pronase/collagenase digestion from the articular cartilage of the metatarso-phalangeal joints of 18 month old calves from a local slaughterhouse. The BPAC and the human chondrogenic cell line C28/I2 were cultured in micromass to promote differentiation and extracellular matrix production. For gain- and loss-of-function experiments, chondrocytes were transfected with a mammalian expression vector encoding for full-length Agrin (FL-Agrin) or with Agrin siRNA's respectively. Accumulation of cartilage-specific extracellular matrix production, rich in highly sulphated glycosaminoglycans (GAGs), was quantified by alcian blue staining at pH 0.2 and alizarin red staining. Chondrogenic potential was measured using pellet analysis as previously described3, BPAC pellets were cultured for 14 days, weighed, sectioned and stained with alizarin red and safranin o. Gene expression analysis of Agrin, Col2A1, Aggrecan, Sox9 was performed by real time PCR. Immunofluorescence was used to determine the expression levels of Agrin at protein level in chondrocytes cultured in monoloayer and in paraffin-embedded tissue sections.

**Results:** Agrin was expressed in healthy adult human and bovine articular cartilage. Agrin was signifcantly downregulated in human osteoarthritic cartilage. This downregulation was also replicated in experimental murine osteoarthritis, indicating that it is a consequence rather than a cause of osteoarthritis. Agrin expression was partially retained in the articular cartilage of sham operated knees, indicating that inflammation alone (present both in DMM and in sham) is not sufficient to induce Agrin downregulation, but cartilage damage is necessary. Endogenous expression of Agrin was confirmed in C28/I2 and in BPAC. Overexpression of FL-Agrin resulted in enhanced differentiation as demonstrated by upregulated the cartilage key transcription factor Sox9. . Knock-down of Agrin by siRNA resulted in reduced GAG production in C28/I2 and chondrocyte de-differentiation as documented by decreased expression Sox9, Col2A1 and Aggrecan mRNA.

**Conclusions:** Agrin expressed in healthy adult articular cartilage and is downregulated in osteoarthritis; - Agrin is anabolic in cultured articular chondrocytes; - Agrin is required for chondrocyte differentiation in vitro.

## 199
## AGE- AND SEX-DEPENDENCE OF FEMOROTIBIAL CARTILAGE CHANGE AFTER ANTERIOR CRUCIATE LIGAMENT (ACL) TEAR – 5 YEAR FOLLOW UP IN THE KANON STUDY

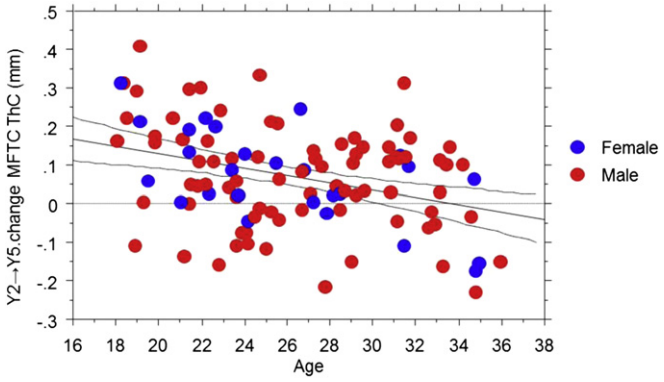
F. Eckstein[†], W. Wirth[†], L. Lohmander[‡], M. Hudelmaier[†], R. Frobell[‡]. [†]*Paracelsus Med. Univ., Salzburg, Austria;* [‡]*Orthopedics, Clinical Sci. Lund, Lund Univ., Lund, Sweden*

**Purpose:** Anterior cruciate ligament (ACL) tear is known to increase the risk of OA, and is associated with acute joint trauma and chronically altered joint mechanics. An increase in medial femorotibial cartilage thickness (ThC) has been described within 1-2 years after ACL tear. However, whether this increase depends on age or sex, whether it represents an early pathological event (caused by trauma), and/or whether it persists (due to a chronic alteration in joint mechanics) is unclear. Hence, we studied ThC change between 2 to 5 years follow-up (Y2→Y5) and between baseline and 2 years (BL→Y2).

