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Biochemical Composition of the Cartilage Matrix in Patients with Type 2 Diabetes Mellitus – Longitudinal Evolution over 24 months in the OAI cohort

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To my mother, Margot

To my father, Albert

To my sister, Marie-Kristin

Introductory remarks

This dissertation is based on data from the Osteoarthritis Initiative (OAI), a longitudinal, prospective, observational and multicenter study of Osteoarthritis of the knee with study protocols available on http://www.oai.ucsf.edu. The OAI is a public-private partnership consisting of five contracts (N01-AR-2-2258; N01-AR-2-2259; N01-AR-2-2260; N01-AR-2-2261; N01-AR-2-2262). Hereby, the OAI is funded by the National Institutes of Health (NIH) as the public partner and GlaxoSmithKline, Merck Research Laboratories, Novartis Pharmaceuticals Corp., Pfizer, Inc. as the private partners.

The author of this dissertation performed the majority of compartment-based cartilage segmentations of knee magnetic resonance imaging scans using a proprietary spline-based algorithm written in MATLAB with the remaining cartilage segmentations being performed by Walid Ashmeik as described in more detail in Chapter 4.3.2. "Quantitative Image Analysis". The conception and design of the research study as a whole was performed by Jan Neumann, MD (TU Munich), the author of this dissertation (Felix Hofmann, TU Munich), Ursula Heilmeier, MD (UCSF), Gabby Joseph, PhD (UCSF), Michael Nevitt, PhD (UCSF), Nancy Lane, MD (UCSF), Charles McCulloch, PhD (UCSF) and Thomas Link, MD, PhD (UCSF). The study participant selection as well as the matching process was performed by Jan Neumann, MD and the author of this thesis (Felix Hofmann). The statistical analysis for the results of this dissertation was performed jointly by the author of this thesis (Felix Hofmann), Jan Neumann, MD and Gabby B. Joseph, PhD with the author of this dissertation (Felix Hofmann) using IBM SPSS Statistics Version 23 to perform the analysis of participants characteristics.

Figures 1, 2, 4, 5, 6, 7 and 8 were created by the author of this dissertation (Felix Hofmann) using Adobe Illustrator Version 24.3 and Microsoft PowerPoint for Mac Version 16.45.

The results that this dissertation is based on have been published in the following journal papers:

1. Neumann J, **Hofmann FC**, Heilmeier U, Ashmeik W, Tang K, Gersing AS, Schwaiger BJ, Nevitt MC, Joseph GB, Lane NE, McCulloch CE, Link TM. Type 2 diabetes patients have accelerated cartilage matrix degeneration compared to diabetes free controls: data from the Osteoarthritis Initiative. Osteoarthritis Cartilage. 2018 Jun;26(6):751-761. doi: 10.1016/j.joca.2018.03.010. Epub 2018 Mar 29. PMID: 29605381; PMCID: PMC5962437.
2. Chanchek N, Gersing AS, Schwaiger BJ, Nevitt MC, Neumann J, Joseph GB, Lane NE, Zarnowski J, **Hofmann FC**, Heilmeier U, McCulloch CE, Link TM. Association of diabetes mellitus and biochemical knee cartilage composition assessed by T_2 relaxation time measurements: Data from the osteoarthritis initiative. J Magn Reson Imaging. 2018 Feb;47(2):380-390. doi: 10.1002/jmri.25766. Epub 2017 May 26. PMID: 28556419; PMCID: PMC5702599.

Furthermore, the following conference paper has been published based on this thesis' results:

1. Neumann, J., Guimaraes, J. B., Heilmeier, U., Joseph, G. B., **Hofmann, F. C.**, Gersing, A. S., Schwaiger, B. J., Nevitt, M. C., McCulloch, C. E., Lane, N. E., Lynch, J. A., & Link, T. M. (2018). Diabetics show accelerated progression of cartilage and meniscal lesions: data from the osteoarthritis initiative. Osteoarthritis and Cartilage, 26, S226. https://doi.org/10.1016/j.joca.2018.02.475

An exhaustive overview of the publications that have been published with contribution of the author of this thesis within the scope of his doctoral research studies can be found in Chapter 11.

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Abbreviations:

