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Metabolic Syndrome impacts T_2 relaxation time at the knee; longitudinal data from the Osteoarthritis Initiative

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ABSTRACT

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longitudinal data from the Osteoarthritis Initiative

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Purpose: To evaluate the association of Metabolic Syndrome and lifestyle factors with severity and two year progression of early degenerative cartilage changes at the knee, measured with T_2 relaxation time values in a middle-aged cohort from the Osteoarthritis Initiative.

Materials and methods: Cartilage segmentation and T_2 map generation was performed in 3T MRIs of the knee from 403, 45-60 year old patients without radiographic osteoarthritis (OA). The influence of Metabolic Syndrome, its individual components and lifestyle factors on T_2 values and progression of disease was analyzed. Statistical analysis was corrected for differences in age, gender and OA risk factors.

Results: In individuals meeting the criteria for Metabolic Syndrome higher baseline T_2 values were found ($P<0.001$). High abdominal circumference ($P<0.001$), hypertension ($P=0.040$), high fat consumption ($P=0.019$) and self reported diabetes ($P=0.012$) were individually associated with a higher baseline T_2 values. The more components of Metabolic Syndrome, the higher baseline T_2 values were detected ($P<0.001$). T_2 progression was non-significantly associated with Metabolic Syndrome ($P=0.617$). T_2 progression increased significantly for beer consumption, but non-significantly with increasing amount of components ($P=0.071$). All components had significant influence on baseline T_2 values ($P<0.05$); if considered in a multivariate regression model higher T_2 remained significantly associated with abdominal circumference ($P<0.001$) and diabetes ($P=0.031$) and high fat consumption showed a trend ($P=0.096$).

Conclusions: Metabolic Syndrome is correlated with higher T_2 relaxation time values, suggesting increased cartilage degeneration caused by metabolic disorders and underlining the importance of public health initiatives for prevention of OA.

INDEX OF CONTENT

1   ABBREVIATIONS

AHA/NHLBI (ATP III)
BMI	body mass index
BME	bone marrow edema
DESS	dual-echo steady-state
DM	diabetes mellitus
dGEMRIC	delayed gadolinium enhanced MR imaging of cartilage
ECM	extra cellular matrix
FLASH	fast low angle shot
FOV	field of view
GAG	Glycosaminoglycan
IDF	
K/L	Kellgren and Lawrence
KOOS	knee injury and osteoarthritis outcome score
LT	lateral tibia
LFC	lateral femur condyle
MFC	medial femur condyle
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MS	Metabolic Syndrome
MSME	multi-slice multi-echo spin sequence
MT	medial tibia
NCEP-ATP-III	National Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults
OA	Osteoarthritis
OAI	Osteoarthritis Initiative
PAT	patella
PG	proteoglycan
TE	echo time
TR	repetition time
TSE	turbo spin echo
WOMAC	Western Ontario and McMaster Universities Osteoarthritis
WORMS	whole-organ MR imaging score

2   INTRODUCTION

Osteoarthritis is one of the most common musculoskeletal disorders and affects a large percentage of the older population, which proportionately in society has steadily risen in the past decades. Today, 27 million people in the United States suffer from clinically symptomatic OA and at least 70 % of the population over the age of 65 show radiological evidence of OA with an increasing trend [55]. The incidence of Osteoarthritis is expected to rise even more due to increased life expectancy and a worsening of risk factors.

OA is a complex disorder with irreversible deformities of the joints, which cause major limitation of activity right up to physical disability. Clinically, it is identified by pain related to structural abnormalities. Apart from other common rheumatic conditions including fibromyalgia, rheumatoid arthritis and gout, primary OA is the most common cause for the gradual destruction of joints. Primary OA (localized or generalized) presents in mainly intact joints without an inciting agent, suggesting an intrinsic disease of cartilage, causing biochemical and metabolic alterations, thus resulting in its breakdown [93]. In contrast, secondary OA is defined as a degenerative joint disease resulting from predisposing factors such as previous trauma or surgery affecting the joint, congenital deformity, infection and inflammation, endocrine, metabolic and neuropathic disorders and obesity. Evidence is growing for the role of genetics, diet, co-morbidity and local biomechanical factors, such as muscle weakness, excessive joint overuse and obesity [31,42,40,95].

At present, OA already represents a fundamental problem for both, individuals and society. Work limitation, frequent health-care appointments and joint replacement surgery increase health costs [70]; decrease in mobility and limitation of daily routines reduce the quality of life for individuals and affected families [67,66].
The knee is the most affected joint of OA (incidence of 240/100 000 individuals/year) [74] and disables more individuals than any other disease [12]. It is followed by a non-weight bearing joint, the hand (100/100 000 individuals/year) and the hip (88/100 000 individuals/year) [74]. OA in non-weight bearing joints strengthens the assertion of OA being a multisystemic disease, sharing common pathogenic mechanisms in patients with Metabolic Syndrome [78]. Attention has been focused on a number of

risk factors mutual to OA, cardiovascular disease and Metabolic Syndrome [91,103,35]. Origin and progression of OA is often attributed to cartilage changes, but alterations in subchondral bone, synovium, capsule, periarticular muscles, sensory nerve endings, meniscus and enlargement as well as deformity of the joint are also noticeable [13].

The current limitation in treatment options for OA increases the urge for effective preventative strategies that address its risk factors, including diet, co-morbidity and obesity [32,81]. Apart from relieving debilitating symptoms with analgesia, currently there is no treatment that targets and inhibits the progressive degenerative structural change that is responsible for OA and its progression. In parallel with growing interest in pharmacological treatments, there has been growing awareness of the medicoeconomic and socioeconomic impact of this disease.

Conventional radiography and MR imaging are used to diagnose and monitor the disease noninvasively. Nevertheless, prior to irreversible morphologic destruction shown on conventional imaging or clinical appearance [15,16], recent studies have shown the potential of new molecular MR imaging techniques to detect early biochemical shifts in the hyaline cartilage matrix, such as increased water content and deterioration of the collagen network [26,28]. These sensitive techniques include diffusion- weighted imaging and delayed gadolinium enhanced MR imaging of cartilage (dGEMRIC), $T_1\rho$ and T_2 quantification [57,59,15]. However, so far the association of Metabolic Syndrome and other lifestyle factors with T_2 measurements and cartilage degeneration has not been well established.

The data used for this project was taken from the OAI public database and image archive. The Osteoarthritis Initiative (OAI) is a longitudinal, prospective observational multicenter study with a cohort of 4796 subjects between 45 to 79 years [75], focusing primarily on knee OA. It was initiated by the National Institutes of Health (NIH) to better understand the natural evolution of OA. The OAI annually obtains data including clinical assessment, serologic samples, and primarily knee joint imaging such as radiographs and MRI, including T_2 mapping sequences.

3   SPECIFIC AIMS OF THIS STUDY

Currently no effective pharmacotherapy for OA is available other than pain relieving medication. Therefore prevention is key and identification of modifiable risk factors is critical. Thus the following goals were identified for this project:

A) To evaluate knee cartilage T_2 relaxation values in relation to Metabolic Syndrome and its individual components (i) abdominal circumference, (ii) hypertension, (iii) diabetes and (iv) fat consumption in 403 patients from the OAI incidence cohort at baseline.

B) To assess whether Metabolic Syndrome and its individual components are correlated with longitudinal changes of cartilage T_2 values in a two-year follow up.

C) To analyze if other lifestyle factors (nicotine consumption, alcohol consumption, burger consumption) are correlated with baseline T_2 values and longitudinal T_2 value changes over 24 months.

4 BACKGROUND

1) ANATOMY OF THE KNEE

Osteoarthritis, or degenerative joint disease, is a highly prevalent joint pathology and the most common form of arthritis [37]. The disease usually occurs late in life and most frequently affects the hands and weight-bearing joints, habitually and notably in the knee and hip joint. Hence evaluating cartilage damage and progression within the knee, which is the major source of reported disability and loss of function, is an essential and ongoing subject in osteoarthritis research.

Significant change in knee cartilage, is a precursor to other anatomical alterations, therefore the anatomy of the knee and its cartilage is fundamental.
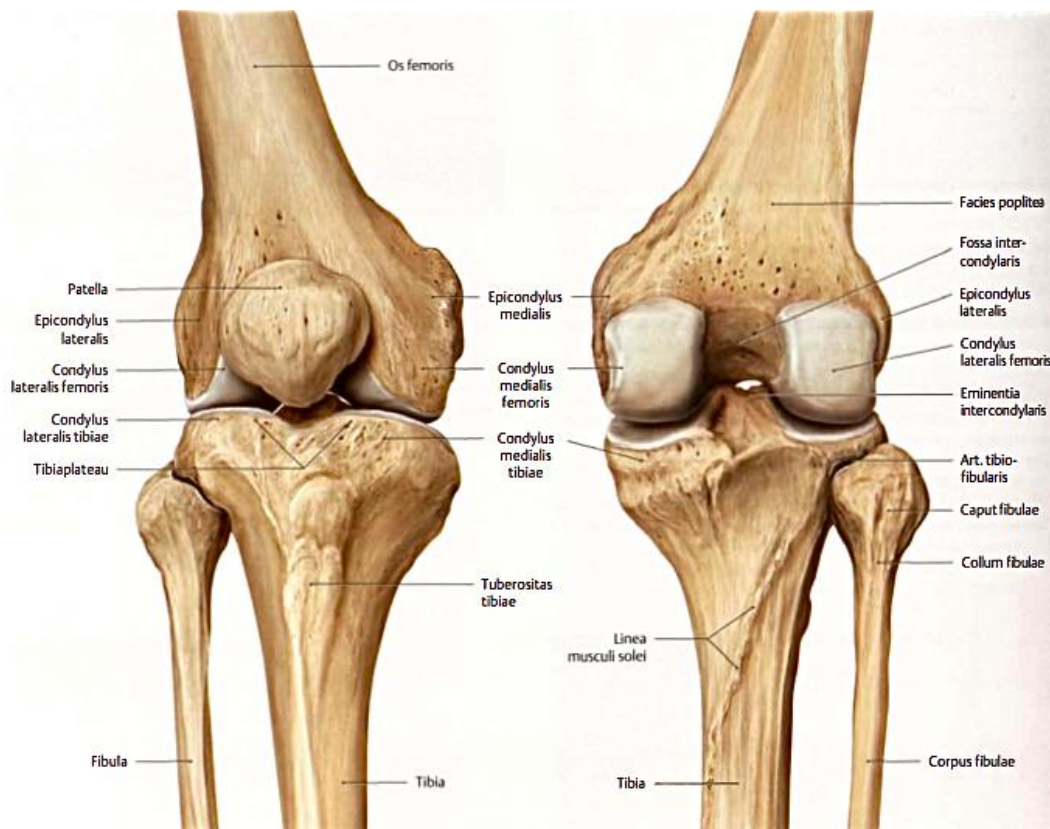

Fig. 1: Anatomy of the right knee front and back view.

(Michael Schünke, Erik Schulte, Udo Schumacher, Markus Voll, Karl Wesker; *Kniegelenk* in *Allgemeine Anatomie und Bewegungssystem Prometheus, LernAtlas der Anatomie*; 2nd edition, Georg Thieme Velag, Stuttgart -New York, 2007 p. 434-447)

The knee joint is formed by two articulating weight-bearing surfaces of the femur and the tibia as well as of the patellofemoral joint. To maintain stability, in tension or torsion, and to distribute the weight evenly onto the tibia, the femorotibial joint has two menisci over its articulating surface. They are situated between the femoral condyles and the tibial plateau of the knee. Just as these surfaces vary in shape amongst themselves and between individuals, so vary the menisci and are attached differently to the knee capsule [51]. The medial meniscus is of greater significance with respect to reducing friction during movement and to weight-bearing. Hence, more radial tears are found in the medial meniscus, which are highly associated with an increased incidence and severity of cartilage degeneration [20]. The menisci consist of water, collagens and proteoglycans akin to the hyaline articular cartilage.
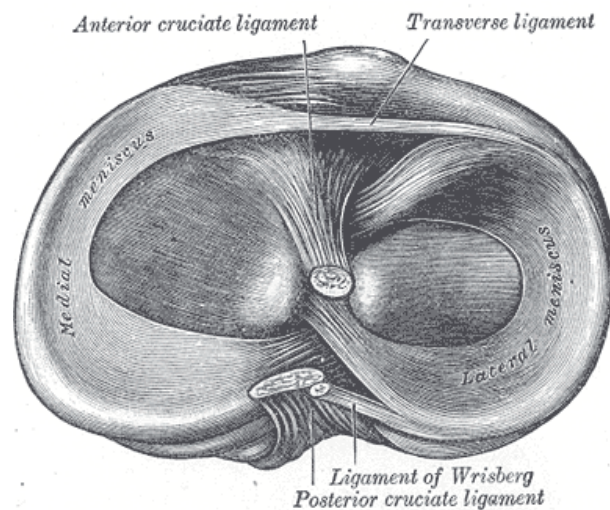

Fig. 2: Anatomy of the right knee, showing the tibial plateau and the menici
(Henry Gray, Anatomy of the Human Body, 20th edition, New York: Bartleby.com, 2000)

Hyaline (from the Greek hyalos, meaning "glass or transparent stone") cartilage is a flexible rigid connective tissue covering the articular surfaces of most notably bones, but it is also part of the nucleus pulposus of the intervertebral disk, the meniscus, the growth plate and other essential anatomical structures. The cartilage matrix consists predominantly of water (66-78%), collagen for mechanical stabilization and structural integrity and proteoglycans (PGs) for physiochemical characteristics of the cartilage [63]. One key proteoglycan of hyaline cartilage is Aggrecan. It has several sulfated carbohydrate side chains and as a result is highly charged, thereby attracting water molecules [100]. These reversible osmotic interactions enable the PGs to expand in

volume to resist compression on motion or at rest and through shifting water (also known as convection) transport of nutrition is given to the chondrocytes.

