Obesity with ACL transection leads to severe collagen matrix disorganization of knee cartilage in a rat model

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INTRODUCTION: Risks to osteoarthritis (OA) are multi-factorial, which include traumatic joint injuries, chronic joint loading abnormalities, joint inflammation, and metabolic disorders. Animal models have been used to investigate the effects of individual risk factors under *in vivo* conditions. For example, the post-traumatic/mechanical OA phenotype has been studied using anterior cruciate ligament transection (ACLx), and the metabolic OA phenotype has been studied using diet-induced obesity[1]. Through animal models of OA, we learned that abnormal joint loading caused by injury leads to proteoglycan (PG) degradation and collagen fibrillation within 4 weeks ACLx in rabbits[2,3]. Similarly, obesity has been found to cause collagen disorganization at 16 weeks following obesity induction in a murine model[4]. Typically, OA phenotypes are studied independent of each other and often using different animal models. It is unclear how different risk factors, such as trauma and obesity, interact with each other in animals exposed to multiple risk factors simultaneously[1]. The objective of this study was to investigate the separate and combined effects of two main OA risk factors, obesity and joint trauma, on the organization of the collagenous network in rat knee cartilage. We hypothesized that obesity and ACLx independently would lead to collagen disorganization in cartilage, with the degree of the disorganization exacerbated when animals were exposed to obesity and ACLx simultaneously.

METHODS: New analyses were conducted on medial and lateral tibial plateaus (MTP, LTP) of the rat knee joints of Collins et al.[5]. 8 – 12 week-old rats (N = 20) were randomized into a lean chow-diet (LF, Lab Diet 5001) or high-fat/high sucrose diet (HF, custom Diet #102412 Dyets, Inc.). 12 weeks into the diet intervention, rats underwent ACLx (N=10) or sham (N=5) surgery. Rats were fed the same diet following surgery and were sacrificed 16 weeks post-surgery at the age of 36 – 40 weeks. The animal groups were: **1)** lean control (**Ctrl**, N = 5), **2)** lean sham (**LF|s**, N = 3) **3)** lean ACLx (**LF|x**, N = 4), **4)** obese sham (**HF|s**, N = 2), and **5)** obese ACLx rats (**HF|x**, N = 6). Knee joints were harvested and processed for histology, before being sliced sagittally into 10 μm sections per animal/joint site. The unstained sections were imaged with polarised light microscopy (PLM) at a wavelength of 630 nm (pixel size: 0.5 μm)[6,7]. From load bearing area of the joint, four to nine 120 μm^2 region of interest (ROI) were imaged per animal group, from which the orientation angle and parallelism index (PI) of the collagen network were measured[6]. Depth-dependent data of collagen orientation and PIs were obtained by averaging across the width of each ROI (i.e., along articular surface, Fig. 1). Orientation angles ranged from 0° – 90°, with 0° being parallel to the articular surface. PI went from 0 to 1, with 0 denoting an isotropic material and 1 denoting a completely anisotropic material. Thus, an increase in orientation and/or a decrease in PI indicate collagen disorganization. A linear mixed model was used for statistical analysis (*$p < 0.05$*).

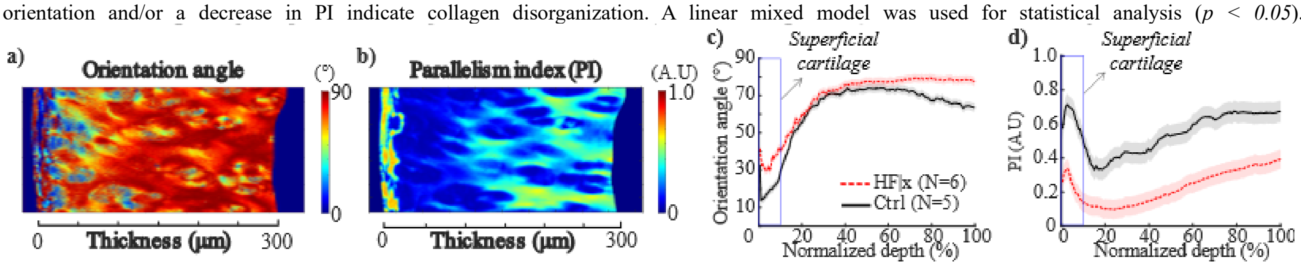

Figure 1: PLM images showing a) collagen orientation angle, and b) parallelism index (PI), with surface of the cartilage on the left and tidemark on the right. Depth-dependent median profiles with 95% CI for c) orientation angle and d) PI were used for the analyses.