3D = three-dimensional

ADAMTS = a disintegrin and metalloproteinase with thrombospondin motifs

AGE = advanced glycation end products

BMEP = bone marrow edema pattern

BMI = body mass index

CI = confidence interval

COR = coronal

CT = computed tomography

CV = coefficient of variation

dGEMRIC = delayed Gadolinium-Enhanced MR Imaging of Cartilage

DESS = dual-echo in steady state

DM = diabetes mellitus

ECM = extracellular matrix

FOV = field of view

FS = fat suppression

FSE = fast spin-echo

GAG = glycosaminoglycan

gagCEST = GAG Chemical Exchange Saturation Transfer

GBD = Global Burden of Disease

Gd-DTPA2 = gadopentate

GLCM = grey-level co-occurrence matrix

GLUT-1 = glucose transporter 1

IL-1α = Interleukin 1 alpha

IL-1β = Interleukin 1 beta

IL-6 = Interleukin 6

IW = intermediate-weighted

JSW = joint space width

KL = Kellgren-Lawrence grading

LF = lateral femur

LT = lateral tibia

ME = multi-echo

MESE = multi-echo spin-echo

MF = medial femur

MMP = metalloproteinase

MR = magnetic resonance

MRI = magnetic resonance imaging

MT = medial tibia

NCCAM = National Center for Complementary and Alternative Medicine

NCMHD = National Center on Minority Health and Health Disparities

NIA = National Institute on Aging

NIAMS = National Institute of Arthritis and Musculoskeletal and Skin Diseases

NIBIB = National Institute of Biomedical Imaging and Bioengineering

NIDCR = National Institute of Dental and Craniofacial Research

NIH = National Institutes for Health

NMSI = Normalized mean signal intensities

NO = nitric oxide

NSAID = non-steroidal anti inflammatory drugs

OA = osteoarthritis

OAI = the Osteoarthritis Initiative

OARSI = Osteoarthritis Research Society International

OR = odds ratio

ORWH = Office of Research on Women's Health

PAT = patella

PG = proteoglycan

RAGE = receptor for AGEs

RKI = Robert Koch Institute

ROS = reactive oxygen species

SAG = sagittal

SD = standard deviation

SE = spin-echo

SI = signal intensity

T = Tesla

T_1 = T_1 relaxation time

T_2 = T_2 relaxation time

TNF-α = Tumor Necrosis Factor alpha

UCSF = University of California San Francisco

US = United States

WHO = World Health Organization

WORMS = Whole-Organ Magnetic resonance imaging Score

YLD = years lived with disability

1 Introduction

Both diabetes mellitus (DM) and osteoarthritis (OA) are chronic conditions with an increasing prevalence that are exerting an enormous global burden on humanity (CDC, 2020a; Dagogo-Jack, 2017; Palazzo et al., 2016). An increase in the years lived with disability (YLD) that amounted 30.1% for DM and 31.4% for OA in the period between 2007 and 2017 was investigated in the Global Burden of Disease Study from the year 2017 (James et al., 2018). Projected further increases in the prevalence of both diseases promote their coexistence in individual patients and make it inevitable to assess the ways in which they potentially interact (King & Rosenthal, 2015).

Osteoarthritis is a chronic, articular disease which is characterized by the deterioration of a joint's hyaline cartilage and the subsequent sclerosis and hypertrophy of the bone as well as inflammation of the synovia (Courties & Sellam, 2016). In developed countries, OA is regarded as one of the 10 most disabling diseases, and an estimation of the World Health Organization (WHO) assumes that almost 1 in 10 men (9.6%) and 1 in 5 women (18.0%) above the age of 60 suffer from symptomatic OA (WHO, 2020a). As one of the most prevalent joint disorders globally, the occurrence of OA may affect any synovial joint, but it is more often observed in the knees, hips, hands and the lower spine region due to their continual stress (Cross et al., 2014; Hunter & Felson, 2006; WHO, 2020a). Osteoarthritis of the weight bearing hips and knees is considered to lead to the highest burden to the population since loss of function in these major joints frequently results in a high grade of disability that requires surgery (Litwic et al., 2013). Clinical manifestations of OA as well as morphologic, molecular and biomechanical alterations differ depending on several risk factors (Hofmann et al., 2018; Sharma et al., 2006; Wise et al., 2012). On the one hand, a range of local risk factors for OA have been identified that include amongst others obesity, high levels of physical activity, severe injuries to the structures of a joint and previous knee surgery (Gelber et al., 2000; Lohmander et al., 2004; Palazzo et al., 2016; Smith et al., 2017; Christoph Stehling et al., 2010). On the other hand, OA has been found to be associated with systemic factors including increasing age, female gender, a heritable, polygenic component and DM

(type 2) (Courties & Sellam, 2016; T. W. O'Neill et al., 2018; Panoutsopoulou & Zeggini, 2013; Spector & MacGregor, 2004; Valdes & Spector, 2011).

Diabetes mellitus is a chronic, metabolic disease that occurs when either the pancreas does not provide enough insulin due to autoimmune destruction (type 1) or the cellular usage of insulin is impaired (type 2) (WHO, 2020b). The resulting disruption of insulin metabolism leads to hyperglycemia, which can cause a condition of chronic systemic inflammation affecting various organs including joints (Alenazi et al., 2020; Atayde et al., 2012). Diabetes mellitus affected approximately 10.5% of the United States (US) population in 2018, making it one of the most common systemic diseases in the Western world with a continuously increasing prevalence (CDC, 2020a; Thareja et al., 2012). Similar to OA, DM poses an enormous economic burden, with the total estimated cost of diagnosed DM reaching $237 billion in the U.S. for the year 2017 having increased by 26% compared with the year 2012 (American Diabetes Association, 2018). Out of all diagnosed cases of DM, DM type 2 represents the vast majority, namely 91.2% compared to 5.6% for DM type 1 with the remainder consisting of other subtypes of DM (Xu et al., 2018).

While previous studies have shed light on the association between OA and DM, identifying DM as an independent risk factor of OA (Courties & Sellam, 2016; Louati et al., 2015; M. F. Williams et al., 2016), the pathophysiological connection between DM and OA has yet to be unraveled in its entirety (Neumann et al., 2018). The metabolic changes that come with DM have been shown to make human, hyaline cartilage softer with lower stiffness indices, more permeable as well as cause non-healing microfractures that potentially disrupt bone mechanics and to result in a promotion of OA (Athanasiou et al., 1999; Janghorbani et al., 2007; King & Rosenthal, 2015; Patsch et al., 2013). In addition to that, hyperglycemia indirectly boosts musculoskeletal pathologies by both impairing tendon homeostasis as well as altering the collagen synthesis and subtype composition of ligaments (Atayde et al., 2012; de Oliveira et al., 2011; Majjad et al., 2018; Nichols et al., 2020; Y.-F. Wu et al., 2017).
While there have been animal studies that demonstrated remodeling of collagen types as well as a decrease in proteoglycans in the cartilage of diabetic rats (Atayde et al.,

2012), investigating the impact of diabetes on human hyaline cartilage proves to be more difficult. One study using a human cartilage specimen obtained from an autopsy was used to demonstrate increased levels of advanced glycation end products (AGEs) in the cartilage of diabetic patients (Steenvoorden et al., 2006).