The hyaline articular cartilage can be divided into four zones (superficial, intermediate, deep and calcified), giving the cartilage a unique microarchitecture. The change in direction of the collagen fibers and chondrocytes give the cartilage its arcadian characteristic. Whereas in the superficial zone for example collagen fibers and elongated chondrocytes are parallel to the articulating surface, which is needed to resist shear forces on joint movement [14].

Counterpart to compressive forces, the collagen fibrils are radially arranged, chondrocytes are arrayed perpendicular to the surface in the intermediate and deep zones [72]. Proteoglycans are numerous in the transitorial and deep zones and show a significant increase from surface to bone [52]. The arcadians end within the calcified zone that attaches the articular cartilage to subchondral bone and is characterized by enlarged chondrocytes. This unique microarchitecture can be pictured using T_2 quantification, since T_2 values correlate with collagen fibril arrangements, also seen in figure 2 [72]. To be more precise, superficial cartilage layers usually show higher T_2 values than deeper cartilage layers. Furthermore an intact and parallel collagen structure shows low T_2 values, while disrupted, damaged and irregular collagen fibers are correlated with higher T_2 relaxation values.
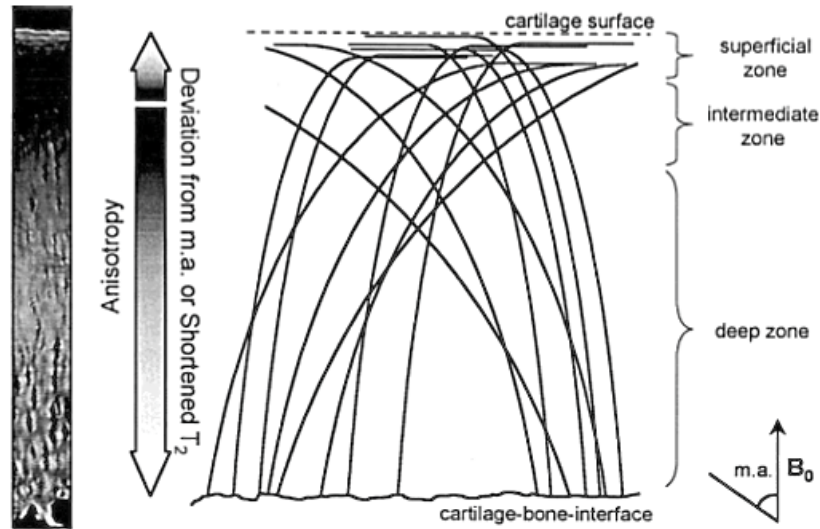
Fig. 3: Schematic diagram of collagen fibrils curving from superficial to deep cartilage. The double arrow indicates the directions of increasing anisotropy and deviation of fibrils from the magic angle (m.a) towards both superficial and deep

tissue resulting in shortening of T_2. Figure from reference [72].

Distinct from many other human tissues hyaline cartilage has a very limited ability to repair itself. Indeed, it is nearly not reparable [99]. Once laid down during development, there appears to be little capacity for chondrocytes to recapitulate the collagen architecture if mature tissue is mechanically injured or goes through advanced stages of degeneration [30]. Chondroblasts are the progenitors of chondrocytes, which cannot migrate to damaged areas, because they are bound in spaces, called lacunae. The chondroblasts ability to divide means these lacunae often shelter small groups of round chondrocytes.

Cartilage, unlike other connective tissue, is notable for its lack of nerves, inflammatory cells, fibroblasts or blood vessel. With joint movement and convection, water is being shifted in and out of the cartilage matrix. Nutrition reaches the chondrocytes through diffusion. The failure of chondrocytes to maintain a homeostatic balance between matrix synthesis and destruction results in cartilage damage and degradation [63]. Those damaged chondrocytes are then typically replaced by fibrocartilaginous scar tissue. At the same time amplified loss of other

components of the cartilage like the PG Aggrecan is common in OA and most physiological or pathological degradation is associated with catabolic actions of the Aggrecanases and MMPs [92,84]. Increased collagenase-3 matrix metalloproteinase (MMP-3) activity is specifically known to play a major role in the pathogenesis of the cartilage destruction in OA [71]. Such a degenerative process leads to irreplaceable cartilage loss and to attrition of the bone, resulting in pain, enlargement and deformity of the joint.

Fig. 4: 3D reconstruction of healthy femorotibial articular cartilage covering the right knee as obtained through segmentation of serial MRIs. Figure from reference [28].

2) PATHOPHYSIOLOGY OF OSTEOARTHRITIS

Osteoarthritis has multiple causes and risk factors and once severe progressive loss of articular cartilage has occurred, the joint fails. OA is an important public health problem, affecting mostly elderly adults in a constantly aging population and is one of the most rapidly growing causes of disability [55]. Overall more women are affected than men. This is thought to be because the broader female hip exerts more long-term stress on the knees.

Increased disorganization and destruction of cartilage with associated subchondral bone changes characterizes OA. Its etiology, although not yet completely understood, appears to result from a complex system of mechanical, biological, biochemical, molecular and enzymatic feedback loops [63].

Healthy joint cartilage consists of 68-85% of water, 10-20% of collagen type II and 5-10% of proteoglycans, while in OA the water content of the cartilage increases by around 10% and the protein component of cartilage reduces [62,58]. Firstly the flexibility of the cartilage decreases, secondly the cartilage thickness lessens, gaps start to form and subchondral sclerosing takes place. Bone erosion, joint space narrowing and ostephyte formation is the result of these changes and can be assed by conventional x-rays. Cartilage degeneration manifests as an intermittent, progressive worsening of pain on movement (especially after overuse or long periods of inactivity), joint swelling and joint fluid accumulation plus enhanced sensitivity to cold, damp or warmth. The stiffness, pain and limited range of movement result in an impaired ability to perform everyday activities, that ultimately requires orthopaedic and prosthetic devices [65].

Table 1: Composition of hyaline cartilage in healthy patients and its tendency in OA.

Component	approximate Fraction (%)	OA tendency
Water	80	⇑ (~10%)
Collagen type II	10	⇓
Proteoglycans	8	⇓
Other cartilage collagens (IX, X, XI)	<1	⇓
Other Proteins	<1	⇓

OA of the knee can be classified as primary/ ideopathic or as secondary, which is related to known medical conditions [3]. Risk factors for OA are predominantly age, heredity, obesity, joint overuse through excessive sporting or indeed no physical activity, traumatic injuries and metabolic/endocrine/neuropathic causes. Factors associated with an increased progression of OA include high body mass index (BMI) and malalignment, particularly varus, less so valgus knee deformity [3,34]. Traditionally, the diagnosis of OA is made by patient´s history and x-rays revealing joint space narrowing, as the most prominent indirect sign of cartilage loss. In doing so, the Kellgren- Lawrence (KL) score is most frequently used as a scaling to categories the severity of knee OA [48]. Clinical symptoms such as pain, stiffness and limited function strengthen the diagnosis. These clinical parameters can be quantified by a well established score: the Western Ontario and MC Master University (WOMAC) osteoarthritis index [21]. The slow degeneration progress, causing pain and joint space narrowing, is illustrated in figure 5.
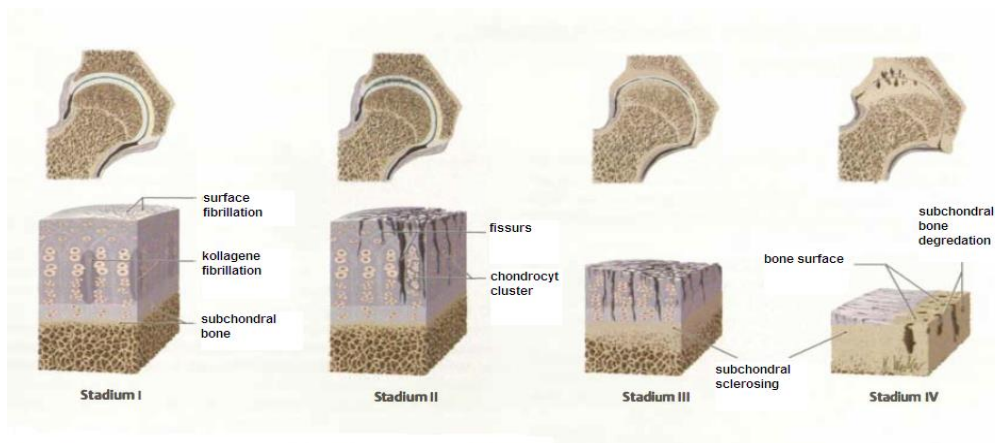

Fig. 5: Cartilage degeneration demonstrated in 4 stages. Figure translated from:
(Michael Schünke, Erik Schulte, Udo Schumacher, Markus Voll, Karl Wesker; *Kniegelenk* in *Allgemeine Anatomie und Bewegungssystem Prometheus, LernAtlas der Anatomie*; , 2nd edition Georg Thieme Velag, Stuttgart -New York, 2007 p. 47)

To secure a reasonable medical treatment, an early and precise diagnosis is crucial. Current drug therapies target symptoms- mainly pain relief. But up to this point, there is no evidence- based therapy that can inhibit or limit degenerative structural changes that are responsible for OA´s origin and progression.

3) DIAGNOSIS OF OA

Osteoarthritis has a large variety of causes with equally numerous different diagnostic methods to assess cartilage degeneration and to monitor its progression. However, core to the diagnosis of OA is the history, physical examination and imaging studies of the patient.

Clinical examination

With reasonable certainty the diagnosis of OA can be made from the patient's history and physical examination, usually conducted by the general practitioner. The main symptoms of OA at the knee are stiffness and pain on movement, typically occurring when the knee is put in motion. The onset of pain is often described as a dull ache, which, in early OA, improves when the affected knee is rested, but then paradoxically increases during the day again. As OA progresses a persistent, often nocturnal pain is described, accompanied by impaired functionality of the joint. Clinical examination should include a complete anamnesis (generally relevant data, e.g. gender, age, BMI, fitness) and should incorporate relevant findings on inspection, palpation, movement range and special functional tests, including ligament stability, menisci tests and gait analysis [65]. Classification criteria, only looking at the physical examination, should include knee pain plus at least 3 of the following 6 clinical findings: age > 50 years, morning stiffness < 30 minutes duration, crepitus on active motion, tenderness of the bony margins of the joint, lack of palpable warmth of the synovium and bone enlargements noted on examination [3].

To validate the diagnosis of OA, and for structural outcome measures, knee imaging, such as x-rays and MRI, are recommended.

Conventional Radiographs of the Knee

Radiography is still standard both for diagnosis and to assess the progression of OA. X-ray imaging studies of the knee are typically obtained in lateral and anteroposterior planes. Primary changes of hyaline articular cartilage and the development of altered joint congruency are not directly visible, whereas gross osseous changes, that tend to

occur late in the disease, can be depicted by X-rays. Radiographs are excellent for evaluating mechanical axis and alignment of bone and joints, as well as revealing secondary changes of OA such as joint-space narrowing between the articulating bones [11], subchondral sclerosis, intraosseus cysts, bone marrow edema (BME) and osteophytes, particularly in the medial compartment. These typical radiological signs of knee OA are incorporated in the staging system of Kellgren- Lawence (KL) - scale, which is today most frequently used by physicians [59].

