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

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SUMMARY

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-transected 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. Over 70% of variation in obese contralateral limb Modified Mankin scores was explained by the variation in 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.

Traditionally, Osteoarthritis (OA) has been thought to be due to the mechanical wear and tear of joints[1,2]. Obesity has been associated with OA due to increased mass and therefore increased joint load[3,4]. However, obese individuals were found to develop OA in hands 5–8 times more frequently than non-obese people[5]. 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 load[6].

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) model[7]. 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 joints[9].

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 cytokines[10]. 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

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severe OA than the contralateral control limbs. The obesity group also demonstrated increased levels of selected serum cytokines, and decreased adiponectin concentrations[11]. Additional studies also found that DIO affects the rate of progression of OA, but not onset[12,13].

Obese patients often present with knee OA without a history of intra-articular knee injury[6]. 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[14]. Rather, it is thought that the obesity epidemic in North America is driven by processed foods consisting of high fats and simple carbohydrates[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 pre-clinical 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 DIOhave 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[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), DL-methionine (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 naïve control group ($n = 6$), and a LFD naïve 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[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®, 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® 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 Axiostar plus, Carl Zeiss Inc., Toronto, Ontario, Canada). Images were digitized using a Zeiss Axiocam® 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[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[19]: 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[20]. The final Modified Mankin score was determined by adding the five site-specific Mankin scores to the two corresponding OARSI scores[19–21].

Cytokine, growth factor, and adipokine measurements

Animals were fasted for 12 h prior to sacrifice and blood collected immediately following sacrifice via cardiac puncture[22]. Synovial fluid was collected shortly after sacrifice using the Whatman chromatography paper method[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[23].

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-1 β, 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,

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