While radiography allows for the non-invasive visualization of cortical and trabecular bone, magnetic resonance imaging (MRI) is unparalleled in the in vivo evaluation of joint tissues such as cartilage, ligaments and menisci (Peterfy et al., 2004). Recently, a study successfully investigated biochemical changes in tendons and cartilage in subjects suffering from DM using quantitative in vivo 7 Tesla (T) sodium MRI (Marik et al., 2016). Additionally, noninvasive MRI has also been employed to semi-quantitatively assess the knee using a multi-feature scoring method, the whole-organ MRI scoring (WORMS), that includes evaluation of cartilage, ligaments, menisci, osteophytes, synovitis, subarticular cysts and bone marrow edema pattern (Peterfy et al., 2004; C. Stehling et al., 2011). Another sophisticated, noninvasive, and well-established way to examine especially cartilage matrix through the analysis of collagen integrity, proteoglycan content and the level of hydratation is prestructural/compositional magnetic resonance (MR)-based cartilage imaging using for example measurements of T2 relaxation time (Link et al., 2017). In addition to plain T2 relaxation time measurements, grey-level co-occurrence matrix (GLCM) texture analysis has proven useful for detecting early changes in the composition of the cartilage matrix and the spatial distribution of the T2 values of cartilage using statistical, second-order texture parameters (Haralick et al., 1973; A. Williams et al., 2017). Additional studies have validated the qualification of T2 measurements and GLCM texture parameters as surrogate markers for both cartilage hydration and molecular collagen structure with elevated and more heterogeneous T2 values in subjects with risk factors for OA or with a pre-morphological stage of OA (Blumenkrantz et al., 2004; Joseph et al., 2011). Patients that do not yet have OA but are developing OA also show altered T2 relaxation times, which underlines the value of this technique as a biomarker for early and developing OA (Liebl et al., 2015, p. 2; Prasad et al., 2013).

In summary, the pathophysiological connection between OA and DM on the molecular level within the hyaline cartilage has not yet been sufficiently investigated in living, human subjects. Magnetic resonance-based, compositional/prestructural imaging represents a technique that may establish a deeper understanding of a potentially impaired cartilage tissue in subjects suffering from DM type 2.

2 Specific Aims of this Study

The purpose of the present study is to explore the longitudinal impact of DM on the degeneration of cartilage over a period of 24 months. To investigate the connection between DM and the composition of hyaline cartilage matrix, prestructural/compositional cartilage imaging is employed.

The predictor variable is the presence of self-reported DM/high blood sugar. Longitudinal changes in measurements of T2 relaxation time over 24 months (also containing laminar analysis and texture parameters) serve as outcome measures. The required, underlying imaging data was acquired using 3.0 Tesla MRI.

Specifically, this study aims to investigate the longitudinal changes in the composition of the knee joint's hyaline cartilage in subjects suffering from DM using measurements of T2 relaxation time as well as T2 GLCM texture parameters and the subsequent comparison of the detected changes with an age-, body mass index (BMI)-, and sex-matched control cohort of subjects not suffering from DM.

3 Background

3.1 Definition of Osteoarthritis

Osteoarthritis constitutes a heterogeneous disease (Kraus et al., 2015). It is considered the most frequent subtype of arthritis (the "wear and tear" form of arthritis), occurs most often in the hips, knees, hands and the lower spine region due to their increased stress and is characterized by the slow destruction of a joint's cartilage with symptoms like stiffness, swelling and pain (CDC, 2020b).

The Osteoarthritis Research Society International (OARSI) describes OA as a disorder that affects various tissues within the joint ranging from cartilage becoming less elastic over bone spurs that grow around the edge of the joint, to breakdown of tendons and ligaments (OARSI, 2016b). The progressive deterioration of the joint's hyaline cartilage may result in inflammation of the synovia and hypertrophy and sclerosis of the bone (Courties & Sellam, 2016).

3.2 Epidemiology of Osteoarthritis

While estimates suggest that OA affects 240 million people globally and over 32.5 million people in the U.S., the prevalence of knee OA increases with age across all global regions (CDC, 2020b; Cross et al., 2014; WHO, 2020a). The Robert Koch Institute (RKI) published a report that showed the self-reported prevalence OA amounting 21.8% for women and 13.9% for men in adults above the age of 18 years with the anticipation of further increases in prevalence (Fuchs et al., 2017). However, prevalence estimates vary because structural changes do not have to cause symptoms and mild radiographic changes are not consistently included or excluded (Lawrence et al., 2008).