**Methods:** 121 young active adults with an acute ACL tear in a previously uninjured knee were included in a randomized control trial, comparing rehabilitation plus early ACL reconstruction (ACLR; n=62) with rehabilitation plus the option of delayed ACLR (n=59). Sagittal MRIs (3D/WATSc) were acquired within 5 weeks of the tear (BL), and at Y2 and Y5 (n=107; 81men, 26 women; median age 25.6y; age range 18-36). ThC in the medial (MFTC) and lateral (LFTC) compartment was measured after segmentation of femoral and tibial cartilages, with blinding to acquisition order and treatment group. Regression analysis (Pearson) and unpaired t-tests were used to explore the relationship of post-tear cartilage changes with age and sex.

**Results:** The increase in MFTC ThC from Y2→Y5 was +1.8% (mean±SD [95% CI]: +70±130μm [45, 95];) compared with +1.3% from BL→Y2 (+49±165μm [17, 80]). The Y2→Y5 MFTC ThC increase did not differ significantly (p=0.94) between men (69±134μm; [40, 99]) and women (71±120μm; [23, 120]), but was significantly (p=0.017) greater in those younger than group median age of 25.6y (99±137μm [62, 137]) than in those older than group median (40±117μm [7, 72]). The correlation (r) of MFTC ThC change from Y2→Y5 with age (Fig. 1) was -0.35 [-0.51,-0.17]. For comparison it was -0.26 [-0.43,-0.07] for BL→Y2, and -0.44 [-0.58, -0.27] for BL→Y5. No significant increase in LFTC ThC was observed and no significant relationship of LFTC change with age. Baseline cartilage thickness in MFTC (but not LFTC) correlated positively with age in men (+0.27 [0.05; 0.46]) and women (+0.30 [-0.10; 0.62]). The annual increase in MFTC ThC (from age 18) estimated from the regression equations was +25μm/y in men and +22μm/y in women.

**Conclusions:** Our findings suggest that the MFTC ThC increase in young adults continues during Y2→Y5 after ACL tear. This increase is stronger in younger than in the more mature adults, with age explaining 12% of the Y2→Y5 and 19% of the BL→Y5 variability. The (baseline) cross sectional findings indicate that there may exist a physiological increase in MFTC ThC with age in early adulthood that correspond in magnitude with those observed after ACL tear, but longitudinal studies will have to confirm this hypothesis. Hence we recommend that young healthy controls be studied longitudinally to differentiate pathological ThC change after ACL tear from physiological maturation. Further, we recommend that analyses comparing ThC changes after early ACLR vs. the option of delayed ACLR adjust for age.




## 200
## SYNERGISTIC EFFECTS OF HYPOXIA AND BMP-2 ON THE HUMAN ARTICULAR CHONDROCYTE PHENOTYPE

J.E. Lafont[†], F.-A. Poujade[†], M. Pasdeloup[‡], E. Aubert-Foucher[†], O. Damour[†], P. Neyret[†], F. Mallein-Gerin[‡]. [†]*Univ. of Lyon, Lyon, France;* [‡]*CNRS, Lyon, France*

Articular cartilage has a very limited ability to regenerate spontaneously after injury. The unique cell type of that tissue (the chondrocytes) produce specific extracellular matrix components, which give to cartilage its biomechanical properties. Beside growth factors, oxygen concentration is considered as an important regulator of the matrix surrounding the chondrocytes. It is known that cartilage is a hypoxic tissue not only during development but also in adult cartilage. A growing number of evidences show that hypoxia influence several chondrocyte functions, but the exact effects and underlying mechanisms of hypoxia on the adult chondrocytes are not fully understood. In addition to explain how the chondrocyte phenotype is controlled, this study could be helpful to improve the cell based cartilage therapies.

**Aim of the study:** we previously showed that hypoxia, through a HIF-2α mediated pathway, up-regulates major chondrocyte markers, unknown chondrocyte-associated genes, and cartilage-specific miRNAs. Here we investigated the effect of hypoxia on the production of collagens by human articular chondrocytes, and if hypoxia could interfere with other chondrogenic factors such as BMP-2.

**Methods:** primary cultures of human articular chondrocytes were assessed for their ability to redifferentiate, under a treatment with the chondrogenic factor BMP-2, either in normoxia (20% O2) or hypoxia (1% O2).

