more, RNA was isolated to evaluate the response to ALK5 or ALK1 signaling on expression of aggrecan, collagen type II and MMP-13. In addition, knee joints were isolated from mice aged 1 or 2 years, OA prone STR/ort mice (aged 3, 6 and 9 months and 1 year) and mice in which OA was induced by destabilizing the medial meniscus (DMM model). Immunohistochemistry for ALK5 and ALK1 was performed and the number of cells staining positive for each receptor in tibial cartilage was measured with a computerized imaging system.

**Results:** TGF-beta signaled in chondrocytes both via Smad2/3 and Smad1/5/8. Transfection of chondrocytes with caALK5 specifically led to Smad2/3 phosphorylation, whereas caALK1 specifically led to Smad1/5/8 phosphorylation. Chondrocytes that over expressed caALK5 showed enhanced aggrecan expression and slightly reduced collagen type II expression. Chondrocytes over expressing caALK1 had elevated expression of aggrecan, collagen type II and MMP-13, thereby displaying an OA-phenotype.

From 1 year old to 2 year old mice ALK5 decreased 88% in the medial tibial cartilage and 76% in the lateral tibial cartilage. ALK1 also was reduced, but only 51% in the medial tibial cartilage and 33% on the lateral side. In the DMM model no change was observed on the lateral side, but a reduction of 91% ALK5 positive cells compared to 75% ALK1 positive cells in the OA developing medial side. In the STR/ort model, the number of cells staining positive for ALK5 was already reduced to 7% in the medial tibial cartilage by 3 months of age. This rapidly declined to less than 1% by 9 months. On the lateral side there was still abundant ALK5 expression at 3 months of age, however, this rapidly decreases in time to less than 10% by 9 months and 1 year of age. ALK1 expression however, although slightly decreased stayed relatively high and even increased again by 1 year of age compared to 9 months of age. Overall a significant increase in the ALK1/ALK5 ratio with age and OA progression was demonstrated.

**Conclusions:** Our data show that ALK1 over expression in chondrocytes induced an OA-like phenotype in chondrocytes. Moreover, a clear switch of chondrocyte ALK5 to ALK1 expression was associated with ageing and OA progression. Our data suggest a role for ALK1 in deviant chondrocyte behavior during ageing and OA development.

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**THE IGF-1 SIGNALING INHIBITOR TRB3 IS INCREASED IN OA CHONDROCYTES**

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**Purpose:** The chondrocyte response to IGF-1 is reduced with aging and in the development of OA, however, the mechanism is not completely known. Activation of the PI3K/Akt pathway is required for IGF-1 stimulation of proteoglycan synthesis and likely also plays a role in survival signaling. Recently it has been shown that TRB3, a tribbles homolog, inhibits IGF-1 activation of Akt in HEK293 cells. The aim of this study was to determine if a)TRB3 is expressed in chondrocytes and b) if it plays a role in the reduced response to IGF-1 with aging or OA.

**Methods:** Adult human articular cartilage was obtained from tissue donors (ages 19 to 83 graded on a 0-4 scale for degenerative changes) or from OA tissue removed at the time of joint replacement. Proteins were either extracted directly from frozen, pulverized tissue or chondrocytes were isolated from fresh tissue by enzymatic digestion and cultured in high density monolayers. Expression of TRB3 was analyzed by RT-PCR, immunoblotting, and immunohistochemistry. Chondrocytes were transfected with a TRB3 expression construct and the effects on cell survival were determined in alginate beads at low density in serum-free media (conditions in which the PI3K/Akt pathway is required for survival).

**Results:** RT-PCR revealed that TRB3 is expressed in human chondrocytes and the expression appeared to increase with age. Immunoblotting of cartilage extracts could not confirm an age-related increase in TRB3 protein in normal tissue, however, TRB3 protein levels were significantly increased in chondrocytes isolated from OA cartilage when compared to age-matched normal tissue. Likewise, TRB3 was detected in OA but not normal tissue by immunohistochemistry. Overexpression of TRB3 in normal chondrocytes by transfection reduced survival by almost 50% when compared to cells transfected with an RFP control plasmid. Overexpression of Akt in cells co-transfected with TRB3 was able to return survival back to control levels.

**Conclusions:** These results are the first to demonstrate that TRB3 is present in human OA cartilage and in isolated OA chondrocytes. TRB3 has previously been shown to inhibit activation of Akt in response to IGF-1 and in the present studies overexpression of TRB3 resulted in increased cell death. Thus, TRB3 could play a role in the increased cell death noted in OA cartilage. Further studies are examining if TRB3 also contributes to reduced proteoglycan synthesis in response to IGF-1.

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**OSTEOARTHRITIS AND CHANGES IN MUSCLE QUALITY**

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**Purpose:** To examine the relationship between osteoarthritis (OA), muscle quality, and peripheral nerve function in the context of a normative aging study.

**Methods:** Data from 184 participants (87 women and 97 men) of a normative aging cohort study were analyzed. Participants were categorized as OA or no OA based on self-reported osteoarthritis of the knee or hip. Persistent and recent knee and hip symptoms were assessed by validated questionnaires. Participants also underwent a standardized exam for presence of abnormalities of the knee and hip. Ten millimeter computed tomography (CT) axial images of the right middle tibia and the middle thigh were obtained and analyzed for whole fat density, muscle density, and cross-sectional area (CSA) (Geanie software, version 2.1). Fat mass and lean mass of the right leg were also measured by dual energy x-ray absorptiometry. The right peroneal nerve conduc tion velocity (NCV) was measured at the popliteal fossa and the fibular head. Data was analyzed using SPSS software. Differences between groups were tested by ANCOVA for continuous variables and chi-square for categorical variables. The relationships between OA and muscle quality were further analyzed using stepwise ANCOVA analyses that included age, sex, race, height, smoking, alcohol consumption, comorbidities, physical activity, and peripheral nerve function as independent variables.