The 2017 update of the Global Burden of Disease (GBD) study showed that in the year 2017 OA accounted for over 9 million YLDs making it the third largest contributor to YLDs due to musculoskeletal disorders (with the two largest being back and neck pain) (James et al., 2018). The same study reported an increase of this burden measured in YLDs amounting 31.4% (95% confidence interval 30.7 to 32.1) in comparison to the year 2007 (Kloppenburg & Berenbaum, 2020). The direct costs of OA refer to the health-

related expenditure including amongst others, conservative treatment, pharmaceuticals, hospital stays and surgery to treat OA and it is estimated that in the US over $185 billion (of which $118 accounted for women) in annual insurer expenditures can be attributed to the care for patients suffering from OA (Hunter et al., 2014; Kotlarz et al., 2009). In Germany, public health insurers paid €7 billion for the treatment of patients suffering from OA in the year 2017 ("Highlights vom deutschen Orthopädie- und Unfallchirurgie-Kongress," 2007). While only one tenth of the direct costs of OA arise from pharmaceutical treatment, the major direct, financial burden stems from hospitals and associated orthopedic surgery (Hunter et al., 2014; Le Pen et al., 2005; Leardini et al., 2004; Loza et al., 2009). Interestingly, researchers in France determined that while hospital admissions accounted for 50% of direct costs, they only appeared in 3% of patients. Furthermore, OA has been found to be associated with losses of productivity, leading to indirect costs reaching approximately 0.4% of gross domestic product due to premature exit from work (Laires et al., 2018).

Regarding OA of the knee, the global prevalence of radiographically confirmed cases was estimated to be 3.8% with a higher percentage for female subjects (mean of 4.8%) compared to male subjects (mean of 2.8%) and a peak of prevalence seen around the age of around 50 years (Cross et al., 2014). In the year 2009, almost 905,000 knee replacements costing $42.3 billion were performed in the US (Murphy & Helmick, 2012).

3.3 Etiology of Osteoarthritis

As with other chronic diseases, the pathogenesis of OA is multifactorial. The disease is supposedly best understood as the result of exceeding mechanical stress occurring in subjects with systemic or local susceptibility (Hunter, 2009). Osteoarthritis may occur when the delicate balance between disruption and regeneration of a joint's tissues is lost (Eyre, 2004). Obesity/increased BMI, previous knee injury, the presence of Heberden's nodes and hand OA, female gender, older age, intensive physical activity, and a genetic susceptibility are recognized risk factors for the onset or progression of knee OA (Blagojevic et al., 2010; Christoph Stehling et al., 2010; Valdes & Spector, 2011).

It is generally agreed upon to split risk factors into either local/mechanical factors on the one hand and systemic factors on the other hand, even though some risk factors

like obesity (which affects local, mechanical loading as well as systemic inflammation) fit in both categories (O'Neill et al., 2018).

Local risk factors for OA encompass meniscal degeneration, joint malalignment, physical activity, severe injuries to the structures of a joint, and previous knee surgery (Gelber et al., 2000; Hunter et al., 2009; Lohmander et al., 2004; O'Neill et al., 2018; Smith et al., 2017; Christoph Stehling et al., 2010). Additionally, OA has been found to be associated with systemic factors including ethnicity, increasing age, female gender, nutrition, smoking, DM and a heritable component of polygenic nature (Courties & Sellam, 2016; O'Neill et al., 2018; Panoutsopoulou & Zeggini, 2013; Spector & MacGregor, 2004; Valdes & Spector, 2011).

The genetic influence on OA is substantial and is transmitted in a non-Mendelian mode, as it is typically seen in multifactorial diseases and is estimated to contribute between 40%-65% depending on joint location, gender, and grade of tissue destruction (Loughlin, 2005; Panoutsopoulou & Zeggini, 2013; Valdes & Spector, 2011). Regarding knee OA, one study estimated that, for women with radiographically confirmed OA, the influence of the genetic component is over 40% (Spector & MacGregor, 2004). Specifically, around 30 loci associated with OA of the knee or hip have been detected, but these only help explain approximately 25% of the overall heritability component of the disease (Gonzalez & Valdes, 2018). It is hypothesized that genetics have an influence on other risk factors of OA like structure and turnover of both bone and cartilage, synovitis skeletal shape and obesity, but these assumptions require to be further investigated (Spector & MacGregor, 2004; Valdes & Spector, 2011).

In spite of the genetic influence on obesity, it is hypothesized that obesity and altered joint mechanics are the two modifiable risk factors accounting for the largest share of disorder development and progression (Gushue et al., 2005; Hunter, 2009). The effect of obesity, which is the most important, modifiable risk factor for the development of severe OA, has long been attributed to mainly gait and altered biomechanical loading of the joint (Coggon et al., 2001; DeVita & Hortobágyi, 2003; D. T. Felson & Zhang, 1998; S. P. Messier, 1994; Stephen P. Messier et al., 2005). However, there is evidence that the presence of systemic inflammation in obese patients also contributes to the progressive degradation of joint cartilage (Das, 2001). Such an inflammatory component would help explain why obese individuals are also at high risk for OA in

the hand, which cannot be explained by mere increased mechanical stress as seen in weightbearing joints (Felson & Chaisson, 1997). Overweight subjects show elevated serum levels of C-reactive protein, which has been explained by the increased release of Interleukin 6 (IL-6) by adipose tissue (Engström et al., 2009; Visser, 1999). Similar to the effects of being overweight, the additional, independent risk factor DM has been discussed regarding its negative impact on joints via an induction of oxidative stress and the promotion of proinflammatory cytokines (Courties & Sellam, 2016). The role of DM in the etiopathogenesis of OA is further discussed in the corresponding Subsection 3.8.