But conventional radiographs alone are insensitive to early biochemical cartilage changes, including proteoglycan loss, increased water content and disorganization of the collagen network. MRI has become the most important modality to assess biochemical pathological changes and is additionally used today, predominantly in early OA [15,16].
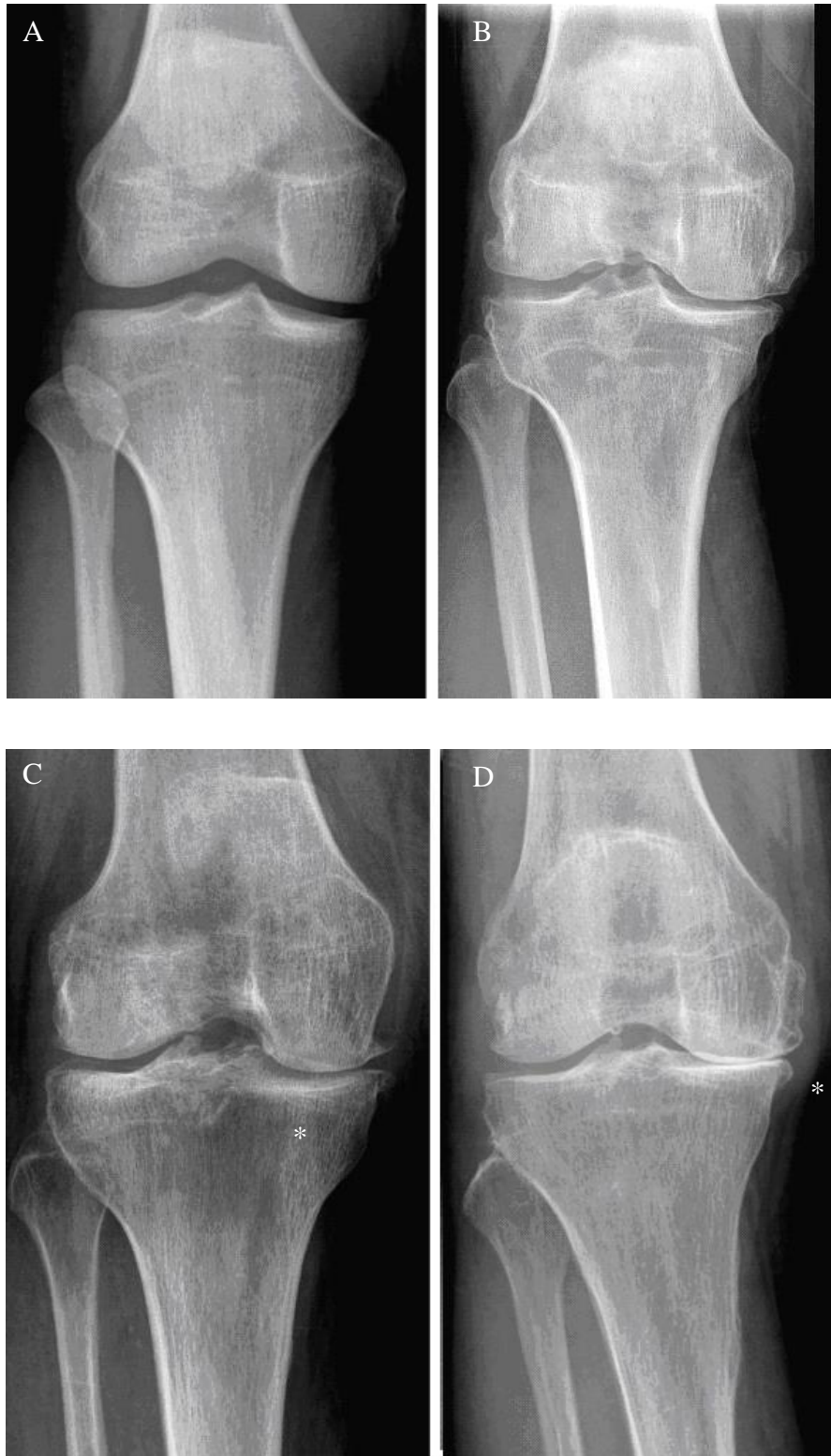
Figure 6: Right knee radiographs of (A) a healthy patient; (B) a patient with moderate OA - mild medial joint space narrowing and osteophytes; (C) more advanced disease; (D) severe medial tibiofemoral joint space narrowing with medial and lateral osteophytes. Figure from reference [4].

Magnet Resonance Imaging (MRI)

Optimized MRI acquisition sequences have shown advantages in pathologic cartilage quantification in clinical and research environments. MRI precisely visualizes structural alterations beyond gross changes in bone or joint space and is free of ionizing radiation. It now allows quantitative assessment of intraindividual cartilage volume/thickness and changes over time in both, healthy individuals and patients with OA [79]. MRI offers advantages for structural diagnosis, disease severity and monitoring OA progression of OA with multiplanar acquisitions of the whole organ [43]. Additional, non- invasive MRI has the feasibility of contrast-manipulation to depict different tissue types [22] and to assess early lesions, a precursor of OA. MRI depicts calcified as well as soft tissue joint components and its tomographic viewing perspective obviates morphololgical distortion, magnification and superimposition [44]. The prevalence of these pathological changes can be estimated by using the semiquantitative whole organ magnetic resonance imaging score (WORMS).

New molecular imaging techniques for quantifying cartilage matrix components (i.e. water, collagen and proteoglycan) have been developed as biomarkers. These include $T_1\rho$ and T_2 quantification, delayed Gadolinium enhanced MRI of cartilage (dGEMRIC) and diffusion-weighted imaging.

T_2 relaxation time measurements

Quantitative T_2 relaxation time is a technique that allows characterization of biochemical cartilage composition and is an imaging biomarker to assess cartilage quality. Spin echo sequences performed at 1.5 or 3 Tesla have typically been used for T_2 quantification. In the MRI protocol of the Osteoarthritis Initiative a multi-slice multi-echo (MSME) SE-sequence had been included.

Degradation of cartilage occurs throughout the stages of knee OA. There is a decrease in proteoglycan content, a reduced concentration and altered orientation of the collagen network [99,101], increased water mobility and increased water content within cartilage [58]. T_2 relaxation times are affected by these pathophysiological processes [11,46] and depict the orientation and integrity of the collagen network. Dardzinski et al. analyzed T_2 measurements in young asymptomatic adults and found a reproducible pattern of increased T_2 that was proportional to the known spatial variation in cartilage water [61] and inversely proportional to the distribution of

proteoglycan [23]. Based on these results, the authors concluded that such regional T_2 differences were a result of restricted water mobility within an anisotropic solid matrix. In a subsequent clinical study, Mosher et al. postulated an asymptomatic increase in T_2 of the transitional zone in senescent articular cartilage. They concluded that age-related cartilage changes differ from damaged cartilage. A later study by Dun et al. compared healthy volunteers with patients suffering from mild and severe OA, and found a correlation of T_2 values with clinical symptoms (cartilage morphology was predominantly found in the medial compartments of the knee) [27].

By using additional texture evaluation techniques, such as laminar analysis, significant longitudinal changes in mean T_2 values were observed within the deep cartilage layer, and were antithetic to superficial cartilage layers [17], as exemplified in figure 7.
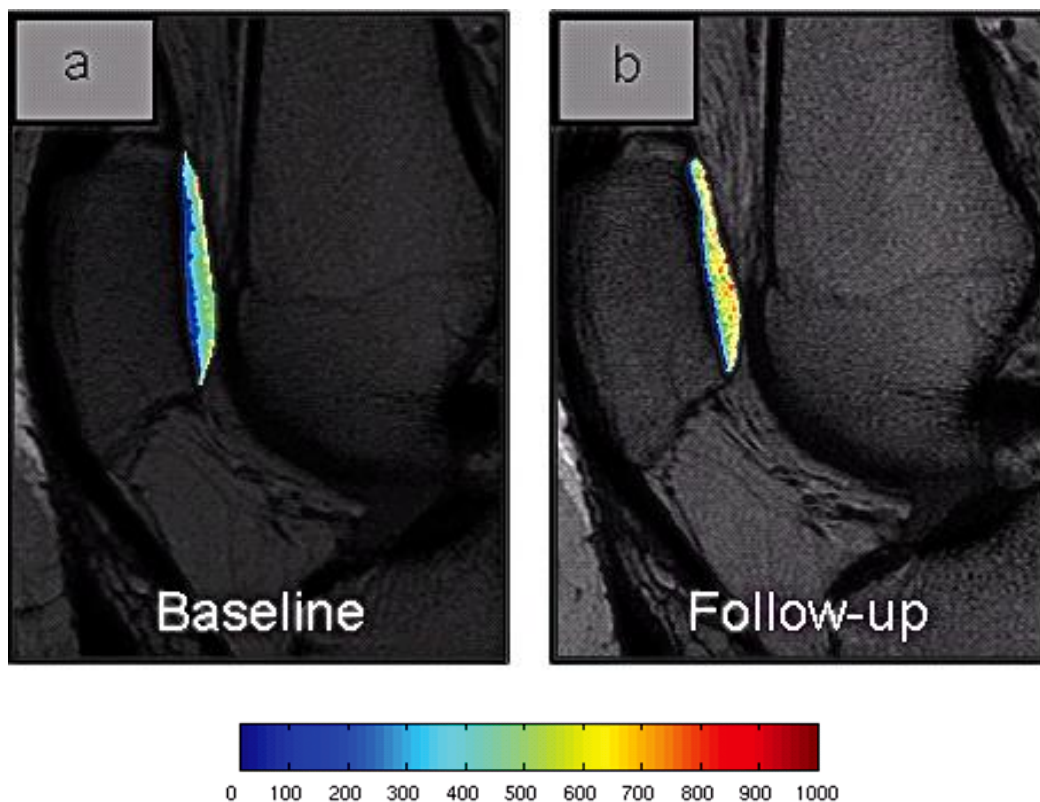

Fig. 7: T_2 MRI color maps of the right patellar cartilage of a patient without morphological cartilage defect at baseline (a) and 2 year follow up (b) from the OAI database. Although there is no clinical symptomatic OA, images show an increase in T_2 values indicating cartilage degeneration. Blue color indicates low, red color high cartilage T_2 values.

Multiple studies have shown the potential clinical relevance of cartilage T_2 relaxation time measurements to detect early stages of disease, quantitatively assess severity, sensitively monitor progression activities and eventually monitor OA therapy. Cartilage T_2 values correlate with pain and are closely related to bone changes due to OA. T_2 measurements also emerged as a potential tool in monitoring different cartilage repair techniques and their efficiency.

$T_1\rho$ measurements

Another promising technique to establish damage to cartilage composition is $T_1\rho$ imaging. Cartilage loss in OA is preceded by damage to the collagen-proteoglycan matrix and by an elevation of cartilage water content. $T_1\rho$ describes the spin-lattice relaxation in the rotating frame [41] and probes the slow motion interaction between motion-restricted water molecules and their local macromolecular environment. It therefore provides unique biomedical information in a low frequency regime (from a few hundred hertz to a few kilohertz) [56]. The change to the extracellular matrix, typically proteoglycan loss, can be reflected in measurements on $T_1\rho$ images. Damaged osteoarthritic cartilage demonstrates higher $T_1\rho$ values than in healthy knees and is arguably said to have a higher sensitivity than T_2 imaging with regard to differentiating between normal cartilage and early-stage OA [89]. Though $T_1\rho$ cannot specify the macromolecular changes, its "nonspecific" sensitivity in the detection of early degenerative lesions may provide valuable etiologic, diagnostic and prognostic information regarding knee OA [22]. This sensitivity is indicated by a larger dynamic range of $T_1\rho$ in comparison to T_2 images [80]. Currently the limited access to $T_1\rho$ measurements and their special pulse sequences, tips the balance towards T_2 weighted image- use in the early detection of cartilage change in OA.

Delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC)

This MRI technique is also used for cartilage morphometry. Hereby less sensitive T_1 sequences are combined with the intravenous contrast agent gadopentate dimeglumine (Gd-DTPA2). This method was specifically designed to image the glycosaminoglycan (GAG) content of cartilage, with the premise that the negatively charged MRI contrast agent would distribute in inverse relation to the negatively charged GAG molecules [50]. Articular cartilage consists approximately of 80% water. The remainder is

primarily collagen type II and the proteoglycan (PG) GAG. Abundant research has revealed that diseased articular cartilage is lacking GAG, which manifests as highly penetrated Gd- DTPA2 areas on MRI [6].

There are additional numerous promising imaging- based biomarkers, for example sodium magnetic resonance or chemical exchange saturation transfer (CEST) magnetic resonance imaging. They exploit the heterogeneity of endogenous PGs and therefore enable quantitative assessment of PGs macromolecular integrity of cartilage.

Table 2: Summary of sophisticated MRI techniques to quantify biochemical changes of cartilage.

MR Imaging technique	Diseased cartilage component assessed	Advantages	Weaknesses
T_2 mapping	-Mobilization of water protons - Damage to collagen-PG matrix - increased water content - collagen structure and orientation	- well validated - high sensitivity for detection of early degeneration	- limited sensitivity for detection of advanced degenerative changes
$T_1\rho$ mapping	- slow motion interactions between motion restricted water molecules and local macromolecular environment - change ECM: PG loss	- robustness - high sensitivity for detection of early degeneration	-special pulse sequence must be applied -acquisition of multiple datasets is time consuming
dGEMRIC	- Glycosaminoglycans (GAGs)	- well validated	- i.v. contrast with time delay - indirect measurement of GAG
Sodium imaging	- GAGs	- direct correlation with GAG content	- low spatial resolution - requires use of special hardware
Diffusion-weighted imaging	- Collagen network - GAGs	- additional information on GAG	- absolute quantification in thin cartilage layers is demanding

5 PATIENTS AND METHODS

5.1 STUDY DESIGN AND POPULATION

The data used in this study was obtained from the Osteoarthritis Initiative (OAI) database. The OAI cohort study is a multi- center, longitudinal, observational study conducted among men and women living in and around the participating recruitment centers (Ohio State University, Columbus; University of Maryland School of Medicine, Baltimore & Johns Hopkins University School of Medicine; University of Pittsburgh School of Medicine; Brown University School of Medicine and Memorial Hospital of Rhode Island, Pawtucket; University of California, San Francisco School of Medicine). The OAI has created an official archive of data, biological samples and joint images, which is available for public access at http://www.oai.ucsf.edu/. The ultimate purpose is to improve public health through the prevention or alleviation of pain and disability caused by OA. It includes 4796 participants with, or at risk of developing knee OA. Specific datasets used are baseline clinical dataset 0.2.2, as well as baseline and two year follow up image dataset 0.E.1 and 3.E.1.

This clinical trial was performed in accordance with the Health Insurance Portability and Accountability Act (HIPPA) and was compliant with the regulations and rules of the University of California Committee for Human Research. Prior to the study, all patients signed written informed consents approved by the local institutional review board.

For this study subjects were selected from the OAI control and incidence cohort. The latter is defined by good health according to past medical history and no signs of clinical symptoms of OA at baseline, but presence of at least one OA risk factor. These included the following eligibility criteria:

(i) preceding knee symptoms or injury without deformity of the knee

(ii) familial predisposition of OA

(iii) Heberden´s nodes or hand OA

(iv) physical activities with frequent knee bending.