**Results:** Data from 58 participants with self-reported knee or hip OA and 126 without OA were analyzed. As shown in Table 1, participants with OA were older (p=0.004) compared to those without OA, and had proportionately higher percentages of individuals with current knee or hip pain (p<0.001) and knee or hip pain on examination (p=0.001). The proportion of females and the average BMI were similar between the two groups. Muscle density of the middle tibia was significantly lower in individuals with self-reported knee or hip OA (Figure 1; p=0.004). This relationship persisted after adjustment for age, self-reported recent knee and/or hip pain, knee and/or hip pain upon examination, and...
Abstract 70 – Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>No OA (N=126)</th>
<th>OA (N=58)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>67.2±1.29</td>
<td>72.6±1.32</td>
<td>0.004</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>68/58</td>
<td>29/29</td>
<td>0.616</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±0.421</td>
<td>27.7±0.603</td>
<td>0.441</td>
</tr>
<tr>
<td>Current Knee or Hip Pain N (%)</td>
<td>11 (8.7%)</td>
<td>19 (32.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Knee or Hip Pain on Examination N (%)</td>
<td>25 (19.8%)</td>
<td>26 (44.8%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lean Mass of Right Leg by DXA (kg) (N=175)</td>
<td>15.6±3.62</td>
<td>15.2±3.48</td>
<td>0.433</td>
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<tr>
<td>Fat Mass of Right Leg by DXA (kg) (N=175)</td>
<td>4.48±1.80</td>
<td>4.77±2.29</td>
<td>0.356</td>
</tr>
<tr>
<td>Peroneal Nerve Conduction Velocity (m/s)</td>
<td>46.0±0.579</td>
<td>44.8±0.791</td>
<td>0.244</td>
</tr>
</tbody>
</table>

Figure 1. Average muscle density by OA status.

peripheral nerve function, but was substantially attenuated after adjusting for total physical activity. Muscle density of the middle thigh was significantly lower in individuals with self-reported knee or hip OA (p=0.014), but this relationship was fully explained by the confounding effect of age (p=0.241). Peroneal nerve conduction velocity was not significantly different between individuals with self-reported OA and those without OA (p>0.05). Muscle CSA, fat density, and lean and fat mass did not differ between participants with self-reported OA compared to those without OA (Table 1).

Conclusions: OA appears to be associated with lower middle tibia muscle density, but not with changes in lean or fat mass. The etiology and functional implications of lower muscle density in OA are currently being evaluated. This study was fully supported by the Intramural Research Program (IRP) of the National Institutes of Health, National Institute on Aging (NIA).

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TARGETED DISRUPTION AND AGING-RELATED LOSS OF THE CARTILAGE SURFACE-SPECIFIC PROTEIN HMGB2 LEADS TO EARLY ONSET OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) typically begins with an aging-related disruption of the articular cartilage surface, followed by progressive destruction and loss of cartilage. Mechanisms leading to the aging-related surface degeneration remain to be determined. In this study we demonstrate that the chromatin protein HMGB2 is uniquely expressed in chondrocytes in the superficial zone of articular cartilage and determine the impact of Hmgb2 deficiency on cartilage homeostasis and OA pathogenesis.

Methods: Immunostaining was used to assess HMGB2 expression in human articular cartilage from younger (17-39 y.o.) and older (61-85 y.o.) donors (N=6); and in C57Bl/6J mouse knee joints at 3, 6, 9 and 15 months of age (N=6), respectively. Using adult Hmgb2-/- mice at 3, 6, 9, 12 months old, knee joints were examined by safranin O staining and OA grades were evaluated by Mankin scoring. Cell death in articular cartilage was examined by immunohistochemistry for apoptosis markers active caspase-3 and parp-p85 and compared with the matrix degradation markers MMP-3 and MMP-13. Superficial zone protein (SZP) in murine joints was examined by in situ hybridization.

Results: In normal human and murine knee articular cartilage HMGB2 is exclusively expressed in the superficial zone. In both species there is an aging-related decline in HMGB2 expression, ultimately leading to its complete absence and this is associated with the development of structural lesions in the cartilage surface. Mice with a targeted disruption of the HMGB2 gene have normal development of the skeleton and joints. However, HMGB2 deficient mice show early onset of osteoarthritis joint pathology. In Hmgb2-/- mice there is increased parp-p85 at 6 months associated with a reduction in cellularity. MMP-3 is increased in Hmgb2-/- at 6 months and reduced at 9 months while MMP-13 is strongly expressed at 9 months. In Hmgb2-/- mice there is also a reduction and early loss of SZP.

Conclusions: These findings identify HMGB2 as a factor specifically expressed in superficial zone chondrocytes and required for cell survival and cartilage homeostasis. The aging-associated loss of HMGB2 may be a novel trigger for the onset of OA. The HMGB2 deficient mouse represents a novel OA model that recapitulates central features of the human disease.