RESULTS: Only results from the superficial zone cartilage (top 10% tissue) are presented here. Compared to control animals (Ctrl), lean sham animals had 93% and 317% greater orientation angles in MTP and LTP (Fig 2. c-d), respectively, whereas obese sham rats had 89% greater orientation angles in LTP. Following ACLx, LF|x animals had 13-331% greater orientation angles than Ctrl animals in the MTP and LTP. When ACLx animals were also obese, HF|x rats had 20-41% lower collagen orientation angles than LF|x in LTP (Fig. 2d). For the PIs, LF|s rats were 41% and 25% lower in PI than Ctrl rats for MTP and LTP (Fig. 2 a-b), respectively. HF|s animals had 25% lower PIs than Ctrl rats only in MTP. When obesity and ACLx were present simultaneously, HF|x rats had 26-61% lower PIs than LF|x for both joint compartments (Fig 2 a-b).

DISCUSSION: We show that collagen disorganization was exacerbated in animals with both obesity and joint trauma, thereby supporting our hypothesis. We also show that sham surgery alone induced cartilage degradation to the similar magnitude as observed for ACLx animals. However, the effect of sham surgery on collagen structure was less severe in obese than in lean animals. If the ACLx animals were obese, the severity of the collagenous degradation was increased (HF|x vs LF|x). This suggests that the degrading effects to collagenous structure resulting from the joint trauma and obesity were additive, though the effects varied for different joint locations. Gait adaptation, under- or over-loading of tissue, increased inflammatory response that occurred for animals of different OA subtype may explain the current findings [5,8,9]. In addition, our preliminary data (not shown here) also show a seemingly beneficial effect of increased proteoglycan content in animals with obesity and ACLx[10]. Therefore, our results highlight the multivariate progression and effects of OA on the structure of cartilage *in vivo.*

SIGNIFICANGE/CLINICAL RELEVANCE: We show that collagen disorganization can be caused independently by ACLx and obesity. The degrading effect to collagenous network was additive when the two OA phenotypes were present simultaneously. Importantly, sham surgeries could cause similar degradation seen in ACLx injury, thus highlighting the importance of using non-invasive injury models[11].

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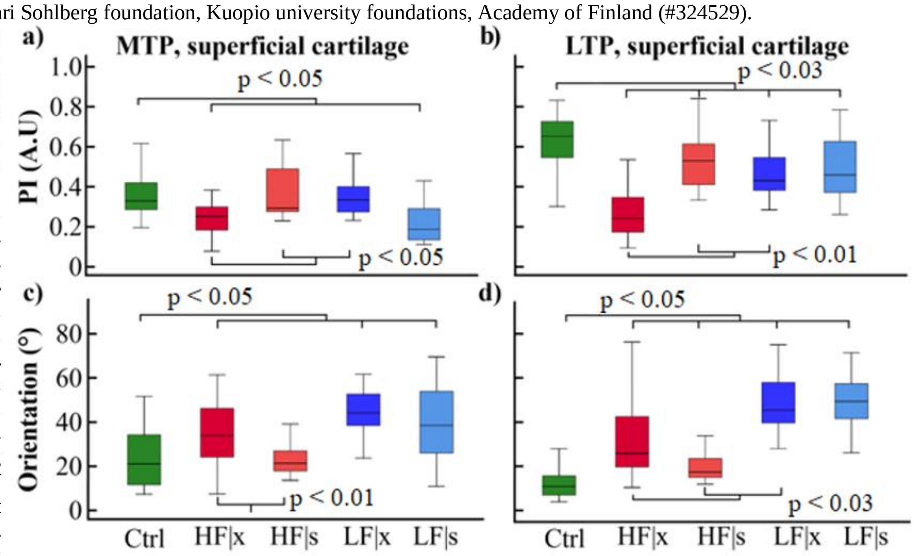

Figure 2: Boxplots of median PIs (a-b) and orientation angles (c-d) of MTP and LTP showing that the HF|x animals had significantly smaller PI while LF|x animals have increased orientation angles. For clarity, only the largest significant differences between the animal groups are shown.

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