We included patients at a relative young age (starting from 45 to 60 years) to assess early degenerative disease stages, which can be quantified by T_2 relaxation time measurements. Equally, the restricted age of the study population allowed us to

minimize the primary risk factor of knee OA, the effect of aging. In addition only patients with a pain WOMAC score of zero at baseline (no pain) and only patients with the OA classification system Kellgren -Lawrence (KL) grade 0 to 1 [48], reflecting no radiographic signs of OA (without joint space narrowing or osteophytes) were included. Subjects with normal to slightly overweight (BMI 19-27 kg/m²) and higher BMI (over 30 kg/m²) were included. There were no subjects with a BMI below 19 kg/m² who would have been excluded due to their propensity to nutritional deficiency and therewith associated metabolic pathologies.

Exclusion criteria were (i) inflammatory arthritis or knee symptoms requiring medication, (ii) injury with deformity of the knee joint and (iii) knee surgery with implantation, causing artifacts on images and (iv) general contraindications for MRI, including pace maker, shell fragments, (v) metallic implants, such aneurysm clips or surgical prostheses and (vi) claustrophobia, (vii) as well as poor MR quality. Subjects with (viii) co-morbid conditions and (ix) use of ambulatory aids were excluded as well as patients with (x) abnormalities in MRI, exemplifying tumor or inflammation. After the inclusion and exclusion criteria were applied, there were 403 incidence cohort subjects eligible and recruited for this project. There were 204 men and 199 women included in the study with an average age of 52.1 years. Particular for this trial data collected over a period of 2 years was used. In addition imaging and clinical data was available for n=381 individuals after 2 years for the longitudinal analysis.

5.2 COMPONENTS OF THE METABOLIC SYNDROME

Patients of the OAI representing Metabolic Syndrome were isolated according to the "National Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults" (NCEP-ATP-III) [94] were used. Adjustment was performed since dyslipidemia, defined as high triglyceride and low high-density lipoprotein (HDL) cholesterol, was not measured in this study; instead corresponding dietary fat consumption per day was used. Increased fat consumption is associated with increasing cholesterol levels [85] and metabolic syndrome [33,60]. Fat consumption

(g/day) was calculated using the Block Brief 2000 Food Frequency Questionnaire administered at the baseline examination (http://www.nutritionquest.com/). Since the recommended fat consumption is less than 78 g per day [86], we used this as threshold in our cohort. Based on the available data from the OAI, we used self-reported diabetes for the factor impaired glucose tolerance. Participants who answered the question `Do you have diabetes (high blood sugar)?´ with yes, were classified as diabetic, since required blood specimens were unfortunately not available to us.

As measurement of central obesity we used abdominal circumference instead of body mass index (BMI), since it has been proven to be stronger linked to visceral adiposity, insulin resistance and cardiovascular disease risk [76,96]. A clinic examiner measured the abdominal circumference (in cm) using a tape measure over bare skin in standing patients. Central obesity was defined according to the NCEP-ATP III cutoff at a waist circumference of ≥ 102 cm in men and of ≥ 88 cm in women.

High blood pressure is the last component missing that is part of the definition of the Metabolic Syndrome. Blood pressure was assessed in sitting patients and was defined as high, when systolic was > 130 mmHg and/or diastolic was > 85 mmHg, according to the "International Diabetes Federation consensus" (IDF) definition [2].

According to NCEP-ATP III, Metabolic Syndrome was diagnosed in our patients if three of the following four components were found: (1) central obesity (clinically measured abdominal circumference ≥ 102 cm in men or ≥ 88 cm in women) and two of the additional components of the self reported questionnaires: (2) diabetes (yes versus no), (3) high fat consumption (calculated ≥ 78 g/day) or (4) clinically measured high blood pressure in a sitting position (systolic > 130mmHg and /or diastolic > 85 mmHg) were found.

5.3 OTHER LIFESTYLE FACTORS

Another aim of this study was to evaluate the relationship between increasing numbers of modifiable risk factors in association with higher cartilage T_2 values. Therefore, not only components of the Metabolic Syndrome but also other modifiable risk factors were categorized and assessed on their association with T_2 relaxation time

values. Overall ten parameters were evaluated: firstly components of the Metabolic Syndrome:

    (i) calculated body mass index (BMI in kg/m²),

    (ii) clinically measured abdominal waist circumference (in cm),

    (iii) clinically measured systolic and diastolic blood pressure (in mmHg),

    (iv) self- reported diabetes,

    (v) calculated fat consumption (g/day)

and also other lifestyle factors:

    (vi) nicotine consumption (current smoker: yes versus no; pack years),

    (vii) self- reported alcohol consumption (none versus <1/week versus ≥ 1/week),

    (viii) self- reported beer consumption (none versus <1/month versus ≥ 1/month),

    (ix) self- reported wine consumption (none versus <1/months versus ≥ 1/month)

    (x) burger consumption (none versus <1/week versus ≥ 1/week).

These additional lifestyle risk factors were chosen from the OAI database and are based on self- reported questionnaires of everyday life and nutrition. Smoking has various effects on the body and may also influence OA, but there is no current consensus. To assess the influence of smoking on OA we divided the patients in a smoking and non- smoking population.

Alcohol consumption was grouped into general alcohol, beer and wine consumption over the past 12 months. Herewith the frequency of drinking grouped the population into three subgroups: (i) for general alcohol consumption: no alcohol, ≤ one alcoholic drink per week, > one drink per week; (ii) for beer consumption: no beer, ≤ one beer per month, > one beer per month; (iii) for wine consumption: no wine, ≤ one wine per month and > one wine per month.

Burger consumption, representing a certain lifestyle, was also grouped on its frequency into none, intermediate consumption of one or less than one burger per week or high burger consumption of more than one burger per week.

5.4 CLINICAL VARIABLES/ QUESTIONNAIRES

All patients completed several standardized questionnaires on their everyday life and limitations caused by OA at baseline.

The **WOMAC** (**Western Ontario McMaster Universities Osteoarthritis**) is a well-established questionnaire focusing on pain, stiffness and knee-related physical function. To complete the assessment of potential symptoms of OA, the WOMAC questionnaire measures through a five-point Likert scale the degree of impairment scale (none, slight, moderate, severe and extreme) [8]. This self – reported questionnaire has been sensitive and validated as an instrument to assess individual changes in OA. It is extensively used in OA trials [9]. Patients were excluded if they reported any knee pain according to WOMAC pain score in the initial clinical visit.

Additional to the enrollment visit, patients were asked to complete a self-administered questionnaire including various supplementary information (weight history, smoking history, current alcohol consumption, co-morbidity index, Block Brief 2000 Food Frequency Questionnaire, SF-12 and further). This questionnaire can be found on the OAI website (http://www.oai.ucsf.edu/datarelease/forms.asp). The Block Brief 2000 Food Frequency Questionnaire was used to assess the variable "fat consumption" derived from the Food Frequency Questionnaire (FFQ) data at baseline and was calculated by NutritionQuest (http://www.nutritionquest.com/). Since the recommended fat consumption is less than 78 g/day [86], we used this as threshold in our cohort.

The **Medical Outcomes Study Short Form 12 (SF-12)** was used to assess general health and functional status of the patient. The SF-12 is a self- administered, health-related quality of life measurement covering eight health domains. This test was designed to be minimally time- consuming. Nonetheless, it covers physical abilities, social function, role-physical, role-emotional, mental health, energy/vitality, pain, and general health perception. The SF-12 is an abbreviated version of the SF-36, yet substantially validate and versatile general health measurement. It facilitates correlating clinical parameters to OA T_2 values and so enables a comparison between OAI participants and other studied populations.

Both, clinical variables and x-rays were taken at the baseline screening visit, lasting approximately 80 minutes. This screening examination included the following

parameters that are important for this trial: measurements of weight, height, BMI, accessory anamnestic information etc. Additional information such as abdominal circumference, blood pressure and resting heart rate were also included but taken on different clinic visits.

5.5 IMAGE TECHNIQUES

5.5.1 Radiographs

Bilateral standing posterior anterior knee radiographs were taken in "fixed flexion" at baseline and 24 months follow up. Both knees were flexed to 20-30 degrees and with an internal rotation of 10 degrees using a plexiglass positioning frame (SynaFlexerTM) to secure a standardized method throughout the cohort. Applying set radiographic techniques, x-rays were taken on a 14 x 17 inch film using a focus-to-film distance of 72 inches. To determine the Kellgren-Lawrence (K/L) grade radiographs were evaluated by two musculoskeletal radiologists separately; if scores were not identical, consensus reading by both radiologists was performed. This OA scale K/L is based on the degree of osteophyte formation, joint-space narrowing, sclerosis and other joint deformity. Patients presenting a K/L score of more than 1 were excluded from this project.

5.5.2 MR Imaging

All examinations were obtained with dedicated identical 3 Tesla MRI scanners (Trio, Siemens, Erlangen, Germany) at four clinical recruitment study centers. In several studies 3.0 Tesla modality was shown to improve the depiction of cartilage lesions to a large extent versus imaging at 1.5 Tesla [22]. An identical standard knee coil and protocol was used to acquire images. As part of the study both knees were examined with standard morphological sequences. T_2 mapping sequences were performed only at the right knee or at the left if the right had contraindication for MRI. Multi-slice multi-echo (MSME) spin echo sequences with seven echos (TEs= 10, 20, 30, 40, 50, 60 and 70ms), repetition time (TR) of 2700ms, field of view (FOV) =12cm and a total acquisition time of 10.6 min was used in this trial [75]. The following five morphological and quantitative sequences were obtained of the right knee, as

described in the OAI protocol [75] and shown in Table 3:

(i) Coronal two-dimensional intermediate-weighted turbo spin-echo (TSE) sequence

(ii) Sagittal three-dimensional dual-echo steady-state (DESS) sequence with water excitation and coronal axial reformations

(iii) Sagittal two-dimensional intermediate-weighted turbo spin echo (TSE) sequence with fat suppression

(iv) Coronal three-dimensional T1-weighted fast low angle shot (FLASH) sequence with water excitation

(v) Sagittal two-dimensional multislice multiecho (MSME) spin echo sequence for T_2 mapping (TR = 2700 ms, seven TEs = 10 ms, 20 ms, 30 ms, 40 ms, 50 ms, 60 ms, 70 ms, FOV = 12 cm, slice thickness = 3mm with 0.5 mm gap, in-plane spatial resolution = 0.313 x 0.446 mm², bandwidth = 250 Hz/pixel)

MR Imaging Parameters

Parameter	Coronal Intermediate-weighted 2D Fast SE	Sagittal 3D Dual-Echo Steady-State with Water-Excitation	Coronal T1-weighted 3D FLASH with Water-Excitation	Sagittal T2-weighted 2D Multiecho SE	Sagittal Intermediate-weighted 2D Fast SE Fat-suppressed
Matrix phase	307	307	512	269	313
Matrix frequency	384	384	512	384	448
No. of sections	35	160	80	21	37
Field of view (mm)	140	140	160	120	160
Section thickness (mm)	3	0.7	1.5	3	3
Section gap (mm)	0	0	0	0.5	0
Flip angle (degrees)	180	25	12	…	180
Repetition time (msec)	3700	16.3	20	2700	3200
Echo time (msec)	29	4.7	7.57	10, 20, 30, 40, 50, 60, 70	30
Bandwidth (Hz/pixel)	352	185	130	250	248
Chemical shift (pixels)	1.3	0	0	1.8	0
No. of signals acquired	1	1	1	1	1
Echo train length	7	1	1	1	5
Phase encode axis	R/L	A/P	R/L	A/P	A/P
Distance factor (%)	0	0	0	16	0
Phase oversampling	20	0	0	0	40
Section oversampling	0	10	0	0	0
Phase resolution	80	80	100	70	70
Phase partial Fourier	1	1	1	0.875	1
Readout partial Fourier	1	1	1	1	1
Section partial Fourier	1	0.75	0.75	0.75	1
X-axis resolution (mm)	0.365	0.365	0.313	0.313	0.357
Y-axis resolution (mm)	0.456	0.456	0.313	0.446	0.511

Note.—A/P = anterior/posterior, FLASH = fast low-angle shot, R/L = right/left, 3D = three-dimensional, 2D = two-dimensional.

Table 3: OAI Protocol acquisition parameters for 3.0- knee Tesla MRI [90].

5.6 IMAGE ANALYSIS

The MSME spin echo sequences were then transferred to a remote workstation (SPARC; Sun Microsystems, Mountain View, California) and T_2 maps were calculated with custom-built software on a pixel-by-pixel basis. To characterize and quantify the cartilage matrix, MSME spin echo sequences were used performing T_2 mapping. Segmentation of the cartilage was used to sense cartilage changes by means of matrix and volume measurements. Segmentation, a visualization of cartilage, allows depiction of cartilage thickness maps overlaid on MRI images in three dimensions. For this project manual cartilage segmentation was performed using in-house software called qbrain.

