rod accelerated to 40 rpm over 5 minutes. In the incremental fixed speed protocol, mice were sequentially tested at 12, 14, 16, 20 and 24 rpm for a maximum of 5 minutes/speed. Mice were moved to the next speed after a 2-minute break. For both the accelerating and fixed speed protocols, the length of time that each mouse was able to remain on the drum was recorded as the "latency to fall".

In the beam walking test, mice were pre-trained to walk from one end to the other on a wooden round beam, 60 cm long, 1 cm diameter, elevated 35 cm above the bench. One week later, mice (DMM, sham, and age-matched naïve control) were made to walk across the beam 3 times with 10 minutes rest between tests. The following measurements were recorded: 1) The time (in seconds) the mouse needed to walk across the beam; and 2) the number of times a hind paw slipped off the beam. Data were averaged for 3 crossings/mouse. The experimenter was blinded to the groups.

Results: Using the accelerating speed protocol, DMM mice had statistically significantly lower latency times to fall compared to age-matched naïve mice at 8 and 16 weeks after surgery (p < 0.05). By 16 weeks after surgery, sham mice also had lower times to fall compared to naïve mice (p < 0.05). At fixed speeds of 16 and 20 rpm, +8 week-DMM mice had statistically significantly lower latency to fall than naïve and sham mice (p < 0.05). Again, by 16 weeks after surgery, sham mice also had lower latency times to fall compared to age-matched naïve mice. The beam walking assay revealed that 16 weeks after surgery, DMM - but not sham- mice had increased numbers of hindpaw slips while crossing the beam (DMM group = 3.42 ± 0.58 slips vs. age-matched naïve 1.22 ± 0.28 slips; p < 0.05). There was no difference in the total time needed to cross the beam among the three treatment groups.

Conclusions: The rotarod and beam walking assays may provide information on motor coordination and balance in the DMM model. Sham-operated mice also develop motor dysfunction, as assessed by rotarod. Current work is focusing on pharmacological testing in order to explore mechanisms driving the dysfunction.

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TRPV1 ANTAGONIST FOR OA PAIN: PRECLINICAL IN VIVO RESULTS PREDICT CLINICAL OUTCOME

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Purpose: Chronic joint pain with pain at walking as major complaint affects a large portion of an aging population. Treatment options for this pain are insufficient, with side effects and less than full efficacy. Many hopes arose after discovery of the TRP channels, especially the TRPV1 receptor which reacts to increases in temperature but also to other stimuli such as acid environment and capsaicin. This lead to research for new drugs, and between 2006 and 2010 more than 20 patents/applications related to TRPV1 blockers for e.g., pain, inflammation and osteoarthritis were filed each year. The TRPV1 inhibitor AZD1386 was one of those, reducing capsaicin-evoked nocifensive response and reversing heat hyperalgesia in models of inflammatory and neuropathic pain, but showed no effect on pain when tested in patients with knee OA. Lack of predictive power for drug effects has been a major criticism against animal pain models and readouts. It is therefore important to define their utility and limitations. The aim of this study was to use gait analysis as a tool to measure effects in a rat model of monoarthritis induced by Freund's complete adjuvant (FCA), and compare efficacy between AZD1386 and drugs targeting cyclooxygenase and nerve growth factor pathways.

Methods: Male rats (Sprague-Dawley or Lewis) injected intra-articularly into one ankle joint with FCA (1.0 mg/mL) were used. Starting one day after monoarthritis induction, they were treated twice daily p.o. over three days with AZD1386, COX inhibitors or pan-Trk inhibitors, or once i.v. with the NGF antibody MEDI-578. Using a walkway with a glass floor where a light source was projected into the long edge, each paw's relative contribution to weight bearing during locomotion was automatically calculated by the PawPrint software, giving the guarding index - difference in percent weight bearing between the hind paws.

Results: Both hind paws had similar weight bearing before monoarthritis, thus giving guarding index ranging from −1.7 to 6.0 %. One day after FCA injection less weight was put on the injected paw and more on the contra-lateral paw, and the guarding index ranged between 25.2 and 39.8 %. The TRPV1 antagonist was without effect during the entire

test period of three days. In contrast, the COX inhibitors reduced the guarding index to values between 18.4 and 18.7 % while the NGF antibody and Trk inhibitors normalized the guarding index to values between 8.0 and 13.2 %.

Conclusions: Our results support the use of gait analysis to assess movement-related anti-nociceptive efficacy of drugs, a measurement readout which resembles a major complaint in patients with joint pain.