3.4 Pathophysiology of Osteoarthritis

Osteoarthritis has to be seen as a disease of the whole joint rather than only a cartilage disease (Courties & Sellam, 2016). The trajectory of disease of OA is characterized by the progressive degeneration and subsequent loss of the cartilage of the affected synovial joint but also involves functional alterations of the subchondral bone, synovial membrane, menisci, and ligaments (Buckwalter & Mankin, 1998a). The trajectory of disease involves micro- and macro-injury resulting in cell stress and extracellular matrix degradation (OARSI, 2016a). One way to understand the progression of the disease once the deterioration of cartilage has begun is to divide it into three general stages (Martel-Pelletier et al., 2004): The proteolytic destruction of cartilage matrix (first stage) is followed by the fibrillation and erosion of the surface of the cartilage (second stage), which finally results in synovial inflammation due to the production of proinflammatory cytokines and proteases, which are themselves produced in reaction to the ingestion of breakdown products by synovial cells (Martel-Pelletier, 2004). As is discussed further in the following subsection on biochemistry and histology (3.5), the eventual damage of cartilage on a molecular level is caused by an elaborate system of proteinases (Iannone & Lapadula, 2003).

While for a long time OA was considered a mainly mechanical, degenerative “wear and tear” disease, it is now well established that it involves a considerable systemic, inflammatory component (Courties & Sellam, 2016, p. 2). Said inflammation combined with structural changes of the joint may lead to pain, stiffness, and impaired function,

which can considerably limit an individual's ability to participate in regular activities of the daily living and cause functional disability of the knee (Cross et al., 2014).

Besides the damage to articular cartilage, OA leads to the remodeling of subarticular bone, osteophyte formation, ligamentous laxity, the weakening of periarticular muscle, joint space narrowing, and sometimes synovial inflammation (Litwic et al., 2013). While it is symptomatic OA that causes suffering, including pain and disability as well as economically expensive usage of healthcare resources, there are many patients that exhibit the required radiographic features to be diagnosed with OA without any symptoms (Oliveria et al., 1995).

3.5 Histology and Biochemistry of Hyaline Cartilage.

Hyaline cartilage is a thin layer of connective tissue with the power to withstand high cyclic loads while experiencing little degenerative wear and tear and serves as a lubricated surface that enables a joint's frictionless articulation (Buckwalter, 1998; Fox et al., 2009; Mankin, 1982). It is a hypocellular, avascular, aneural, alymphatic tissue that only contains a single type of differentiated cell, namely chondrocytes, which have the function of producing and keeping up the extracellular matrix (ECM) (Fox et al., 2009; Palukuru et al., 2014). Both functionally and structurally the layered macrostructure of cartilage can be split into the four zones (1) calcified layer, (2) deep zone, (3) middle zone and (4) superficial zone as displayed in Figure 1.

