DIO and LFD animals. Furthermore, increased concentrations of 2 of the 27 serum markers (serum leptin and serum IP10/CXCL10) were significantly correlated with increased Modified Mankin scores in DIO animals. These markers have previously been implicated in OA\(^\text{34,35}\). Three synovial fluid mediators were elevated in DIO compared to LFD animals: leptin, IL-1α, IP10/CXCL10.

Systemic leptin is generally proportional to body fat\(^\text{36}\), and may affect chondrocytes through NoSynthase-2, IGF-1, TGF-beta, IL-8 (GRO-KC), or synovial fibroblasts through TIMPQ-1\(^\text{37,38}\). Systemic leptin could be the initiator of the downward cascade of joint degradation\(^\text{42}\). In a DIO murine model, 90% of the variance in proteoglycan staining loss was explained by body fat percentage, supporting a case for body composition and adipose-derived catabolic cytokines in OA disease pathology\(^\text{33}\). In the present study, there was a strong relationship between DIO contralateral limb Modified Mankin score and body fat. It is possible that the reduced variance explained in Modified Mankin Score by body fat in our study is due to the confounding effect of ACL-X. Others have shown that leptin deficient mice gained weight but did not develop OA\(^\text{39,40}\), which suggests that mass increase in the absence of leptin signaling is not sufficient to cause OA. In humans, the ratio of plasma leptin and synovial fluid leptin may be a marker of OA severity\(^\text{41}\). In this study, synovial fluid leptin was higher in all DIO compared to LFD animals, regardless of limb and surgery.

Likely, an inflammatory mediator downstream of leptin is involved in Metabolic OA\(^\text{32}\), IP10/CXCL10, a chemo-attractant for monocytes and macrophages selectively activated by leptin, is associated with angiogenesis, and increases in the numbers of monocytes and macrophages in fat and muscle\(^\text{42}\). Obese OA patients have lower lean mass, decreased muscle strength, and a relatively higher fat mass with low-level systemic inflammation, so IP10/CXCL10 may provide insight into the link between muscle, fat, and cartilage damage\(^\text{34}\). Together, serum leptin and synovial fluid IP10/CXCL10 may explain more variance in OA damage than serum and synovial fluid leptin.

Also, IP10/CXCL10 stimulates subchondral bone progenitors in vitro, which may be one mechanism of joint damage in Met-OA\(^\text{42}\).
However, synovial fluid IP10/CXCL10 concentrations in OA patients have been reported to be lower than synovial fluid IP10/CXCL10 concentrations in rheumatoid arthritis patients. The synovial fluid in that study was not obtained exclusively from obese patients, so increases in IP10/CXCL10 may have been missed. In rheumatoid arthritis, IP10/CXCL10 is expressed by infiltrating macrophages and the synovium, leading to osteoclastogenesis. It is possible that IP10/CXCL10 has been missed in the past due to a lack of OA subtyping masking increases in IP10/CXCL10. However, the strong associations here warrant future investigation into the role of IP10/CXCL10 and metabolic OA.

VEGF has also been shown to induce IP10/CXCL10. Moreover, serum VEGF, a marker for angiogenesis and critical for adipose signaling, has been associated with inflammatory arthritis. Here, we observed increased serum VEGF in DIO animals compared to LFD. Plasma and synovial fluid VEGF has been shown to correlate positively with radiographic knee OA severity, but a non-significant trend toward increased synovial fluid VEGF in DIO animals was observed here.

In agreement with previous work, there was no difference between LFD and DIO in serum IL-1α, IL-1β, IL-4, IL-10, TNF-α, or IL-6 in this study. However, we observed increases in synovial fluid IL-1α and IL-6 in DIO animals compared to LFD animals, which is thought to contribute to matrix degradation and cartilage catabolism. Increases in synovial fluid IL-1α have been shown to be associated with mild and moderate OA. We did not find a significant association between OA damage and IL-1α.

Although the mechanical data presented here are limited, we believe these data suggest a non-mechanical driver for metabolic OA. Previously, we and others demonstrated a side to side loading disparity after ACL-X, that is restored over time in cats. Here, LFD animals follow that paradigm. Load was reduced in all DIO experimental limbs at 8 and 16 weeks compared to DIO contralateral limbs, regardless of ACL-X or sham surgery. This finding is of interest because the similar average Mankin scores were observed in both experimental and contralateral DIO limbs at 28 weeks. Therefore, we suggest that these preliminary kinetic data support the notion that different drivers (biological in contralateral, mechanical in experimental) may affect OA, as these limbs with similar damage are loaded differently. However, as joints were not scored for OA at each time point, we cannot conclusively say that the joint damage was similar throughout the duration of the study. Differences in the time-course of experimental limb unloading may affect OA progression. These inclusion criteria may delineate a subset of animals that demonstrate this pattern. This phenomenon warrants further investigation.

This cross-sectional study does not evaluate OA progression, or structural damage due to DIO and surgery over time, which is critical to our understanding of the mechanism of metabolic OA. Future work elucidating mechanisms of Met-OA should be conducted over several time points, and involve serial time-course measurements, to characterize the effects of high fat/high sucrose DIO on OA progression.

Conclusion

From the results of this study, we conclude that obesity from a high fat/high sucrose diet is an independent risk factor for the onset of OA in the rat ACL-X knee. These phenomena may be clarified in future work by investigating the role of advanced glycation end-products from the enhanced dyslipidemia and hyperglycemia from the diet used here. Body fat percentage may be crucial to this metabolic subtype of OA, stimulating inflammatory changes due to DIO that may differentiate a metabolic subtype from an LFD post-traumatic OA model. The suggested subtype of Met-OA, and how it relates to OA onset, may be studied by using a high fat/high sucrose DIO model with post-traumatic OA.

Author contributions

Acquisition of Data: Collins, Seerattan, Leonard, Herzog.
Analysis and Interpretation of Data: Collins, Reimer, Seerattan, Leonard, Herzog.
Statistical Analysis: Collins.

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Competing interests

No conflicts of interest to report.

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Supplementary data

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