Cartilage abnormalities were analyzed for the whole knee and for distinct regions. Specific compartments were defined for Patella (PAT), lateral femur compartment (LFC), lateral tibia (LT), medial femur compartment (MFC) and medial tibia (MT). One whole compartment of artifact free cartilage was segmented on all slices simultaneously, then moving on to the next. To exclude artifacts occurring at the bone-cartilage interface, cartilage-only-segmentation was carefully done without including chemical shift artifacts. This was specifically noticeable at the lateral tibia. Cartilage was contiguously segmented up to the menisci, excluding fluids, which were well detectable on the T_2 maps. Interfering fluid flow artifacts from the pulsating popliteal artery subsequently caused the exclusion of the trochlea segmentation. In order to exclude fluid and water-fat shift artifacts from the regions of interest (ROI), a technique was used that allows adjustment of the ROIs simultaneously in T_2 maps and first echo of the multiecho sequence by opening both image panels and using a synchronized cursor, slice number, and zoom. To enable the calculation of the mean T_2 values from the ROI in the T_2 maps, an IDL (Interactive Data Language, Research Systems, Boulder, CO, USA) routine was used, as per several previous studies [90].

At baseline and at the two-year follow-up, mean T_2 values were calculated individually for each compartment and globally (mean of all compartments) from the segmented areas. For better understanding the T_2 progression, the individual longitudinal increase over the two years time period was calculated as an absolute value ($T_{2\,follow-up}$ - $T_{2\,baseline}$).

Mean T_2 relaxation values were computed after the segmentation of articular cartilage was performed in 5 compartments:

A Lateral Femur
B Lateral Tibia
C Medial Tibia
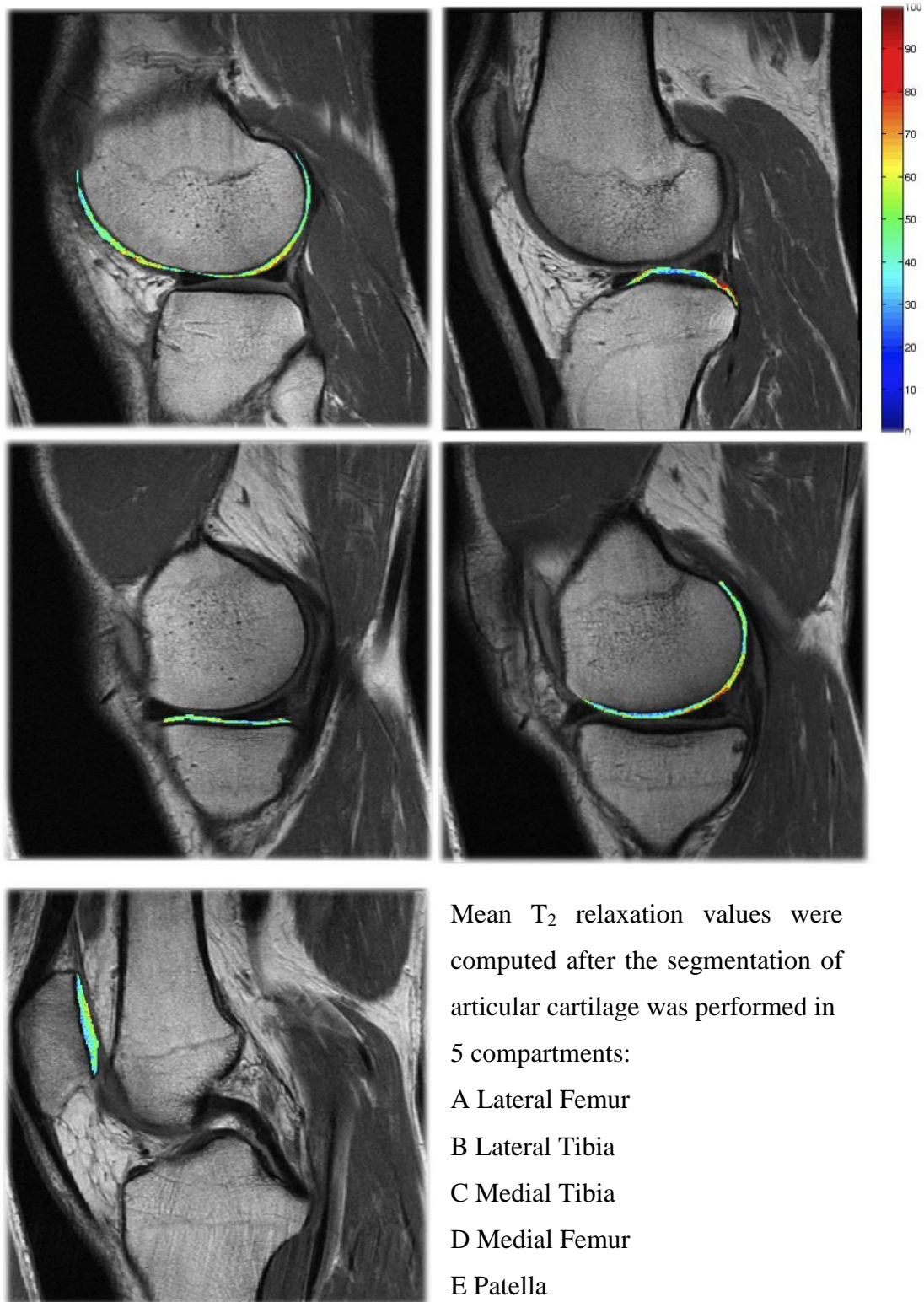D Medial Femur
E Patella

Fig. 8: T_2 MRI color maps of the right knee overlaid with the first-echo images of MSME sequences from the OAI database. Blue color indicates low, red color high cartilage T_2 values. All five compartments of the right knee are shown after segmentation and color maps.

Cartilage matrix and volume measurements require segmentation of cartilage and this visualization allows depiction of cartilage thickness maps, as demonstrated with an overlaid color map in figure 8. For segmentation T_2 maps were run using a command line existing of 7 echos, which do not account for noise. Manual cartilage segmentation of the five compartments and analysis was performed by a blinded assessor (M.S.K.) after undergoing one week of training with an experienced investigator (H.A.). The segmentation and analysis was continuously supervised by two experienced radiologists (T.M.L. (more than 20 years of experience) and P.M.J.). After segmentation T_2 maps were then calculated with custom-built software on a pixel-by-pixel basis using 6 echoes (TE= 20-30 ms) and parameters accounting for noise. The first echo (TE = 10 ms) was skipped in the T_2 fitting procedure to reduce potential errors from stimulated echoes. Additionally a noise-corrected exponential fitting was implemented based on results from recent studies demonstrating increased accuracy and precision of T_2 relaxation time with this algorithm (by G.B.J.).

5.7 REPRODUCIBILITY MEASUREMENTS

The reproducibility of the cartilage T_2 measurements of each compartment was determined in baseline T_2 maps. Intra-observer reproducibility for T_2 measurements was calculated in a randomly selected sample of 10 OAI image data sets for each compartment. These subregions were manually segmented three times by the same individual and were analyzed using the same techniques, as per the training. Intra-class correlation coefficients (ICC) were used to compare global T_2 measurements by treating the data as a continuous variable. Coefficient of variation (CV) for T_2 measurements was calculated (1.17%) for intra-reader reproducibility. Inter-observer agreement in our group was assessed to have an inter-reader reproducibility error of 1.57%, respectively 0.53ms [87].

Covariates were defined for measurement and information was then obtained from the participant through questionnaires (yes/no answers). Patients were for example asked about familial predisposition defined as total knee replacement for OA in parents or siblings, a history of knee injury resulting in difficulty walking over at least 2 days and a history of knee surgery. On examination Heberden´s nodes were considered if

bony enlargements were found in three or more distal interphalangeal joints in either hand, and isometric strength for knee flexion and extension was measured using a Good Strength Chair (Metitur, Jyväskylä, Finland; www.oai.ucsf.edu/datarelease/OperationsManuals.asp).

5.8 STATISTICAL ANALYSIS

All statistical processing was performed with JMP software Version 7 (SAS Institute, Cary, NC, USA). Parametric tests, such as linear regression or t-tests, were used when the distribution was approximately normalized. To analyze the association of potential risk factors with T_2 baseline data and its change, descriptive statistics were obtained applying two-sided t-test and one-way analysis of variance (ANOVA). Additionally, a multivariate linear regression model was used to correct for effects of known OA risk factors, including age, gender, history of knee injury, history of knee surgery, family history of knee replacement and Heberden's nodes in hands. For progression analysis, data was adjusted for baseline T_2 values of the corresponding compartment, which excluded the negative correlation of baseline T_2 values on progression data. Means ± standard deviation (SD) of T_2 values are presented if not otherwise stated. The statistical significance was defined for all calculations if $*P < 0.05$.

6   RESULTS

6.1 *Baseline Subject Characteristics*

Table 4 shows baseline characteristics for all participants subdivided into sex (male and female): age, BMI, abdominal circumference, systolic and diastolic blood pressure, daily fat consumption, diabetes, smoking habits, risk factors for OA and potential associated lifestyle factors.

Mean age of subjects analyzed in this study (n=403) was 52.1 years (±standard deviation 3.9), ranging from age 45 to 60 years at baseline. While there were no gender related differences in age (men n=204; 52.0 ± 3.8 years) and women n=199; 52.2 ± 4.0 years), women had a slightly lower BMI (28.1 ± 5.6 kg/m²) than men (28.9 kg/m² ± 4.2 kg/m²). Mean BMI lay with 28.5 ± 4.9 kg/m² in the overweight range (25-30 kg/m²) as classified by the World Health Organization (WHO). Mean abdominal circumference of 101.1 ± 15.2 cm for women and 102.5 ± 12.3cm for men was above the threshold for Metabolic Syndrome. When considering BMI and abdominal waist circumference as continuous variables, the correlation was 0.87 ($p<0.001$).

Mean blood pressure was 118.5 ± 13.5 mmHg systolic and 76.9 ± 9.5 mmHg diastolic. Both measurements of systolic and diatolic blood pressure were higher in men than in women (women: systolic 116.7 ± 13.8 mmHg; diastolic 74.6 ± 9.3 mmHg; men: systolic 120.2 ± 13.0 mmHg; diastolic 79.1 ± 9.2 mmHg). Likewise daily fat consumption was slightly lower in women (52.0 ± 27.0 g/day) than in men (62.0 ± 32.3 g/day), both within daily recommended rages (< 78 g/day). Six women and three men reported to suffer from previously diagnosed diabetes. Table 4 also shows the proportion of patients who smoked regularly, risk factors for OA and modifiable lifestyle factors such as alcohol consumption. 10.7% (n=43) of the analyzed cohort reported to be a current smoker, to be more precise 4.6 ± 8.9 pack years for women and 7.0 ± 15.1 pack years for men. 11.7% (n=47) of patients reported drinking alcohol more than once per week; 31.0% (n=125) and 34.5% (n=139) drank beer and wine more than once per month respectively. Burger consumption of more than once a week was reported in 132 subjects (32.8%)

Individual components of the Metabolic Syndrome (NCEP-ATP-III) were

(1) abdominal circumference ≥ 102 cm for men or ≥ 88 cm for women (n=298; 73.9%)

(2) hypertension ≥ 130 mmHg systolic or/and ≥ 85 mmHg diastolic (n=113; 28.0%)

(3) presence of diabetes (n=9; 2.2%)

(4) fat consumption of ≥ 78 g/day (n=74; 18.4%)

Only one of these four components was present in n=164 subjects (40.7%), two components in n=89 subjects (22.1%), three components in n=24 subjects (6.0%) and all four components in n=2 subjects (0.5%).

Since the definition of NCEP-ATP-III includes all patients who have 3 (n=24) or more (n=2) components, this means that consequently n= 26 patients were found to suffer from Metabolic Syndrome.

Table 4: Epidemiological parameters of the analyzed cohort. Mean values for all, female and male subjects are presented (± standard deviation). OA risk factors, diabetes prevalence, smoking are given in absolute numbers of the cohort as n.

Parameters	mean all subject (n=403)	mean female (n=199)	mean male (n=204)
Age (years)	52.1 ±3.9	52.2 ±4.0	52.0 ±3.8
Body mass index (kg/m²)	28.5 ±4.9	28.1 ±5.6	28.9 ±4.2
Abdominal circumference (cm)	101.7 ±13.8	101.1 ±15.2	102.5 ±12.3
Systolic blood pressure (mmHg)	118.5 ±13.5	116.7 ±13.8	120.2 ±13.0
Diastolic blood pressure (mmHg)	76.9 ±9.5	74.6 ±9.3	79.1 ±9.2
Fat consumption (g/day)	57.2 ±30.2	52.0 ±27.0	62.0 ±32.3
Diabetes*	n=9	n=6	n=3
Smoking*	n=43	n=14	n=29
OA risk factors*:			
History of badly knee injury*	n=112	n=44	n=68
History of surgery*	n=46	n=9	n=37
Family history of knee replacement surgery*	n=60	n=27	n=33
Heberden nodes*	n=69	n=46	n=68
Lifestyle factors*:			
Alcohol °*	n=47 (11.7%)		
Beer ª*	n=125 (31.0%)		
Wine ª*	n=139 (34.5%)		
Burger °*	n=132 (32.8%)		

* Data are absolute numbers of patients
° Consumption with <1 drink/ week
ª Consumption with <1 drink/ month

6.2 Baseline T_2 Measurements

Correlation of baseline T_2 mean maps of all compartments with age and gender

Figure 8 shows the correlation between global baseline knee cartilage T_2 values and age. The factor "age" was significantly correlated with both global baseline T_2, as well as with T_2 values in each compartment (P<0.001, except for patella in which P=0.004). T_2 values increased with age as shown in Figure 9.
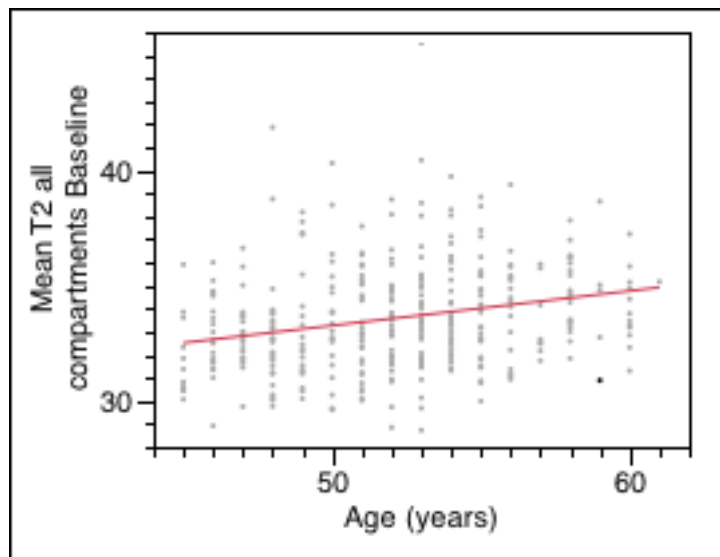

Fig. 9: Correlation of age with baseline knee cartilage T_2 mean values for all compartments within the cohort.

