These results provide interesting insight into the nature of the SCB, which is the subchondral bone (SCB) in this model of OA. Imaging modalities often only provide high-resolution information about one type of tissue, thus limiting the radiologic evaluation of complex inter-tissue interactions involved in OA. This study uses bone morphometry in order to identify SCB osteosclerosis (OS), TB osteosclerosis (OS), and the mineralization of the AC. We hypothesize that the contrast agent presents a viable means of identifying the loss of articular cartilage using \( \mu \text{CT} \). Further, we hypothesize that \( \mu \text{CT} \) can be used to detect significant osteosclerotic changes during OA, such as increases in bone density, hydroxyapatite (HA) density, cartilage mineralization, osteophyte formation, and trabecular thickness.

**Methods:** 10-week (W) old male C57BL/6 mice underwent destabilization of the medial meniscus (DMM) or sham operation surgery to induce OA. 12W and 20W after surgery, mice were euthanized and dissected to isolate the tibiae. Tibiae were fixed for 48 hours in 4% PFA, rinsed in PBS, and immersed for 24 hours in the Cysto Conray II (CC2, Covidien) contrast agent. Samples were then rinsed in PBS and \( \mu \text{CT} \)-scanned (SCANCO \( \mu \text{CT} \)40, 55 kVp, 145 \( \mu \)A, 8W, Voxel 6 \( \mu \)m). Bone morphometry (SCANCO software) was performed in three regions of interest: the AC, the SCB, and TB of the medial condyle (Fig. 1). Bone volume, HA density, and trabecular thickness were used to describe the OS typical of end-stage OA. Coronal sectioning allowed for osteophyte identification.

**Results:** Our data indicate OS was evident in all three regions of interest in DMM compared to SHAM samples (Fig. 2). In the SCB, OS was evident at 12W in DMM compared to SHAM by means of bone volume per total volume (Fig. 2). An increase in HA per total volume and per unit bone volume was also observed in the SCB, indicating that this OS was dominated by mineralization. These differences became more pronounced 20W. The AC was effectively visualized using CC2, as seen in SHAM samples (Fig. 1b, 2c), but was completely mineralized by 12W DMM. In the TB, there was an increase in trabecular thickness in the medial tibial plateau 20W after surgery. Further, osteophyte formation (Fig. 2i) was visible 20W after surgery.

**Conclusions:** These results suggest the feasibility of using \( \mu \text{CT}/\text{CC2} \) to identify the loss and subsequent mineralization of articular cartilage as well as OS within the subchondral and trabecular bone. The increase of HA density in the subchondral bone in this model of OA indicates that this region not only undergoes OS, but also a change in composition. These results provide interesting insight into the nature of the SCB, which is currently being reported as a critical structure in OA progression.
were significantly decreased in the severity of the OA-like changes. The level of microtubule-associated protein 1 light chain 3 (LC3), a main marker of autophagy, was increased in articular cartilage of Bach1−/− mice compared with wild-type mice. The rate of apoptotic chondrocytes in Bach 1−/− was lower than that of wild-type mice.

**Conclusions:** Bach1−/− mice reduces the severity of age-related OA-like changes by activation of autophagy and anti-apoptosis. The maintenance of HO-1 expression may play an important role in a protective effect or homeostasis in joints. These results suggest that autophagy via HO-1 is a novel mechanism in OA prevention. The inducer of HO-1 may be an effective therapeutic molecule for OA prevention.

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**433 DEVELOPMENT OF OSTEOARTHRITIS AND DISRUPTION OF KNEE JOINT MORPHOLOGY IN A CARTILAGE SPECIFIC MITOGEN INDUCIBLE GENE 6 DELETION MOUSE**

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**Introduction:** Osteoarthritis (OA) is a degenerative joint disease afflicting over 1 in 10 individuals in Canada. Current treatments focus on relief of pain and disability, rather than slowing or reducing the underlying joint damage. Previous studies by our lab have shown that the signalling molecule transforming growth factor alpha (TGFα) is upregulated in both animal models of OA and in a subset of human cases (Appleton et al, 2007;2010). TGFα signals via epidermal growth factor receptor (EGFR), which mediates mitogenicity. Mitogen inducible gene 6 (Mig-6) has been shown to attenuate signalling by EGFR through blockade of autophosphorylation and induction of receptor internalization. Ubiquitous deletion of Mig-6 in mice causes the rapid development of joint deformities and OA (Zhang et al, 2005; Jin et al, 2007), however it is not known which cell type is responsible for these defects.

**Purpose:** To assess the consequences of increased EGFR signalling specifically in cartilage by removing negative regulation via Mig-6 using a transgenic in vivo mouse KO model.

