**Purpose:** Partial Meniscectomy causes dramatic changes in knee joint biomechanics. As a consequence, this surgical intervention generally leads to the development of osteoarthritis (OA). Furthermore, the magnitude of the effect is dependent on age since many of the most important risk factors for OA development are after partial meniscectomy. However, the mechanisms through which changed loading patterns lead to OA and how age related changes in articular cartilage influence its response for increased loading remains largely unknown.

Our previous findings showed that in young and healthy articular cartilage, when subjected to compression, TGF-beta signals via type 1 receptor ALK5 causing activation of downstream genes specific for Smad2/3 signaling pathway. Since in aged articular cartilage there is loss of type ALK5 leading to shift towards Smad1/5/8 signaling pathway, we wanted to investigate how TGF-beta signals in aged articular cartilage when subjected to physiological and excessive loading.

**Methods:** Full-thickness articular cartilage specimens were cored from metacarpophalangeal joint surface of two different age groups of cows (juvenile of 1 year old and aged of 8-12 years old). All explants were allowed to equilibrate for 48 h in static, serum free culture conditions. Explants were divided into two stimulation groups: 30 min stimulation with 3 MPa (physiological load) and 30 min stimulation with 12 MPa (excessive load). Unloaded cartilage was used as controls. Stimulation with 3 MPa (physiological load) and 30 min stimulation conditions. Explants were divided into two stimulation groups: 30 min were allowed to equilibrate for 48 h in static, serum free culture conditions. After 2 and 6 hours cartilage samples were frozen and mRNA levels of selected genes were examined using real-time polymerase chain reaction (RT-PCR).

**Results:** In 2 hours after the stimulation there was significant upregulation of TGF-beta in both age groups when stimulated with 3 MPa but also with 12 MPa compared to the static control. However, in young articular cartilage this upregulation was more pronounced than in old. In 6 hours after the stimulation, in young cartilage, TGF-beta was upregulated in control group (when compared to 2 hours time point), therefore the effect of stimulation was less pronounced. This was not the case in aged cartilage where in both stimulation groups TGF-beta was upregulated when compared to static control. Examination of TGF-beta signaling pathways downstream genes expression demonstrated that in 2 hours after the stimulation in both age groups, 3 MPa and 12 MPa stimulation caused upregulation of PAI-1. The level of upregulation was higher in young articular cartilage but this could be related to lower PAI1 expression in static controls of young cartilage. In aged articular cartilage, PAI-1 upregulation was caused only by 12 MPa stimulation. Stimulation with both physiological (3 MPa) and excessive loading (12 MPa) showed no effect on ID1 expression when measured in 2 hours in both age groups. However in 6 hours after the stimulation in 12 MPa compression group ID1 was down-regulated in both age groups.

**Conclusions:** Unexpectedly many changes in expression of genes caused by repetitive loading did not depend on the amount of force that was applied in our experiments. Mechanical compression with physiological and excessive forces affected similar TGF-beta-signaling related genes in young and old cartilage, however, in young cartilage with a greater magnitude. Although ALK5 expression is decreased in aged articular cartilage, loading is still able to activate PAI-1, indicating activation of the Smad2/3 signalling pathway. This indicates similar but not equal stimulation of TGF-beta signaling in different age groups. Since activation of protective TGF-beta signaling by loading was higher in young cartilage this might indicate that the protective effects of loading are more pronounced in young cartilage than in old cartilage.

**188 AMINO ACID RACEMIZATION REVEALS A HIGH STATE OF REPAIR IN KNEE COMPARED WITH HIP OSTEOARTHRITIC CARTILAGE**

J. Catterall, R. Zura, M. Bolognesi, V. Kraus. Duke Univ, Durham, NC, USA

**Purpose:** After synthesis, proteins undergo age dependent non-enzymatic post-translational modifications such as amino acid racemization and deamidation. Many cartilage proteins have long half-lives, so are prone to accumulation of post-translational modifications with age. During previous studies we quantified protein racemization in whole cartilage; osteoarthritic (OA) cartilage appeared to accumulate racemized amino acids at a slower rate than normal cartilage suggesting higher turnover of cartilage with OA. Our recent work with deamidated COMP showed greater accumulation of this post-translationally modified protein in hip OA than knee OA suggesting higher turnover of cartilage extracellular matrix in knee joints. The goal of this study is to confirm and expand upon our earlier investigations of protein turnover in health and disease through investigation of protein racemization in soluble (mainly aggrecan) and insoluble (mainly collagen) cartilage fractions of knee and hip non-OA and OA joints.