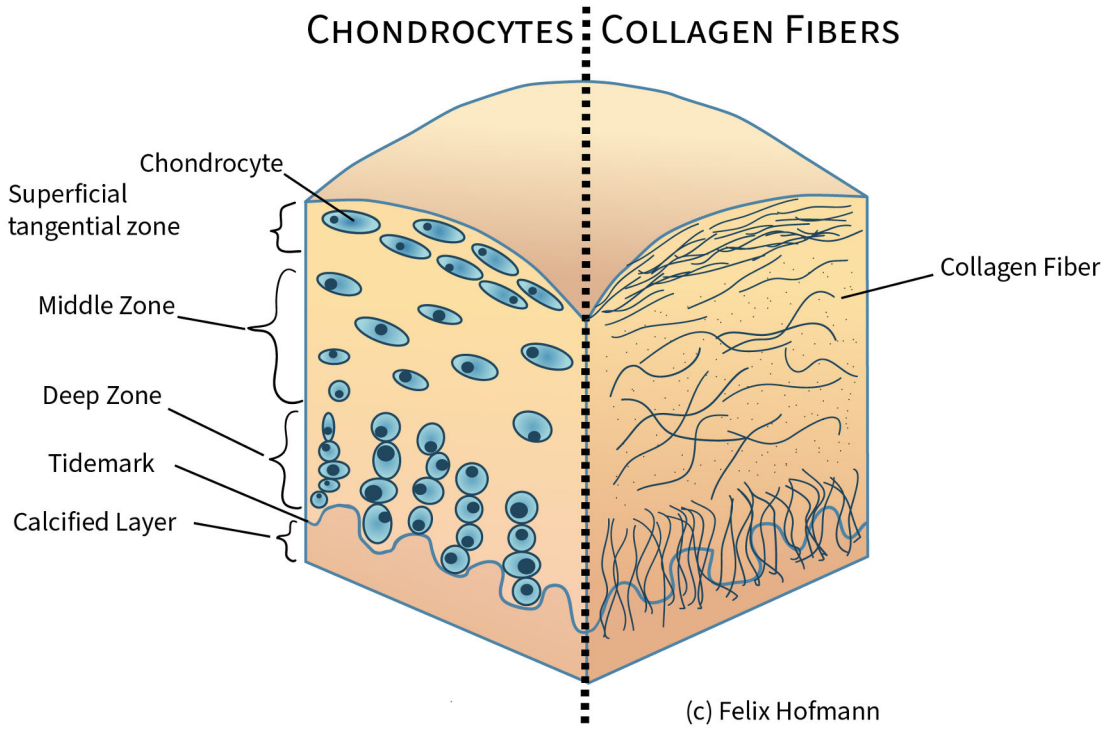

Figure 1: Schematic diagram of the zonal architecture of healthy cartilage

Within the superficial zone, which makes up roughly 10%-20% of the cartilage, collagen fibers are oriented parallelly to the surface, flattened chondrocytes are stretched horizontally, and the proteoglycan fraction is low (Flik et al., 2007). Compared to the superficial zone, the middle (or transitional) zone which makes up roughly 40%-60% of the cartilage, contains diagonally organized collagen fibers with a higher diameter and fewer chondrocytes of rounder shape as well as more proteoglycans (Fox et al., 2009). The following deep zone harbors again thicker collagen fibers with a vertical orientation in relation to the surface and displays the lowest amount of water and highest concentration of proteoglycans (Flik et al., 2007). The subsequent "tidemark" indicates the transition from the deep zone to the calcified layer (Cohen et al., 1998).

Chondrocytes construct the macromolecular framework of the ECM using the three molecular substances proteoglycan, collagen, and non-collagenic proteins (Buckwalter & Mankin, 1998b). Hyaline cartilage's wet weight is made up of 10% chondrocytes, 60%-85% water and electrolytes, 3%-10% proteoglycans (PG) and 10%-30% of collagen

type II fibrils (Cohen et al., 1998). Regarding the dry weight of adult, articular cartilage, collagen makes up two thirds with the cartilage's robustness heavily depending on the remarkably cross-linked architecture of the collagen network in addition to its fibrillar organization (Eyre, 2004). The collagen type in hyaline cartilage which accounts for over 90% of articular collagen, is type II, even though types VI, IX, X, and XI can be detected as well (Buckwalter & Mankin, 1998b). Proteoglycans, the other major molecular component of articular cartilage, consist of one or more linear glycosaminoglycan (GAG) chains (which can be composed of over 100 monosaccharides) covalently connected to a protein core (Fox et al., 2009). GAGs split into chondroitin sulfate and keratin sulfate in a ratio that has been estimated to be 2:1 (Hardingham, 1981). A range of different proteoglycans are found in articular cartilage, with the largest by individual size and most substantial measured by overall weight being aggrecan (Fox et al., 2009). Proteoglycans are often organized as large proteoglycan aggregates made of numerous aggrecan molecules that in turn stick to a hyaluronic acid backbone connected via link proteins (H. Muir, 1983; Palukuru et al., 2014). Figure 2 depicts the organization of the ECM with proteoglycans being embedded in a robust network of collagen fibers.

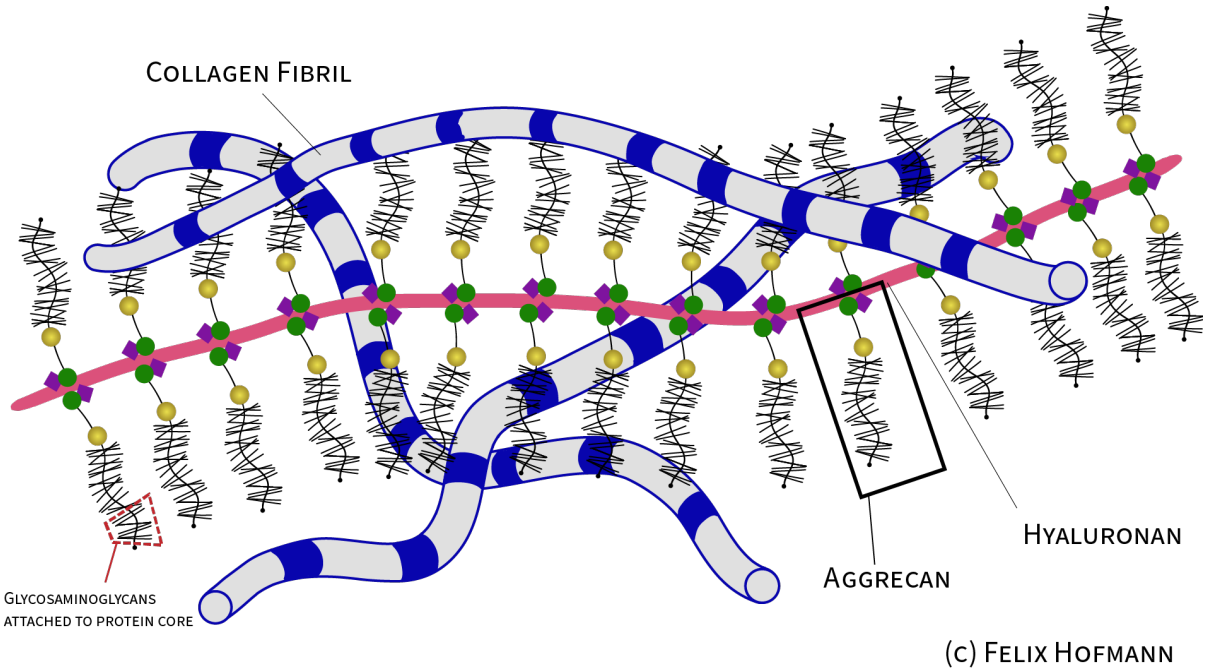

Figure 2 Extracellular maxtrix of hyaline cartilage. Proteoglycans form large aggregates sticking to a hyaluronic backbone embedded in a network of collagen fibrils.