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OXYGEN REGULATES LIPID PROFILES IN HUMAN PRIMARY CHONDROCYTE CULTURES

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Purpose: Articular cartilage is generally exposed to a finely regulated gradient of relatively low oxygen percentages (from 8% at the surface to 1% in the deepest layers). While most cartilage research is performed in supraphysiological oxygen levels (19−21%), culturing chondrocytes under hypoxic oxygen levels (≤8%) promotes the chondrogenic phenotype and cartilage-specific matrix formation. Although hypoxia-inducible factor-1α is identified as a key mediator of these beneficial effects on chondrogenesis, the underlying mechanisms remain unclear. A recent study in primary chondrocytes and chondrogenically differentiating mesenchymal stem cells showed that the cholesterol metabolism is altered in hypoxic conditions. Moreover the lipid metabolism is changed in osteoarthritic cartilage and intracellular lipid accumulation is correlated with osteoarthritis (OA) severity. In this study we assessed whether healthy and OA chondrocytes have distinct responses in normoxia or hypoxia with respect to their lipid composition.

Methods: Human primary chondrocytes were isolated from cartilage knee biopsies of patients (n = 5) undergoing total knee replacement. From each donor cells were isolated from macroscopically healthy and OA damaged areas. Cells were expanded in monolayer in normoxia (21% oxygen) or hypoxia (2.5% oxygen) and subsequently cultured in 3D pellets in normoxia or hypoxia for 7 days. Lipid profiles were assessed with Matrix Assisted Laser Desorption Ionization mass spectrometry imaging (MALDI MSI). Cell pellets were cryosectioned (10 μm section) and sprayed with α-Cyano-4-hydroxycinnamic acid (CHCA) matrix 5 mg/ml in methanol/water/trifluoroacetic acid (70:30:0.01) using the Suncollect (Sunchrom). A Synapt HDMS MALDI-Q-TOF (Waters) instrument was used to perform the MSI experiments with a spatial raster size of 100 μm. Principal component analysis (PCA) and discriminant analysis (DA) were used to search for spectral similarities and differences between the conditions. Biomap software was used to visualize molecular distributions. The Lipid Maps database was used for lipid assignment and tandem MS for the identification.

Results: Chondrocyte cells cultured under different oxygen tensions were discriminated by MALDI MSI followed by discriminant analysis. Discriminant function 1 (DF1) described the lipid profiles specific to each oxygen tension (Figure 1). In hypoxic pellets the intensity of phosphatidylcholine (PC) 16:0/18:1 (m/z 798.5) and sphingomyelin (SM) d18:1/16:0 (m/z 741.5 and 725.5) (Figure 1A) was higher compared to normoxic pellets. Moreover, while various phosphatidylinositol (PI) were present at both oxygen tensions, the intensity of the PI's in normoxia (m/z 865.5 and 885.5) was much higher (Figure 1B). The molecular distribution of SM d18:1/16:0 at m/z 741.5 and PC 18:0/18:1 (m/z 810.6) showed a higher expression at the edges of the pellet (Figure 2). Interestingly, we found that the lipid profiles of chondrocytes harvested from either OA or healthy cartilage showed a more pronounced difference when cultured in hypoxia. In both positive and negative ion modes the second DF separated healthy from OA chondrocytes in hypoxic conditions.