The factor "gender" (204 male and 199 female) was not associated with global T_2 at baseline (P= 0.879). There was barely a discrepancy between both sexes in mean T_2 (Figure 10). Although, if compartments were considered individually, significantly higher T_2 values were found in the medial compartment in women (medial femur compartment MFC: P=0.006; medial tibia MT: P<0.001), as well as in the lateral tibia plateau (P=0.013).
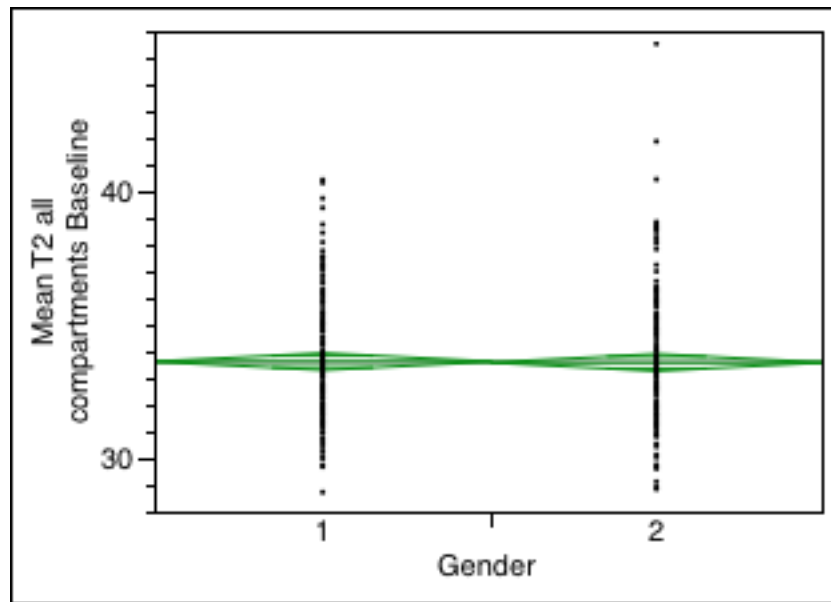
Fig. 10: Correlation of gender with baseline knee cartilage T_2 mean values for all compartments within the cohort. (1= male subjects, 2= female subjects)

Impact of Metabolic Syndrome on baseline T_2 Relaxation time

Patients suffering from Metabolic Syndrome, according to the NCEP-ATP-III definition, presented significantly higher global T_2 values at baseline (35.3 ±2.3 ms; 3 or 4 components of Metabolic Syndrome; table 5) than patients who did not fulfill the Metabolic Syndrome criteria (33.5 ±2.2 ms; P<0.001; less than 3 components of Metabolic Syndrome). Considering the individual compartments, the strongest association was found for MT and LT (P<0.001) and MFC (P=0.011). LFC showed a statistical tendency for an association with Metabolic Syndrome (P<0.1), while PAT cartilage did not have a significant association (P>0.1).

Table 5: Mean T_2 relaxation time values± standard deviation for subjects with Metabolic Syndrome (NCEP-ATP-III definition) compared to subjects who did not fulfill the criteria. (P values are adjusted for OA risk factors. *P<0.05; §P<0.1)

Baseline	Healthy patients (n=377)	Metabolic Syndrome (n=26)	P
Global *	33.5 ±2.2	35.3 ±2.3	<0.001 *
PAT	32.4 ±3.3	33.5 ±3.8	0.200
MFC *	37.7 ±2.6	38.8 ±2.9	0.011 *
LFC §	34.7 ±2.3	35.5 ±1.9	0.075 §
MT *	31.8 ±3.1	35.4 ±4.6	<0.001 *
LT *	30.6 ±3.3	32.9 ±2.4	<0.001 *

The prevalence of high T_2 relaxation time values escalated significantly with increasing components of the Metabolic Syndrome: An increase in the number of qualifying Metabolic Syndrome variables (1-4) correlated to an increase in mean T_2 values (P<0.001, adjusted for OA risk factors, see figure 11). The values increased stepwise from 32.8 ms (± 1.9) for none (0) and 35.3 ms (± 0.9) for four components of the Metabolic Syndrome (4). Considered individually, mean global T_2 value of patients with presence of two (P<0.001), three (P<0.001) or four (0 versus 4: P=0.014; 1 versus 4: P=0.046) components was significantly different from the value of individuals with 1 or no components (as asterisks indicate in figure 11). The presence of 2 or 3 components was significantly different (P=0.024), whereas the value of 4 components was not significantly different to 2 or 3 components. Considering the individual knee compartment T_2 values increased with the number of components of the Metabolic Syndrome, but only for both tibial compartments did significance remain after adjustment for other OA risk factors (P<0.001).
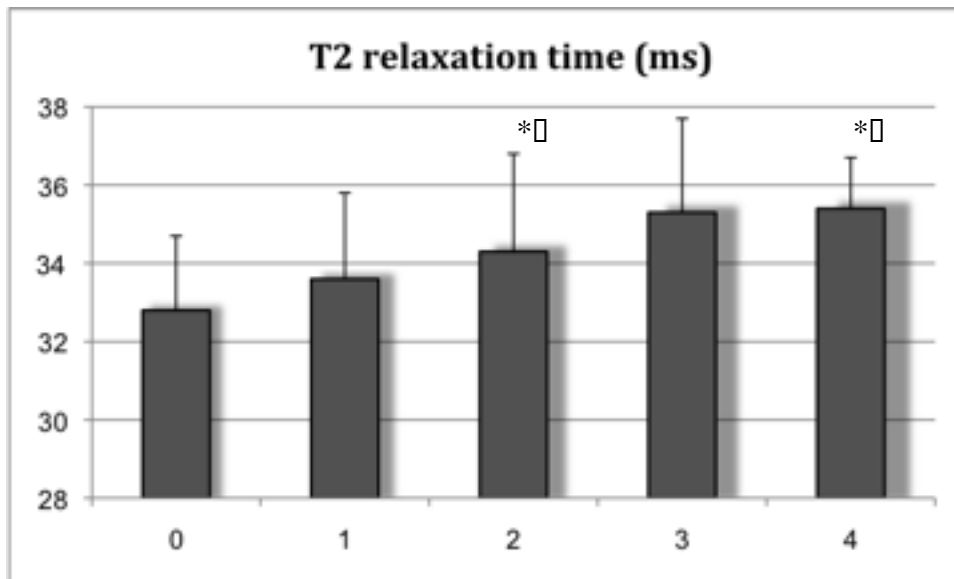
Fig. 11: Mean baseline T_2 relaxation time values (ms) and standard deviation are illustrated in subgroups, classified by the number of components of Metabolic Syndrome. Metabolic Syndrome components consisted of obesity (abdominal waist circumference), hypertension, high fat consumption and presence of diabetes. Asterisks indicate significant difference from one or no component, towards two or more, moreover there was a significant difference if only 2 and 3 components.

Individual facets of the Metabolic Syndrome and baseline T_2

Each individual facet of the Metabolic Syndrome, defined by fat consumption, hypertension, abdominal waist circumference and presence of diabetes, had a significant influence on T_2 baseline values for all compartments if individually adjusted for other OA risk factors: Daily total fat consumption of more than 78 g/day, deriving from food, effected T_2 baseline values significantly (P=0.019). High blood pressure (systolic >130 and/or diastolic >85) showed significance (P=0.046). When comparing systolic with diastolic blood pressure, both considered as a linear parameter, a greater and more significant influence was found for systolic (P=0.046) versus non-significant diastolic blood pressure (P=0.753).
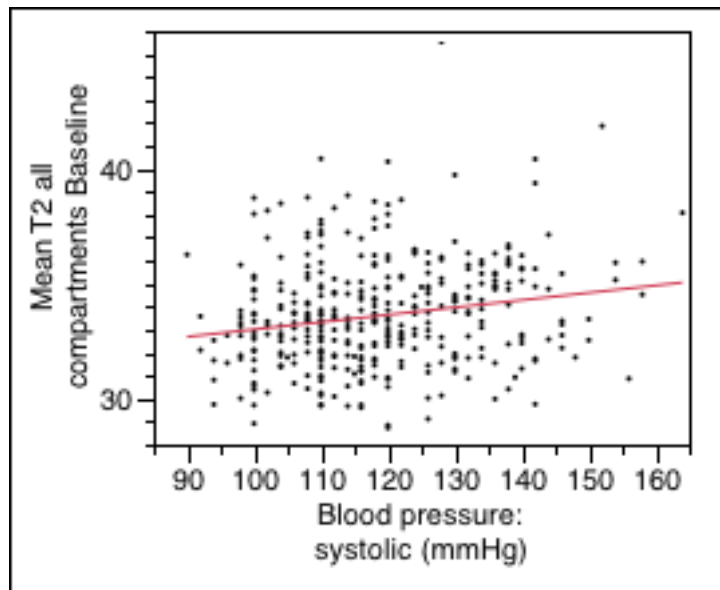
Figure 12: Correlation of systolic blood pressure, considered as a linear parameter, with baseline T_2 mean values.

Whilst all individual facets showed a significant impact on T_2 values, main influences on the different compartments varied. Generally, the main influence of abdominal circumference and blood pressure was seen in tibial T_2 values (P<0.05). For fat consumption MT (P=0.011) and MF cartilage showed significance (P=0.010) and LT showed a trend (P=0.071).

To analyze the impact on global baseline T_2 values in a multivariate regression model all four factors of the Metabolic Syndrome (defined according to NCEP-ATP-III) and other OA risk factors were included. Hereby abdominal waist circumference (P<0.001) and diabetes (P=0.031) remained significant and fat consumption showed a trend (P=0.096). In a regression model with all four components of the Metabolic Syndrome, blood pressure was not significant (P=0.351), due to adjustment for abdominal circumference, see table 6. If additionally adjusted for BMI in the multivariate regression model, only diabetes showed a statistical trend (P=0.065). The distribution of impact on various compartments in the multivariate regression model was similar to the overall analysis, with respect to the Metabolic Syndrome component analysis. Significance for abdominal circumference and blood pressure was noticeable for both tibial plateaus. Significance for the parameter "fat

consumption" was found at the medial femoral condyle, as indicated through asterisks, as well as a trend at the medial tibia.

Table 6: Individual facets of the Metabolic Syndrome and their influence on T_2 values. All components showed significance after adjusted only for OA risk factors (*P<0.05). If all parameters were analyzed in a multivariate regression model, abdominal circumference and diabetes remained significant. Fat consumption showed a trend (°P<0.1) and blood pressure was no longer significant.

Component	P adjusted. for OA risk factors	Multivariate regression with all OA components and OA risk factors					
	Global T_2	Global T_2	PAT	MFC	LFC	MT	LT
Abdominal circumference	<0.001 *	<0.001 *	0.282	0.161	0.586	<0.001*	<0.001*
Blood pressure	0.040*	0.351	0.396	0.275	0.593	0.036 *	0.0295*
Diabetes	0.012*	0.031 *	0.743	0.223	0.719	0.191	0.655
Fat consumption	0.019*	0.096°	0.691	0.015*	0.191	0.084°	0.306

Association of BMI and baseline T_2 Measurements

Figure 13 shows the correlation between T_2 mean baseline values for all compartments and Body Mass Index (BMI in kg/m²). Since BMI is sometimes considered as an alternative component within the Metabolic Syndrome instead of abdominal circumference, these two parameters were compared. Both linear parameters significantly influenced baseline T_2. The correlation was obtained for the whole data set of this study and was found to be significant (P< 0.001), as described in several studies before. Men had a distinctly higher mean BMI than women, yet the tendency of higher T_2 mean values were shown in both sexes.

