was associated with greater odds of overall, medial, and nonsignificantly with lateral hamstring co-activation compared with those in the highest tertile (Table). Similar but mainly nonsignificant associations were noted when limited to only those with radiographic knee OA. No associations were noted for sensitization with the middle vs. lowest tertiles of muscle co-activation.

Table: Relation of sensitization to muscle co-activation

Central Sensitization measure (Exposure)	Adjusted* OR (95% CI) for Hamstring muscle Co-activation (Outcome): Highest tertile vs Lowest tertile**		
	Overall Hamstring	Medial Hamstring	Lateral Hamstring
Among all participants (N=1633)			
Temporal Summation	1.3 (1.0-1.8)	1.3 (1.0-1.8)	1.2 (0.9-1.7)
PPT (kg/cm^2):			
Lowest Tertile	1.5 (1.0-2.3)	1.5 (1.0-2.3)	1.4 (0.9-2.2)
Middle Tertile	0.9 (0.6-1.4)	1.1 (0.7-1.7)	1.0 (0.7-1.6)
Highest Tertile	1.0 (ref)	1.0 (ref)	1.0 (ref)
p for linear trend	p=0.04	p=0.01	p=0.06
Among only participants with radiographic knee OA (N=612)			
Temporal Summation	1.8 (1.1-3.0)	1.1 (0.7-1.9)	1.5 (0.9-2.6)
PPT (kg/cm^2):			
Lowest Tertile	1.2 (0.6-2.3)	1.4 (0.7-2.7)	1.5 (0.7-3.0)
Middle Tertile	0.8 (0.4-1.5)	1.1 (0.5-2.1)	1.0 (0.5-2.0)
Highest Tertile	1.0 (ref)	1.0 (ref)	1.0 (ref)
p for linear trend	p=0.9	p=0.2	p=0.2

*Adjusted for age, sex, BMI, depressive symptoms, clinic site, radiographic OA (only in analyses including all subjects)
** Measures of central sensitization were not significantly associated with middle vs lowest tertiles of hamstring co-activation

Conclusions: In this initial evaluation, presence of sensitization as assessed by temporal summation and lower PPT was generally associated with greater hamstring co-activation during knee extension. This provides support to the possibility that peripheral/central nervous system alterations may not only affect pain sensitivity, but also motor function.

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IDENTIFICATION AND DIFFERENT LOCALIZATION OF PROTEINS IN THE SUPERFICIAL AND THE DEEP HUMAN OA CARTILAGE BY IMAGING MASS SPECTROMETRY

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Purpose: The knowledge of the distribution and the modulation that proteins suffer in the different phases of the rheumatic pathologies is essential to understand the development of these diseases and to find the best targets for treatment. Imaging mass spectrometry (IMS) allows us to study the spatial distribution of different components with a high resolution, through a section of tissue. It permits also the identification of many proteins at the same time without labeling. In this study, we have studied the localization of proteins associated with OA pathology in different areas of normal and OA human cartilage by MALDI-imaging.
Methods: Human control and OA cartilage samples were cut in triplicate obtaining 10 μm thick sections and deposited on indium tin oxide (ITO) high conductivity slides. For MALDI imaging experiments different washing and enzymatic steps were performed. For imaging purposes spots 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 was used to perform the imaging-MS and MS/MS experiments with a spatial resolution of 150 μm. To perform the peptide identification, the Mascot algorithm was employed after profiling MS/MS and imaging-MS/MS experiments. Biomap software was used to study the localization and the intensity of the different peptides. Hematoxylin-eosin staining was performed to complement the spatial information. Principal component analysis (PCA) and discriminant analyses (DA) were used for data interpretation.
Results: We have created specific peptide/protein maps in control and OA cartilage slides identifying and localizing proteins by profiling MALDI-MS/MS and imaging MS/MS experiments on tissue. For the first time, well known proteins such as Biglycan, Prolargin, Aggrecan core protein or Decorin have been identified directly from the tissue with this technique in the human control cartilage samples. In addition to this, important OA biomarkers have been identified and localized. Different peptides of Cartilage oligomeric matrix protein (COMP) (m/z 1613.83, 2256.15), Fibronectin (m/z 1349.72, 1401.71, 1591.87, 1913.03) or Cartilage intermediate layer protein 1 (CILP1) (m/z 1954.00) were found in the OA tissues. MS/MS experiments of digested peptides in solution confirmed these results. After normalizing the intensity of these peaks using the Biomap software, we observed that the presence of these peptides was higher in OA than in normal samples as we expected. Interestingly, their distribution seemed to be more evident in the deep cartilage than in the superficial area. By means of PCA and DA analyses between control and OA groups we created a list of peaks that differentiate OA samples. The larger part of these OA-related peaks, were localized again in the deep area.
Conclusions: We have localized and identified proteins in normal and OA human cartilage by MALDI-imaging. Classical biomarkers of OA are mostly distributed in the deep area. These results support that the cross-talk between the cartilage and the bone could be relevant in the OA development.