Conclusions: Our MALDI-MSI data show that oxygen tension modulated the lipid composition of chondrocytes. Furthermore, culturing OA or healthy chondrocytes in hypoxic conditions resulted in more pronounced differences in lipid profiles as compared to culturing in normoxia. Since glycerophospholipids, including PC, SM, PS, and PI, are key components of the lipid bilayer and involved in metabolism and cell signaling, we will next investigate how these lipids influence chondrocyte metabolism and cell signaling.
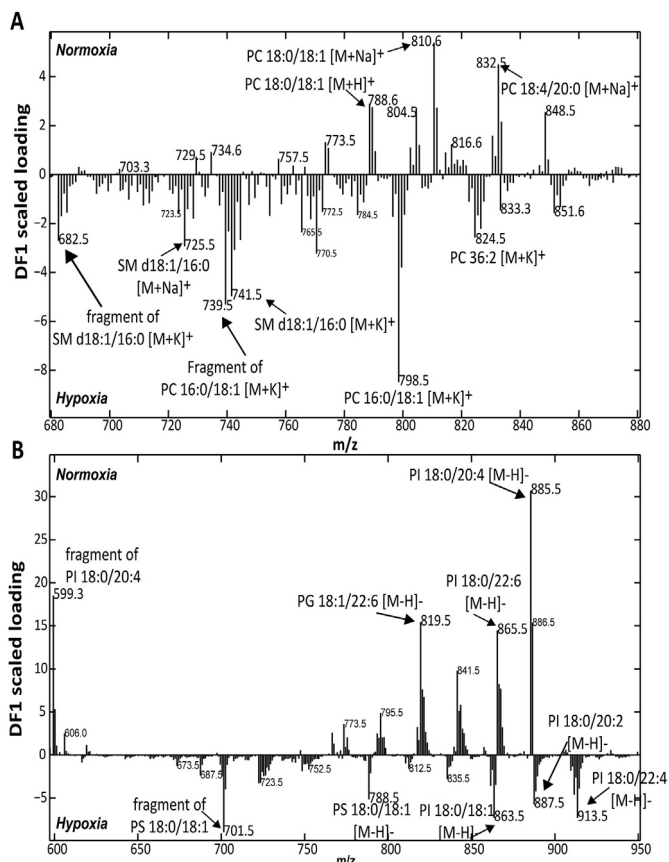
Figure 1. Spectral mapping of positive (A) and negative (B) lipid ions in chondrocyte pellets cultured in normoxia (DF1+) and hypoxia (DF1-).DF:Discriminant function, PC: phosphatidylcholine, SM: sphingomyelin, PG: phosphatidylglycerol and PI: phosphatidylinositol, PS: phosphatidylserine, m/z= mass/charge.
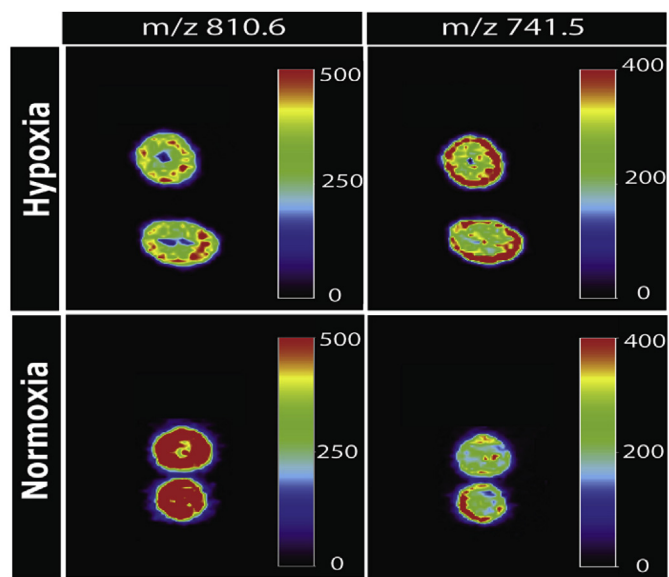

Figure 2. Lipid distribution and presence in chondrocyte pellets.Sphingomyelin dl8:l/16:0 (m/z 741.5) expression is higher in hypoxic pellets and phosphatidylcholine 18:0/18:1 (m/z 810.6) is higher in normoxic pellets.

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MTOR INHIBITION REMODELS EXTRACELLULAR MATRIX COMPONENTS OF HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: The mammalian target of rapamycin (mTOR) is a conserved serine-threonine kinase that regulates cell growth, cellular proliferation and metabolism in response to metabolic signals. It has been demonstrated that mTOR plays an important role in osteoarthritis (OA) progression. However, the specific mediators involved on mTOR signaling pathway in OA cartilage are poorly understood. The objective of this study is to determine the modulation of protein profiles of OA cartilage after mTOR inhibition by using Mass Spectrometry Imaging.

Methods: OA cartilage slices were treated with mTORC1 selective inhibitor Rapamycin (Rapa, 10 μM), and mTORC1 and mTORC2 inhibitor Torin 1 (75 nM) for 24 hours. Mankin score was performed for histopathological evaluation of OA cartilage. Sections of 10 μm thickness of each condition were deposited on high conductivity slides. Then, microspots of trypsin were deposited by a high-accuracy position automatic chemical inkjet printer. Alpha-Cyano-4-hydroxycinnamic acid matrix (HCCA) was deposited by a vibrational sprayer system. A Synapt HDMS MALDI-Q-TOF instrument was used to perform the Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) and MS/MS experiments with a spatial raster size of 150 μm. Principal Component Analysis (PCA) and discriminant analysis (DA) were used for data interpretation. Localization and quantitation of the intensity of the different peptides was evaluated with Biomap.

Results: Peptide profiles of OA cartilage treated with Rapamycin and Torin 1 are univocally distinguished by MALDI-MSI coupled to PCA and DA. In fact, the peptide profile of cartilage treated with Rapamycin or Torin 1 was discriminated from Control condition. In untreated OA samples, we identified peptides from Prolargin (PRELP; m/z 1044.5, m/z 1590.8), Fibromodulin (FM; m/z 1352,7, m/z 1361.7), Fibronectin (FN; m/z 1349,7, m/z 1401,6, m/z 1431,7) and Cartilage Oligomeric Matrix Protein (COMP; m/z 1613,8). These identified peptides are structural proteins involved in extracellular matrix organization. FN and COMP are considered potential biomarkers of OA involved in inflammation and tissue remodeling, respectively. In addition, it has been demonstrated that under pathological conditions, FM plays a role in joint