Obesity and anterior cruciate ligament transection minimally alter knee cartilage collagen content

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INTRODUCTION: Risk factors, such as traumatic injuries, loading abnormalities, inflammation, and metabolic disorders, contribute to the development of osteoarthritis (OA). Animal OA models have provided insights into the effects of various risk factors on knee joint health. For example, through a rabbit anterior cruciate ligament transection (ACLx) model, we learned that joint instability leads to proteoglycan (PG) degradation and collagen fibrillation in cartilage as early as four weeks post-surgery. 1, 2 Through a murine model, obesity is found to cause collagen disorganization within 16 weeks. 3 Our group previously used a rat model and found that obesity and joint injury cause significant structural disruption to the collagen network in cartilage. 4 However, little is known if the collagen content in joint knee cartilage is similarly affected. The objective of this study was to investigate the independent and combined effects of obesity and joint trauma on the depth-wise collagen content in cartilage. We hypothesized that obesity and ACLx independently lead to a loss of collagen cartilage content, which is exacerbated when both risk factors are simultaneously present.

METHODS: The histological slides of rat knee joints of Collins et al. 6, 10, 11 were used for new measurements. 8 – 12 week-old rats (N = 20) were randomized into a lean chow-diet (LF, Lab Diet 5001) or a high-fat-high sucrose diet group (HF, custom Diet #102412 Dyets, Inc.), 12 weeks into the diet intervention, rats underwent ACLx or sham surgery. The same diet continued following the surgery and animals were sacrificed at 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|x, N = 3), 3) lean ACLx (LF|x, N = 4), 4) obese sham (HF|x, N = 2), and 5) obese ACLx rats (HF|x, N = 6). Medial and lateral femoral condyles (MFC, LFC), and tibial plateaus (MTP, LTP) of the knee joints were harvested and chemically fixed before being cut sagittally into 10 µm thin sections. Cartilage regions of interest (ROIs, 140×140 µm²) were selected from the unstained knee joint sections and imaged by Fourier Transform Infrared spectroscopy with 80 scans/pixel at the Amide I peak region (1720–1759 cm⁻¹) to assess the collagen content. 12 Depth-dependent profiles of collagen content were obtained by averaging the data across the ROI width (Fig. 1.A.2) A linear mixed model was used for statistical comparisons between the different animal groups (p < 0.05).

RESULTS: The depth-dependent median profiles of collagen content are presented in Fig. 1B. In the most superficial cartilage (i.e., 1 – 10% depth), obese sham and transected animals at LFC and LTP had ~30% higher collagen content than Ctrl group animals. In the most superficial tissue of MFC and MTP sites, obese transected animals had ~20% lower collagen content compared to the Ctrl group. In the middle cartilage zone (11 – 30% depth) of MFC, both obese transected and lean transected rats had 15 – 20% lower collagen content in comparison to the Ctrl group. At the middle zone of MTP collagen of lean transected and lean sham animals, a 20 – 30% higher collagen content was observed compared to the Ctrl group animals.

DISCUSSION: The differences in collagen content found here are consistent with the literature that decreases in cartilage collagen content are characteristic of late-stage OA. The collagen loss in the superficial and middle zones of MFC and MTP with obese ACLx animals indicates that collagen content loss is increased when joint trauma and obesity are combined, compared to collagen damage from obesity or ACLx alone. Nevertheless, at some joint sites and cartilage depths, both obese sham and obese ACLx animals (LFC, LTP) and lean sham and lean ACLx animals (MTP), had increased collagen content compared to Ctrl animals. Gait adaptation, under- or over-loading of tissue, increased inflammatory response in animals of different OA subtypes, may explain these small differences. 13, 14 However, in contrast to our hypothesis, the small differences in the depth-wise analyses suggest that the OA models combining obesity and surgery do not accelerate collagen content loss in vivo in the whole joint.

SIGNIFICANT/Clinical RELEVANCE: This study improves our understanding about the effects of OA risk factors to knee cartilage. We show that ACLx and obesity may lead to minimal collagen (~20%) content loss regardless of whether these two risk factors are combined or separately present. However, the small differences found for cartilage collagen content in different treatment groups support the current view that collagen loss characterizes late rather than early OA without concurrent injury.

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Fig.1: Workflow of the FTIR-imaging with illustrations of A.1) the ROI selection on the histological sections, A.2) the chemical map of Amide I for collagen content measurements and A.3) the depth-dependent analysis, with comparisons performed in discrete intervals. On right, are median depth-dependent collagen content profiles as a function of normalized tissue thickness at medial (B.1 and B.3) femur and tibia, respectively, along with lateral (B.2 and B.4) femur and tibia. Shaded area around each profile represents a 95% confidence interval obtained with a linear mixed model for the whole tissue thickness.