**Results:** compared to normoxia, hypoxia environment dramatically enhanced the BMP-2 effect on Sox9 and type II collagen expressions.

Analysis of BMP-2 signaling under hypoxia showed an unexpected inhibition of Smad1/5/8 phosphorylation, whereas p38 MAPK was activated. Inhibition of p38 MAPK with SB202190 prevented Sox9 binding onto the type II collagen promoter and subsequent type II collagen production.
**Conclusions:** in addition to the HIF pathway, hypoxia could help to promote the chondrocyte phenotype through p38 MAPK signaling. These results suggest that hypoxia environment could be a not only an anabolic signal per se, but also a physiological signal that boosts the action of anabolic growth factors on the resident chondrocytes in human cartilage. Thus, combination of hypoxia and BMP-2 could be considered for a better control of the chondrocyte phenotype during the amplification step used in the cell therapy protocols such as Autologous Chondrocyte Implantation.

## 201
## HYPERCHOLESTEROLEMIA AND LIPID ACCUMULATION IN CHONDROCYTES

K. Kuroki, C. Bozynski, A. Stoker, M.H. Laughlin, J.L. Cook. *Univ. of Missouri Columbia, Columbia, MO, USA*

**Purpose:** Patients with primary OA commonly have cardiovascular disease (CVD) and it has been reported that cardiovascular mortality is directly proportional to the extent of OA in affected individuals. Although the high incidence of concurrent OA and CVD may be merely an independent feature of advanced age and/or obesity (major risk factors for both), one can speculate that there is a direct link between the two. In an attempt to investigate the effects of hypercholesterolemia and exercise on lipid accumulation in chondrocytes, we examined articular cartilage from hypercholesterolemic pigs. Our hypotheses were that lipid accumulation in chondrocytes would be elevated in hypercholesterolemic pigs and lipid accumulation in chondrocytes would be altered by exercise.
**Methods:** All procedures were approved by the institution's animal care and use committee. Familial hypercholesterolemic pigs (FMH, n=6) were randomized to either a sedentary (Sed, $n = 3$) or exercise (Ex, $n = 3$) group. The Ex group was trained between 20 and 24 months of age using an established progressive treadmill-based training program. The Sed group was caged alongside the Ex animals but did not undergo training. Blood and medial femoral condyles were obtained after euthanasia at 24 months of age. Articular cartilage was obtained from the weight bearing portion of the medial femoral condyle of the left knee for quantification of glycosaminoglycan (GAG) content using DMMB assay. Medial femoral condyles of the right knees were fixed in 10% neutral buffered formalin fixative, decalcified in 10% EDTA solution, cryosectioned, and stained with oil red O for examination of lipid accumulation. The remaining right medial femoral condyles were routinely processed, sectioned and stained with H&E and Toluidine blue for histopathologic examination. Blood and medial femoral condyles obtained from size matched regular crossbred pigs served as a normocholesterolemic control (n=3). Three consecutive images of mid-central articular cartilage of each section were captured using a microscope with a 40x objective lens and a digital camera. The number of lipid particles, the total area of lipids, and the number of chondrocytes were quantified using the computer software Image-Pro Plus, ver. 7 (Media Cybermetrics, Inc. Bethesda, MD). Data from each group were combined and mean $\pm$ S.D. determined. Significance was set at $p < 0.05$.
**Results:** Total cholesterol and low-density lipoprotein in FMH (both Ex and Sed) were significantly higher than in controls ($P<0.005$). None of the plasma lipid indices were altered between Ex and Sed. The number of lipid particles per 100 chondrocytes was significantly higher in the Sed ($15.9 \pm 6.9$) and Ex ($7.9 \pm 2.6$) compared to controls ($4.6 \pm 3.1$) ($P<0.005$). While total lipid area was largest in Sed, followed by Ex and controls, there were no statistical differences between the groups ($P=0.096$). GAG content [μg/dry weight (mg)] was significantly higher in the controls ($216.8 \pm 19.2$) compared to Ex ($126.4 \pm 18.2$) and Sed ($129.5 \pm 23.2$). There was not a statistically significant difference between Ex and Sed for GAG concentration. Subjectively, the articular cartilage of the FMH groups had reduced toluidine blue staining compared to controls.
**Conclusions:** Loss of GAG, which is often the earliest histology finding associated with osteoarthritis, was evident in FMH pigs. This study also demonstrated increased chondrocyte lipid accumulation in hypercholesterolemic animals providing further evidence for a potential link between cardiovascular disease and osteoarthritis. Exercise may be associated with benefits to chondrocyte lipid metabolism at the cellular level, however, further research is necessary to delineate these findings.