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HIGH ADAMTS4 OR 5 ACTIVITY AND SEVERE ARTICULAR CARTILAGE AGGRECAN DEPLETION ONLY DO NOT LEAD TO OA IN YOUNG MICE UNDER STANDARD LABORATORY CONDITIONS

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Background: Joint diseases such as osteoarthritis (OA) result in destruction of articular cartilage and loss of joint function. The main structural components of articular cartilage are, besides water, type II collagen and aggrecan. Aggrecan is considered the molecule responsible for the load-resistant characteristics of cartilage. The two main enzymes that degrade aggrecan are identified as ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2).
Purpose: We studied whether local over-expression of human ADAMTS4 or 5, only in the knee joint, was possible and if this resulted in OA development.
Methods: Two unique transgenic mouse strains (Balb/c background) that express inducible active ADAMTS4 or ADAMTS5 were developed. These strains contain floxed human ADAMTS4 or ADAMTS5 genes and transcription is only activated after tamoxifen exposure. Transgenic mice and control littermates were injected in the right knee joint with 4-hydroxytamoxifen (4-HOT), highest dose 5 microgram/joint. Whole knee joints of mice sacrificed at 2 and 42 days after 4-HOT injection were sectioned and stained.
Results: Intra-articular injection of 4-HOT in ADAMTS transgenic mice resulted in expression of ADAMTS, solely in the injected knee joint. Injection of 4-HOT in control littermates did not lead to any observable effect. In ADAMTS mice, on day 2 after 4-HOT injection, the articular cartilage showed clear aggrecan loss. Loss of aggrecan progressed over time and after 42 days severe aggrecan depletion was present in the femoral-tibial and patella-femoral cartilage. In aggrecan-depleted cartilage, a few chondrocytes were surrounded by an intensely safranin O-stained halo. Remarkably, apart from aggrecan depletion no other indication of pathology, such as inflammation or tissue damage, was observed. Neither human ADAMTS expression nor aggrecan loss was seen in any other joints of the 4-HOT injected mice. To our great surprise, no structural sign of OA, such as surface fibrillation or erosions, were present in the severely aggrecan-depleted cartilage at day 42, although aggrecan depletion in the knee joints of these mice was severe for weeks.
Conclusion: ADAMTS4 or 5 can be locally over-expressed in the knee joint of inducible transgenic mice. This leads to severe aggrecan depletion but not to development of OA-like cartilage lesions within 6 weeks. Merely elevated ADAMTS activity and loss of aggrecan is not sufficient to induce damage in young mice. Apparently, an additional trigger, damaging the collagen matrix such as increased loading, is essential to cause OA in aggrecan-depleted cartilage in young ADAMTS4 and ADAMTS5 transgenic mice under standard laboratory conditions.