**Methods:** Mig-6 was selectively knocked out in cartilage using flexed transgenic mice (Mig-6fl/fl) by the Col2α1 driven expression of Cre (Col2-Crefl/fl). Animals were kept on a normal diet and unregulated activity levels. Littermate matched controls were used in all experiments. Male and female mice were aged up to one year to assess spontaneous development of OA and joint deformation. Behavioural changes indicative of joint pain and disability were assessed through gait analysis and grip strength measurement. Evaluation of bone changes indicative of joint pain and disability were assessed through spontaneous development of OA and joint deformation. Examination of individual lymph nodes showed that exercise is an important systemic inhibitor of inflammation and its actions are mediated via suppression of NF-κB activity (Figure 1).

**Results:** Mice exposed to exercise following LPS injection showed a significant systemic inhibition of inflammatory cytokine induction. More importantly, the suppressive effects of exercise are sustained and for how long. In these experiments mice were either exercised every day or only for one day post induction of inflammation. In mice exposed to exercise 7 days prior to LPS injection showed a significant systemic inhibition of LPS-induced NF-κB activation. However, mice exposed to exercise following LPS injection showed more than 90% suppression of NF-κB activation. These observations indicated that exercise is an important systemic inhibitor of inflammation and its actions are mediated via suppression of NF-κB activity (Figure 1).

**Conclusions:** Mig-6 is a novel mechanism in OA prevention. The inducer of Mig-6 may be an effective therapeutic molecule for OA prevention.

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**434 FOCAL CHANGES IN TIBIAL BONE STRUCTURE AND OSTEOCYTE INTEGRITY IN A MOUSE SURGICAL MODEL OF OSTEOARTHRITIS**


**Purpose:** Inflammation is integral to cartilage damage and bone erosion observed in joints afflicted with osteoarthritis (OA). We have earlier reported that physiologic levels of exercise are anti-inflammatory and suppress local inflammation in joints in experimental models of OA in vivo. The abrogation of pro-inflammatory signals by mechanical stimulation is mediated by suppression of NF-κB activity. Here we examined whether the observed effects of physiological levels of exercise are mediated via its local or systemic actions on inflammation.

**Methods:** All protocols were preapproved by the Institutional Animal Care and Use Committee at OSU. Transgenic BALBc female mice (12-14 wks old) containing firefly luciferase cDNA in NF-κB response elements (NFKB-RE-luc mice; Caliper Life Sciences, MA) were used to study transcriptional regulation of the NF-κB gene to examine the effects of exercise (treadmill walking at 8 M/min) on inflammation. Inflammation was triggered by injection of lipopolysaccharide (LPS; 1 μg/gm body weight) or IL-1β (10-50 ng/30 g body weight) in the right ankle of mice. Mice received following treatments, (i) no treatment (ii) exercise alone, (iii) LPS injection alone, (iv) pre-exercised for 7 days prior to induction of inflammation, (v) exercised only post induction of inflammation, or (vi) exercised pre and post induction of inflammation. Activation of NF-κB was assessed 2 hrs, 24 hrs, 48 hrs or 5 days post induction of inflammation by examining luciferase activity by digital imaging (IVIS 100). The induction of proinflammatory cytokines in the serum samples of the same mice was assessed by Multiplex ELISA assays (Biorad Labs, CA).

**Results:** Control NFKB-RE-luc mice and those exposed to exercise alone did not exhibit significant NF-κB activation. LPS injection in right ankle provoked a systemic and local inflammatory response that was 6-8 fold greater within 2 hours of LPS administration. Mice exposed to exercise 7 days prior to LPS injection showed a significant systemic inhibition of LPS-induced NF-κB activation. However, mice exposed to exercise following LPS injection showed more than 90% suppression of NF-κB activation. These observations indicated that exercise is an important systemic inhibitor of inflammation and its actions are mediated via suppression of NF-κB activity (Figure 1).

**Conclusions:** Further analysis of NF-κB activation revealed that LPS activated NF-κB predominately in axillary and inguinal lymph nodes, spleen and mesentery. Examination of individual lymph node showed that exercise was effective in suppressing LPS-induced NF-κB activation in all of these lymph nodes and the site of injection. Further immunofluorescence analysis of NF-κB in all of these tissue confirmed that exercise inhibited NF-κB nuclear translocation and its synthesis. Exercise may not only suppress inflammation, but its effects may also be systemic through global inhibition of NF-κB activation in leukocytes following acute inflammation.

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**435 INCIDENCE OF SEVERE KNEE AND HIP OSTEOARTHRITIS IN RELATION TO THE METABOLIC SYNDROME AND ITS COMPONENTS: A PROSPECTIVE COHORT STUDY**

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