**Methods:** With IRB approval, cartilage was collected at the time of joint replacement for OA or trauma surgery. OA tissue was split into macroscopically lesioned and normal (remote from lesion) regions. Cartilage was pulverized and extracted in 4M guanidine HCl to isolate soluble proteins; these extracted residual cartilage represented the insoluble protein fraction. Samples were hydrolyzed in 6M HCl, fluorescently labeled and both the non-racemized (L) and the racemized (D) forms of the amino acids Asx (Asp and Asn) and Ser were quantified using reverse phase HPLC. Data were expressed as a ratio of D/(D+L). The association of racemized protein with age was determined by linear regression; statistical differences in racemized protein content between joint sites was analyzed using a multivariable model with age as a covariate.

**Results:** In non-OA cartilage amino acid racemization was associated with age: insoluble Asx (p = 0.0006, r² = 0.61) and Ser (p = 0.01, r² = 0.39) and a trend for soluble Asx (p = 0.05, R² = 0.35). Remote OA cartilage from both joint sites showed similar associations with age: insoluble Asx (p = 0.0002, R² = 0.32) and Ser (p < 0.0001, R² = 0.40) and a trend for soluble Asx (p = 0.06, R² = 0.09). Lesioned OA cartilage however only demonstrated a weak association with age based on insoluble Ser (p = 0.03, R² = 0.16). While hip OA fractions demonstrated significant associations of racemized amino acids with age, no significant associations with age for any of the knee fractions: hip remote insoluble Asx (p = 0.06, R² = 0.19) and Ser (p = 0.01, R² = 0.34), lesion soluble Asx (p = 0.03, R² = 0.66) and Ser (p = 0.04, R² = 0.62). Accounting for age, hip had a significantly higher content of racemized amino acids compared with knee: remote insoluble Asx (p = 0.0001) and Ser (p = 0.0001), remote soluble Asx (p = 0.001).

**Conclusions:** Our data on OA lesions cartilage demonstrates a loss of the normal age-related increase in racemization that is particularly striking for the knee. Compared with knee, the hip soluble and insoluble cartilage fractions had a higher content of racemized amino acids. Taken together, these data strongly suggest that protein turnover increases in OA, particularly at sites of OA lesions. These data further demonstrate that hip OA cartilage turns over more slowly than knee OA cartilage; conversely, these data are compatible with a less robust reparative anabolic response in hip OA compared with knee OA. These results suggest that effective treatment of hip OA may require anabolic stimuli in addition to anti-catabolic stimuli. Effective treatment of knee OA may require mainly anti-catabolic treatment strategies given the apparent unchanging evidence of the ongoing repair and high turnover state in knee OA.

This work was supported by Claude D Pepper OAIC P30 AG028716 and P01 AR05245.

**189 PERSISTENT EXPRESSION OF COL2A1 EMBRYONIC ISOFORMS DISRUPTS CARTILAGE MATRIX ASSEMBLY AND ALTERS BONE PROPERTIES**

S. Ravindran1, L. Wirthlin1, U. Hansen1, M.J. Silva1, R.J. Fernandes1, A. McAlinden1. 1Washington Univ., St. Louis, MO, USA; 2Univ. of Muenster, Muenster, Germany; 3Univ. of Washington, Seattle, WA, USA

**Purpose:** To determine how the transition from embryonic (IIA) to mature (IIB) Col2a1 isoform expression affects skeletal development and homeostasis. A transgenic mouse model was utilized that has been engineered to express only embryonic IIA isoforms of Col2a1 during pre- and post-natal development. The clinical significance of this model is apparent from observations that IIA collagen is expressed in engineered cartilage tissue and re-expressed in human osteoarthritic articular cartilage.

**Methods:** A splice site targeting approach was used to generate a global knock-out mouse model to inhibit the developmentally-regulated IIA-to-IIB splicing event and drive persistent expression of IIA collagen isoforms in vivo. RT-PCR and immunohistochemistry (IHC) was carried out to determine the Col2a1 splicing patterns in heterozygous (ki+) and homozygous (ki/ki) knock-in mice at different time-points of development (E12.5 – F70). IIA procollagen processing patterns were analyzed by Western blotting using an antibody recognizing the exon 2-encoded site.