After adjustment for gender, age and OA risk factors (finger nodes, pre-injury, pre surgery and family correlation), the correlation between baseline mean T_2 relaxation time values and BMI still remained significant with P< 0.001.
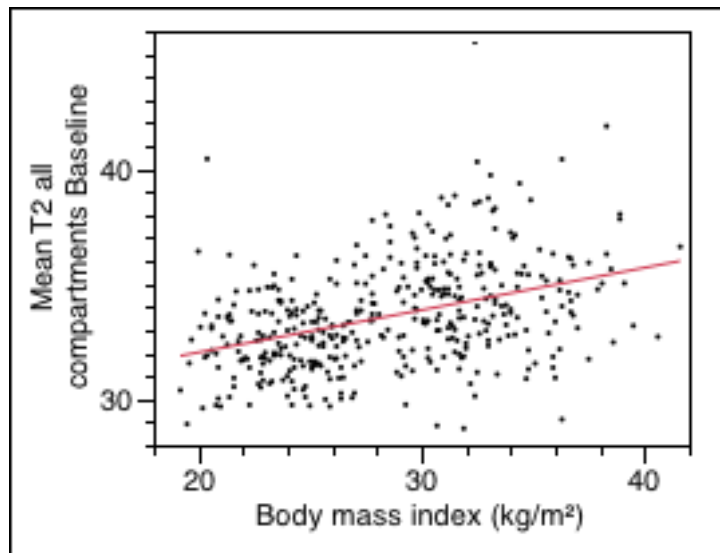
Fig. 13: Correlation of BMI with baseline knee cartilage T_2 mean values for all compartments within the cohort.

Other lifestyle factors and their influence on baseline T_2 values

Other lifestyle variables considered in this study were nicotine abuse, alcohol and burger consumption. Alcohol intake was grouped into general alcohol, beer and wine consumption. Multiple tendencies were found when considering an effect of different lifestyle factors on global T_2 relaxation times, as shown in figure 14.

Mean global T_2 relaxation values at baseline were non-significantly higher for smokers (33.5 ± 2.3 ms) than for non-smokers (33.9 ± 2.2 ms) if adjusted for other OA risk factors (P=0.162).

Burger consumption of <1/week, representing the intermediate of three groups, was associated with the lowest T_2 values (33.5 ± 2.2 ms; P=0.026), compared to none and high burger consumption (34.4 ± 3.6 ms and 34.2 ± 2.2 ms). Self reported alcohol consumption and wine consumption did not show significant differences, whilst beer consumption showed a tendency (P=0.060). Adjusting additionally for BMI, burger consumption was not significant any more (P=0.214), whilst beer consumption was even more significant (P=0.003).
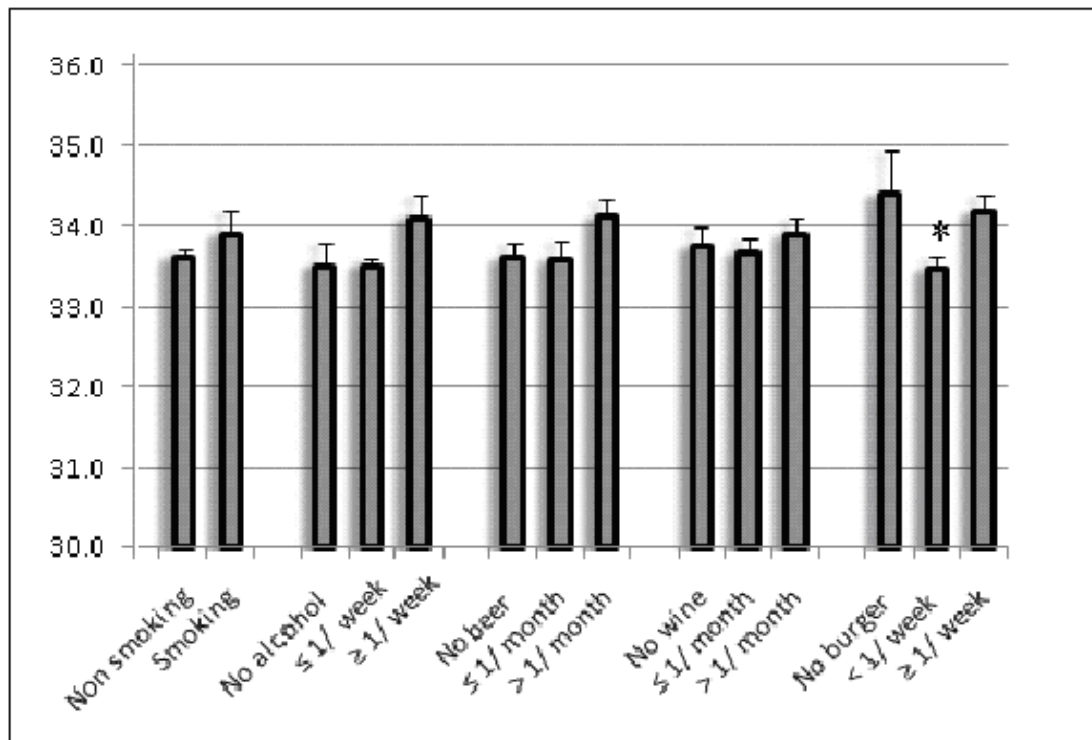
Fig. 14: Mean global T_2 values (ms; ±SD) for categorized parameters (*=P<0.05)
Before adjustment for OA risk factors, significant difference remained for
intermediate burger consumption (P=0.026).

6.3 T_2 progression analysis after 24 months

Two-year changes in T_2 relaxation values from baseline to the 24 months time-point
were evaluated (n=381). Most parameters showed a trend in the progression analysis,
but significance only after additional adjustment for baseline T_2 values. Mean (±SD)
longitudinal change of global T_2 in all subjects was $3,5 \pm 5,3\%$ (1.1 ± 1.8 ms).

An important influencing factor in the T_2 progression analysis was age. It correlated
significantly (P<0.002) with global T_2 progression, if additionally adjusted for
baseline T_2 values and OA risk factors. Considering every compartment separately,
the strongest influence of "age" was found in the tibial cartilage (P<0.001), PAT and
LFC were also significant (P=0.005), while no increased progression was found for
MFC (P=0.348). Gender did not have an influence on progression in various

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compartments or on global T_2 progression relaxation times (P=0.568).

The individual metabolic factors were not significantly associated with change in global T_2 values (P=0.130 to P=0.977). With increasing numbers of metabolic risk factors, a statistical trend for the association of global T_2 progression values was present (P<0.071 without and P=0.191 with adjustment for BMI). Though individuals with Metabolic Syndrome had slightly greater increase in global and especially in the medial tibia compartment mean T_2 values than the control cohort, none of these differences was significant after adjustment for OA risk factors and BMI.

Table 7: Mean T_2 baseline and progression (T_2 follow-up/ T_2 baseline) ± standard deviation for subjects with Metabolic Syndrome, defined by NCEP-ATP-III, are compared with subjects who did not fulfill the criteria. (*P<0.05; §P<0.1)

Baseline	T_2 No Metabolic Syndrome (n=377)	T_2 Metabolic Syndrome (n=26)	P
Global *	33.5 ±2.2	35.3 ±2.3	<0.001 *
PAT	32.4 ±3.3	33.5 ±3.8	0.200
MFC *	37.7 ±2.6	38.8 ±2.9	0.011 *
LFC §	34.7 ±2.3	35.5 ±1.9	0.075 §
MT *	31.8 ±3.1	35.4 ±4.6	<0.001 *
LT *	30.6 ±3.3	32.9 ±2.4	<0.001 *
Relative Progression			
Global	1.035 ±0.054	1.041 ±0.047	0.617
PAT	1.033 ±0.088	1.076 ±0.100	0.129
MFC	1.011 ±0.061	1.026 ±0.071	0.857
LFC	1.028 ±0.063	1.046 ±0.051	0.332
MT	1.029 ±0.079	1.060 ±0.093	0.024 *
LT	1.057 ±0.046	1.064 ±0.081	0.543

An increase of global longitudinal T_2 progression value was discovered with an increase of components defining the Metabolic Syndrome, however it did only show a statistical trend (P=0.071; Figure 15).

When taking a closer look at the individual components of Metabolic Syndrome, all T_2 values increased significantly over time. When all components were analyzed together in a multivariate regression model, only abdominal circumference was

significant for progression analysis (P=0.006). However, this significance was lost, when adjustment for other OA risk factors and BMI was performed. Thus there was no significant influence on longitudinal T_2 value progression in the multivariate regression model.
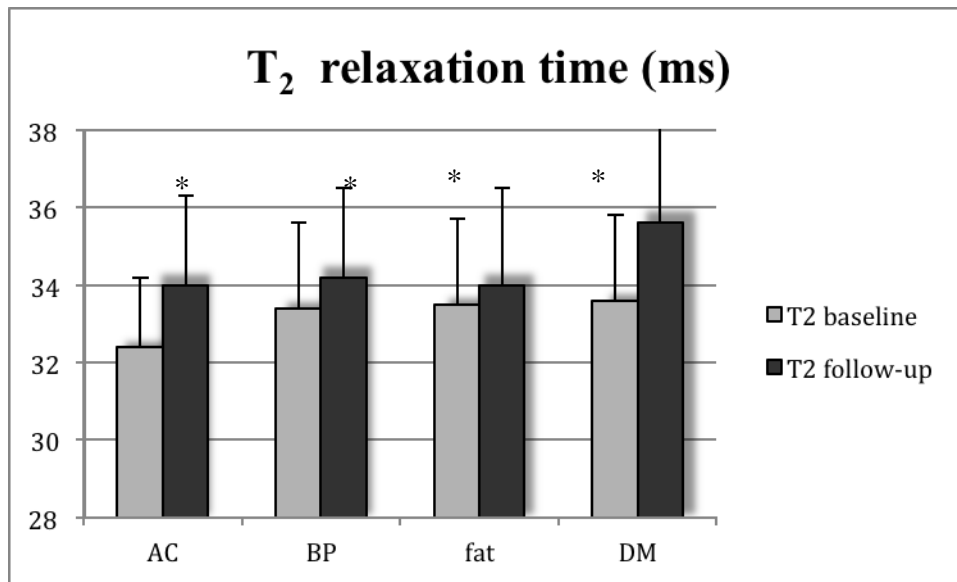

Fig. 15: T_2 relaxation time value ± Standard deviation at baseline (light grey) and two-year follow-up (dark grey) for patients with presence of individual Metabolic Syndrome components. High abdominal circumference (AC), high blood pressure (BP), high fat consumption (fat) and presence of diabetes melltius (DM) showed a significant increase over time (adjusted for age, gender and OA risk factors; *P<0.05). In the baseline multivariate regression analysis, AC and DM stayed significant, fat consumption showed a tendency (P<0.1), blood pressure was not significant.

A trend for an increased, longitudinal T_2 change was observed for individuals, who consumed beer (P= 0.067 with adjustment for BMI), while other lifestyle factors did not demonstrate significant, longitudinal T_2 value changes (P>0.05), especially when additionally adjusted for BMI (table 8).

Table 8: Mean T_2 values $\pm$ standard deviation of different groups regarding the self-reported lifestyle parameters. Smoking, alcohol, beer, wine and burger consumption were included. P-values are adjusted for other OA risk factors (P) and additionally for BMI (P adjusted for BMI) for T_2 baseline and progression analysis. Hereby high beer consumption showed significance (P*<0.05) at T_2 baseline and progression value.

Lifestyle factor Consumption of...	Mean T2 ± SD Group 1	Mean T2 ± SD Group 2	Mean T2 ± SD Group 3	P baseline	P baseline adj for BMI	P progression	P progression adj for BMI
Nicotine	No (n=360)	Smoking (n=43)	-				
	33.6 ±2.3	33.9 ±2.2	-	0.162	0.616	0.788	0.781
Alcohol	No alcohol (n=60)	≤ 1/ day (n=294)	>1/day (n=47)				
	33.4 ±2.1	33.5 ±2.4	34.1 ±1.9	0.272	0.058	0.082	0.076
Beer	No beer (n=128)	≤1/month (n=108)	>1/month (n=125)				
	33.6 ±2.4	33.6 ±2.3	34.1 ±2.1	0.067	0.003 *	0.002 *	0.004 *
Wine	No wine (n=80)	≤1/month (n=142)	>1/month (n=139)				
	33.7 ±2.2	32.7 ±2.4	33.9 ±2.3	0.446	0.248	0.330	0.291
Burger	No burger (n=19)	<1/week (n=206)	≥1/week (n=132)				
	34.4 ±3.6	33.5 ±2.2	34.2 ±2.2	0.026 *	0.214	0.318	0.162

BMI had a significant influence on longitudinal T_2 progression, if adjusted for OA risk factors and baseline T_2 values (P=0.032). If BMI was categorized in normal weight (BMI<25), overweight (BMI 25-30) and obese (BMI>30), P-value was smaller than 0.001. However, significance was lost if not adjustment for baseline T_2 value was performed (P>0.05).

7   DISCUSSION

This study demonstrated that middle-aged asymptomatic individuals with presence of components of Metabolic Syndrome had significantly higher baseline T_2 relaxation time values, indicating early degenerative cartilage changes. The more components of Metabolic Syndrome (including (i) high abdominal circumference, (ii) hypertension, (iii) high fat consumption and (iv) diabetes) were present, the higher T_2 values were detected at baseline. This suggests that individuals with an accumulation of metabolic risk factors have more severe cartilage degradation. All individual components had a significant influence on baseline T_2 values, if considered individually. The association between two-year progression of T_2 values with Metabolic Syndrome was not significant. T_2 progression increased with increasing amounts of components, showing a statistical trend. In addition, the life-style parameter "high beer consumption" correlated with high baseline T_2 values and showed a trend for T_2 progression.