## 202
## ABERRANT EXPRESSION OF AUTOPHAGY MARKERS DURING OSTEOARTHRITIS

Y. Zhang, F. Vasheghani, M. Blati, J. Martel-Pelletier, J. Pelletier, M. Kapoor. *Univ. of Montreal, Montreal, QC, Canada*

**Objectives:** Recent studies suggest that the process of autophagy, a form of programmed cell survival, is impaired during osteoarthritis (OA) and may contribute towards decreased chondroprotection in the articular cartilage associated with OA pathophysiology. To further explore the role of autophagy in OA, we determined the expression of known autophagy genes in human OA, mouse and dog model of OA.
**Methods:** Human normal and OA cartilage was subjected to human autophagy PCR array and Heatmap was generated. The expression of key autophagy markers was further investigated by quantitative PCR (qPCR) and immunohistochemistry and compared with mouse and dog OA model. The effect of major OA pro-inflammatory cytokine (IL-1β) on the expression of autophagy markers was also determined.
**Results:** The Heatmap and GEDI images obtained from human autophagy PCR array demonstrated a down-regulation of 16 and up-regulation of 17 autophagy genes in human OA cartilage versus normal cartilage, with a fold change of $<$-1.5 or $>$1.5 respectively. Data further demonstrated a significant up-regulation in the expression of autophagy master regulator mTOR, and significant reduction in the expression of key autophagy markers including ULK1 (most up stream autophagy inducer), LC3B (critical factor for autophagy vacuole formation), ATG5 (required for autophagosome formation) and BNIP3 (interactor of LC3) in human OA compared to normal cartilage. Similarly, a significant up-regulation in the expression of mTOR and downregulation of autophagy-specific genes (LC3B and ATG5) was observed in mouse and dog experimental OA. Treatment of normal human cartilage explants with IL-1β resulted in a significant reduction in the expression of LC3B, ATG5 and BNIP3.
**Conclusions:** This study is the first to provides a global view of dysregulation in the expression of mTOR and various autophagy-specific genes in human OA compared to experimentally-induced OA in mouse and dogs. Targeting autophagy could open up new therapeutic avenues for OA treatment or prevention.

## 203
## MITOCHONDRIAL DNA REPAIR ENZYME OGG1 IS ESSENTIAL FOR PROTECTION AGAINST THE DOWNREGULATION OF CHONDROCYTE ACTIVITY IN OA

K. Yudoh, R. Karasawa, N. Yui. *St. Marianna Univ. Sch. of Med., Kawasaki City, Japan*

**Purpose:** During the development of osteoarthritis (OA), mechanical and chemical stresses on articular cartilage change the stable cellular activities of chondrocytes and produce excess amounts of reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines. Previous studies have provided ample confirmation of the generation of ROS and the depletion of cellular antioxidants in degenerated articular cartilage.
An oxidized form of guanine, 8-oxo-7,8-dihydroxyguanine (8-oxoguanine), is a major causative lesion for mutagenesis by ROS, because it can cause a stable base pair with adenine or cytosine during DNA replication. These mutations are thought to be involved in the pathogenesis of a variety of diseases, including degenerative diseases. In our previous study, we have found that 8-oxoguamine level was increased in the degenerated articular cartilage in OA, suggesting that the oxidative damage may accumulate in the OA cartilage. 8-oxoguanine DNA glycosylase (Ogg1) repairs 8-oxoguanine, one of the most abundant DNA adducts caused by oxygen free radicals. We postulated that depletion of cellular antioxidant, Ogg1, in degenerated articular cartilage participates in the development of cartilage degeneration. The aim of the study was examined the potential involvement of accumulation of 8-Oxoguanine and impairment of mitochondrial DNA repair enzyme Ogg1 in the pathogenesis of OA.
**Methods:** The expressions of 8-oxoguanine and Ogg1 were immunohistologically investigated in articular cartilage samples from patients