A solid matrix build by proteoglycan tied in collagen contains water, which is the most voluminous component of hyaline cartilage (Flik et al., 2007). Proteoglycans, however, do not necessarily have to form large proteoglycan aggregates. Aggrecan and its siblings from the group of large-sized proteoglycans (e.g. versican, agrin, perlecan) as well as the small-sized proteoglycans (e.g. syndecan, glypican, decorin, biglycan) can fulfill their function of binding water and absorbing mechanical loads either linked to a hyaluronic backbone or non-attached (Grässel & Aszódi, 2017a, p. 1). While collagen provides tensile strength due to its robust, cross-linked architecture, aggrecan-hyaluronate complexes offer enormous compression strength as well as elasticity due to their high water binding capacity (Haubeck, 2019).

Regardless of whether biochemical overload or inflammatory stimulus leads to the onset of cartilage destruction, both pathways involve the crucial role of pro-inflammatory cytokine secretion which in turn leads to an increased production and activity of destructive proteinases (Berenbaum, 2013; Grässel & Aszódi, 2017a; Houard et al., 2013). Specifically, the stress-related activation of chondrocytes and synovial cells induces a complex system of proteinases produced by both cell types (Iannone & Lapadula, 2003). The proteolytic proteinases involved in cartilage degeneration include mainly metalloproteinases (MMPs), a disintegrin and metalloproteinase with

thrombospondin motifs (ADAMTS) and cathepsins (Flik et al., 2007). Especially the MMP and ADAMTS families take the leading role in cartilage degradation, wherefore present thesis will concentrate on these two agents (Grässel & Aszódi, 2017a). In cartilage of healthy individuals a low pace of reorganization of the ECM is achieved by a strictly regulated ADAMTS and MMP enzyme production which can be disrupted by the aforementioned inflammatory mediators, especially tumor necrosis factor alpha (TNF-α), interleukin (IL) 1- α and IL-1β (Goldring et al., 2011; Goldring & Otero, 2011; Grässel & Aszódi, 2017a, p. 2). Degradation of type II collagen fibers is performed by a subgroup of MMPs which are called collagenases with collagenase-3 (MMP-13) being the most efficient (Eyre, 2004). The enzymes involved in the degradation of aggrecan, the aggrecanases, are mainly MMP-3, ADAMTS-4 and ADAMTS-5 (Houard et al., 2013). Some research suggests that degraded aggrecan can be replaced much better than degraded collagen type II, which indicates that cartilage can tolerate only a limited amount of collagen type II breakup before being irreversibly damaged (Grässel & Aszódi, 2017a; Stoop et al., 1999).

3.6 Clinical and Radiological Assessment of Osteoarthritis

3.6.1 Clinical symptoms

There are various ways in which OA can clinically manifest itself. Primarily, symptoms include joint pain, swelling of the joint, stiffness of the joint, impaired movement and weakness of muscles (Gui-Xing, 2010). Other than in rheumatoid arthritis, pain in OA is inclined to get worse with activity, which is called “gelling phenomenon” (Sinusas, 2012). Generally, the degree of radiographic OA has been found to only weakly correlate with pain severity, which has been hypothesized to be a result of limited radiological variables studied (Sanghi et al., 2011; Shimura et al., 2013) . However, compared to other diseases like kidney disease or liver disease that clinically manifest themselves rather late, even minor cartilage losses have been shown to correlate with pain worsening, which in principle suggests a potentially low threshold to clinical illness (Kraus et al., 2015; Wluka et al., 2004). While in the early stages of OA a dull, mild pain appearing at intervals that gets better with rest is typical, the advanced stages of OA may be associated with continuous pain even occurring at night (Gui-Xing, 2010). The stiffness is often describes as occurring after periods of rest or

temporarily in the morning (Hunter & Felson, 2006). The swelling occurring in subjects suffering from OA is a clinical symptom resulting from synovitis, inflammation and associated effusion (Berenbaum, 2013). While there are numerous patients that suffer from radiographically diagnosed OA, primarily symptomatic OA inflicts pain on patients and leads to individual disability and costs for healthcare systems (Oliveria et al., 1995). Nevertheless, the presence of joint symptoms can have multiple potential causes and therefore have to be treated with caution as they do not definitely prove OA when there is a lack of structural, anatomical alterations (Kraus et al., 2015).

3.6.2 Radiographs

Plain radiography remains an integral component in the diagnosis of OA. Generally, asymmetrical joint space narrowing (JSN), bone cysts, sclerosis of subchondral bone and osteophytes are amongst the typical findings in radiographs of subjects suffering from OA (Gui-Xing, 2010). The Kellgren-Lawrence (KL) scale represents a common, standardized metric of OA grade and is well established in both clinical decision-making and research for the assessment especially of radiographic knee OA (Emrani et al., 2008; Kohn et al., 2016). Regarding the knee, AP radiographs are used to determine a grade between 0 and 4 with 0 equaling no proof of OA and 4 corresponding to severe OA as shown in Figure 3 (Kellgren & Lawrence, 1957).