Because preventive interventions and therapies may potentially prove more effective in preventing irreversible destruction of cartilage, this study focused on young and middle-age asymptomatic individuals without radiographic OA and without knee pain. To detect early pathological cartilage abnormalities, T_2 relaxation time mapping has shown promising use and was therefore included in the OAI magnetic resonance imaging protocol. It is sensitive to a wide range of water interactions in tissue and, eminently, depends on the content, anisotropy and orientation of collagen; particularly disorganization of the latter induces higher water mobility [88]. T_2 relaxation time can detect early stages of the disease, quantitatively assess disease severity and monitor its progression through cartilage hydration, orientation and integrity of the collagen network. This means that high T_2 relaxation time values are associated with increased severity of cartilage defects and at the same time can predict cartilage loss [46,77,7]. In contrast, $T_{1}\rho$ relaxation time is more sensitive to the proteoglycan content of cartilage. These quantitative MR techniques have been proven to allow for insight into the biochemistry and function of the cartilage [87]. Since sequences for T_2 measurements are multi-echo sequences, there is a need for stable calibration algorithms to be able to provide reproducible and precise data across different MRI scanners.

A number of studies have examined the influence of age on T_2 relaxation time and findings have shown an age dependency of cartilage T_2 maps [69,68]. Mosher et al indicated, that the diffuse increase in T_2 in senescent cartilage alters more in appearance than focally increased T_2 observed in damaged articular cartilage [69]. The authors concluded, through the location of T_2 elevations, that senescent changes of cartilage collagen begin near the articular surface and progress to the deeper cartilage with advancing age [69]. This coincides with the findings in the present study, where an age dependency of T_2 relaxation time was demonstrated. T_2 progression values were significantly increased if adjusted for baseline T_2 values. Patient´s age is commonly accepted as a risk factor for OA. This is reflected by increased T_2 values in our study and also explains a significant T_2 increase over two years.

In this study, gender neither influenced global baseline nor global follow up T_2 values, however for tibial compartments higher values in females were detected. In literature, gender was often used as an adjustment factor and gender-specific effect estimates were rarely reported. Mosher et al performed cartilage T_2 measurements in young healthy subjects and concluded that the magnitude and spatial dependency of cartilage T_2 does not differ with gender [69]. Additional studies with T_2 comparison of individuals in later life are required, since generally there is agreement, that females are more likely to develop knee problems than males [10].

There is mounting evidence that OA is not simply a disease related to aging or mechanical stress but rather a multisystemic disease in which interrelated metabolic disorders contribute to the initiation and progression of cartilage degradation. The fact, that OA is as common in non-weight-bearing joints, like interphalangeal hand joints, supports the association of OA being related to a systemic proinflammatory disease with several risk factors [32,18]. A number of studies have demonstrated a key role of neuropeptides in producing inflammatory cytokines, indicating that secreted inflammatory mediators influence the matrix homeostasis of articular tissue cells by altering their metabolism [63]. Previously, OA has been linked not only to obesity but also to other cardiovascular risk factors, diabetes, dyslipidemia, hypertension, and insulin resistance, characterizing the Metabolic Syndrome [94,78,97]. This is in agreement with our findings, since T_2 continuously increased

with accumulation of metabolic risk factors and demonstrates an increase of T_2 values at baseline and at progression in patients with Metabolic Syndrome.

Indeed, Metabolic Syndrome is characterized by a combination of various risk factors that additionally imply emergence as well as aggravation of OA and supplementary cardiovascular morbidity that is greater than the sum of the risks associated with each individual component [82]. Epidemiological and clinical studies have proven the coexistence of obesity and OA of the knee as well as of the hand, but also other numerous lifestyle factors, such as hypertension and diabetes, are frequently observed in OA patients [83,102,35]. In the 1960s early investigations reported a significant association of high cholesterol levels and hand OA [47]. Followed by Lawrence´s findings of correlations between knee osteoarthritis and hypertension: diastolic blood pressure [54]. Over the years several studies were dedicated to this subject also with contradictory findings regarding the correlation of metabolic risk factors and OA. Independent of obesity, prevalence of hypertension, hypercholesterolemia and high blood glucose in OA was found by Hart et al. Based on their findings, they suggested that OA has an important systemic and metabolic component in its etiology [39].

Additionally, we evaluated the accumulation of components of Metabolic Syndrome. Since evidentially T_2 values continuously increased with the amount of components of Metabolic Syndrome, a higher risk for OA with presence of more components can be assumed; confirming previously reported findings that utilized radiographic magnification [102]. Of note, the analyzed variables do not exclusively have to fulfill the strict criteria for Metabolic Syndrome and also each in their own right is more prevalent in a population with OA [78]. For example, a high abdominal circumference increases the risk for OA even if not passing the obesity threshold; or diabetes is likely to increase T_2 values independently of presence of obesity, although usually each accompanies the other.

In a model including all four metabolic risk factors, only abdominal circumference and diabetes remained significantly associated with elevated T_2. This might be due to the effect of each risk factor being attenuated when including all factors at once and at the same time a putative measure of a single concept is encountered by all factors. Also the expectancy to be interrelated, might explain some of the variation in other factors. For example, BMI and abdominal circumference are both measure of obesity and individually both highly correlated with T_2 values, but when considered in a

common model, neither remained significantly associated with T_2.

Although recent studies have shown, that abdominal obesity correlates higher with cardiovascular disease than BMI, BMI is still part of the rather old definition of the WHO for Metabolic Syndrome from 1999 [5]. In most Metabolic Syndrome definitions abdominal circumference is implied instead of BMI, which is why we chose abdominal circumference too. There is limited evidence on whether this remains accurate for OA [36]. Heavy bones can easily influence BMI, as can a muscular figure. Therefore we additionally assessed those two parameters in our study.

Both, BMI and abdominal circumference were found to influence T_2 values. T_2 progression was influenced if adjusted for baseline T_2 values. Even though abdominal circumference has shown to have a higher correlation with cardiovascular disease than BMI, this has not been equally confirmed for OA [36]. Replacing one another showed similar results and was strengthened by a high correlation of 0.87 of these two parameters. We confirmed, that besides age, increased BMI as well as abdominal circumference are the most influential known risk factors for early degenerative cartilage changes, representing precursors of radiographic OA [73]. J. Martel-Pelletier et al postulated that high plasma levels of leptin, encoded by an obese gene and mainly produced by adipocytes, have been found to be related to increased susceptibility to the development of OA [63]. Although leptin might be a contributing factor, a clear link between circulating leptin levels and OA has yet to be further investigated, since local leptin levels in the joint may be more important than circulating leptin for OA progression.

Self reported diabetes was identified to be one of the parameters, which had a significant effect on early degenerative changes but not on their progression. This may be due either to small numbers, or a relatively short follow-up interval of 2 years. An equally plausible explanation is that patients with diagnosed diabetes are treated to achieve normal blood glucose levels and therefore diabetes correlated risk for OA progression is reduced. Once glycemia was controlled, risks for cardiovascular complications, diabetic retinopathy or chronic kidney disease have shown to be reduced [64,53]. In 2007 Rojas-Rodriguez et al showed that systemic pathologic pro-inflammatory mechanisms in OA involve the association of insulin resistance [83].

But also matrix synthesis and mitotic activity controlling Insulin-like growth factors (IGF) are seen to be upregulated in osteoarthritic cartilage [63]. However, for a definitive conclusion, this and related parameters need further investigation, including examining a bigger sub-cohort.

Past studies have revealed mixed results on the subject of hypertension and knee OA, despite being studied since the early 1960s. An association of hypertension and knee OA, independently of body weight, was reported [39], but was not verified by other groups [25]. Our study revealed an influence of hypertension on T_2 relaxation time values, but it was attenuated after adjustment for abdominal circumference and risk factors.

Fat consumption was analyzed as a representative variable of dyslipidemia, since correlation with blood lipids is known [85]. Fat intake correlated with increased baseline cartilage degeneration, reflecting a consensus with dietary recommendations. Normalized blood lipids seem to have not only beneficial effects on cardiovascular diseases and overall mortality but also for prevention and worsening of OA [49]. Indeed, it has been demonstrated that fatty acid intake influences adipose tissue expression of leptin, which plays a key role in osteoarthritis by influencing the metabolism and promoting nitric oxide synthesis in chondrocytes [45]. Fat intake may therefore influence OA directly as well as indirectly, also through increased obesity.

In keeping with the recommendation that a moderate meat intake is healthy, our study revealed the lowest baseline T_2 value in subject with intermediate burger consumption (less than one burger consumption per week) [19]. An explanation of this result might be, that exogenous amino acids from meat consumption lead to the formation of inflammatory eicosanoids correlating, for example, with inflammatory severity in rheumatoid arthritis [1]. Both nutrition parameters (fat intake and burger consumption) did not effect OA progression, which might be due to the observation interval being too short.

Concurring effects of nicotine have been observed to date. It has a toxic effect on chondrocytes, delays chondrogenesis, induces degeneration and is correlated with a higher incidence of cartilage defects [24,29]. Although an increased cartilage volume

loss in a longitudinal two-year follow up study has been observed before [24], in our study there was no significant increase in T_2 values of smokers compared to non-smokers. In addition and by contrast, a positive influence of nicotine on glycosaminoglycan and collagen syntheses of chondrocytes has been observed previously [38]. This was not substantiated in our study given that, if it were present, it would decrease T_2 values in our biochemical analysis (the measurement quantifies cartilage collagen and water content).

Controlled alcohol consumption is said to have a positive effect on cardiovascular diseases and was therefore of particular interest. Higher alcohol consumption increased the two-year progression of cartilage deterioration by trend, for which mainly beer consumption was responsible. Wine consumption did not show a negative effect. Placing OA again in the context of a multifactorial disease, this observation remains true for cardiovascular disease: Red wine, containing antioxidants, has been reported to be especially cardioprotective [98].

Multiple risk factors have been linked to OA including age, female sex, obesity, previous injury, genetic elements and also sports activities. Stehling et al revealed that physically active individuals show significantly more cartilage and meniscal abnormalities and higher T_2 measurements [90]. Light and non weight- bearing exercise is associated with low T_2 values, whereas both moderate/strenuous exercise in women were associated with high T_2 values, as were frequent knee-benders [42]. There are several other risk factors that need to be considered and it is of great importance to take them and their influences into account when analyzing cartilage degeneration.

There are several limitations of this study that need to be considered. First of all, the study was observational and clinical trials yet need to determine if modifying risk factors can protect healthy cartilage and reduce the potential of OA.

Another important limitation was in our use of indicating parameters of daily fat consumption and glucose tolerance. We used imperfect proxies for these factors, relying on the patients declarations, for example earlier diagnosed diabetes, which to

be precise do not allow a definition of "Metabolic Syndrome". The focus of our study therefore also lay in the association of different individual risk factors rather then specifically concentrating on the Metabolic Syndrome. Then there are several definitions of the Metabolic Syndrome with differences in thresholds for each parameter. To give an example, in our study according to the IDF definition n=27 patients were found to suffer from Metabolic Syndrome, due to the slightly lower threshold for central obesity than the NCEP-ATP-III (abdominal circumference for men ≥ 94 cm or ≥ 80 cm for women). Also, this study did not directly compare cohorts of patients under treatment versus non-treated patients, nor analyzed if treatment reduces the risk of OA.

Third, a relative short follow-up interval of two years was used to evaluate time course of T_2 measurements and its progression, which may be too short.

Fourth, Metabolic Syndrome only showed a significant association with longitudinal T_2 progression in additionally adjusted baseline T_2 values. Since an inverse natural correlation of baseline T_2 values with T_2 progression was found, it is likely that missing significance may partially be explained by this statistical artifact and a possible ceiling effect.

Fifth, a limitation of our study was that we only used T_2 mapping for assessing biochemical composition of the articular knee cartilage. A detection of cartilage changes as well through promising sophisticated techniques, e.g. $T_1\rho$ and dGEMERIC, would have been of great value and may be useful to implicate in further studies. Stahl et al pointed out that $T_1\rho$ is well suited besides T_2 to differentiate healthy subjects and early OA changes and is even more sensitive than T_2 relaxation times. This needs further investigation [89].

Sixth, cartilage segmentation was done manually, which is a time-consuming process, taking up to one hour per knee. Therefore currently a major limitation of potential clinical application of T_2 quantification is the lack of a time-efficient and reliable segmentation. In future a more feasible technique, like a fully automated segmentation algorithm, will need to be developed helping to establish changes in

articular cartilage.

Finally, the comparison of baseline and 24 month follow-up T_2 measurements requires reliable and accurate MR imaging, which was obtained longitudinally with rigorous quality assurance of the OAI to allow high quality and reproducibility of cartilage T_2 measurements.

8   SUMMARY

In conclusion, the results of this study demonstrate that middle-aged asymptomatic individuals with risk factors for Metabolic Syndrome and other lifestyle factors have an elevated prevalence of high knee cartilage T_2 relaxation time values on MR images. We found that T_2 relaxation time at baseline was significantly increased in subjects representing the Metabolic Syndrome. There was a trend seen in higher elevated T_2 values after 24 months. All individual components, abdominal circumference, blood pressure, fat consumption and diabetes, showed increased T_2 values at baseline. The more components were present, the higher T_2 measurement was found, suggesting an abnormal biochemical composition of cartilage in these individuals.

Further investigations, along with continued longitudinal surveys are needed to provide evidence on how to prevent and influence the development of early osteoarthritis and reduce devastating economic costs of OA disability for the public and each individual. These results underline the need for imminent awareness and the importance of public health initiatives to address the growing amount of individuals suffering from Metabolic Syndrome in the modern Western society, in order to decrease the risk of early OA in young individuals.

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11 ACKNOWLEGEMENTS