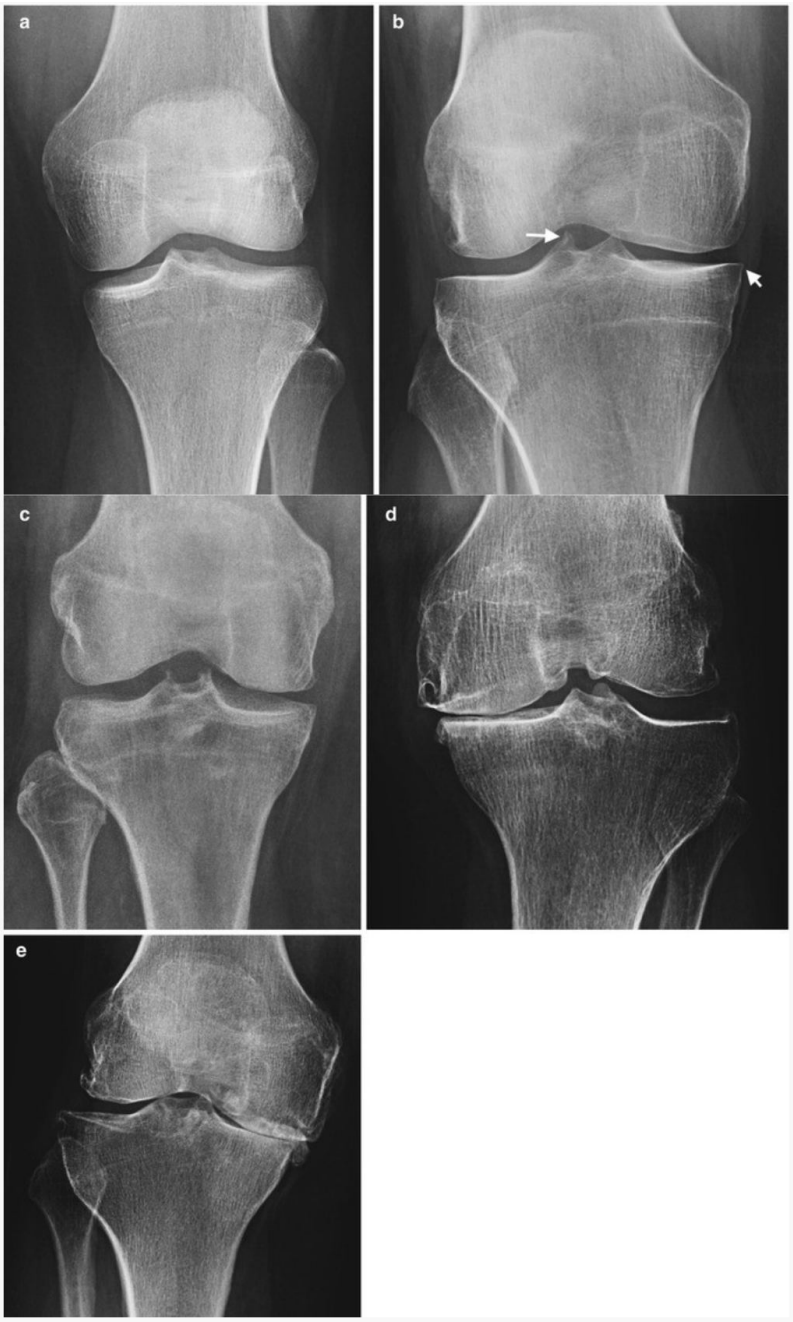

Figure 3 KL scoring in AP radiographs of the knee. "a" depicts a normal left knee radiograph with grade 0. "b" demonstrates a grade 1 knee radiograph. "c" shows a grade 2 knee, "d" is a KL grade 3 and "e" is a KL grade 4. Figure adapted from O'neill et al. with permission from Springer Nature (Cividino & O'Neill, 2015).

The radiologic features of osteoarthritis in the original publication comprised: (1) "narrowing of joint cartilage associated with sclerosis of subchondral bone", (2) "formation of osteophytes on the joint margins or, in the case of the knee joint, on the tibial spines", (3) periarticular ossicles, (4) "small pseudocystic areas with sclerotic walls situated usually in the subchondral bone" and (5) "altered shape of the bone ends, particularly in the head of the femur" (Kellgren & Lawrence, 1957). Certainly, the

KL system has its limitations, however, KL grading fulfills a clear, cheap, quick and thus important purpose within the initial assessment of patients that present themselves with clinical symptoms consistent with OA (Kohn et al., 2016).

3.6.3 MRI

In the last two decades MRI has shown an incredible pace of improvement that allows highly precise imaging of joint tissues and especially cartilage with volumetric evaluation, high-resolution three-dimensional (3D) isotropic sequences, semiquantitative MR-based scores, cartilage sensitive sequences and sophisticated cartilage-sensitive sequences (Gahunia et al., 2020). In contrast to both conventional radiography and computed tomography (CT), MRI offers superior contrast of soft tissue and allows better assessment of subchondral cancellous bone (Fox et al., 2009). Great anatomic detail as well as contrast between subchondral bone and cartilage is achieved with T1-weighted spin echo sequences (Hayes et al., 1990; Karvonen et al., 1990; Pilch et al., 1994).

3.6.4 Quantitative compositional MRI of cartilage

While quantitative MRI-based measurement of cartilage morphology like cartilage volume, surface area and thickness constitute valuable surrogate markers for structural disease (progression) in patients with OA, quantitative compositional MRI techniques aim to detect alterations of the ECM of cartilage tissue before damage has occurred that cannot be repaired (Eckstein & Wirth, 2010; Link et al., 2017). To achieve this aim, quantitative MRI techniques have to accurately analyze the components of articular cartilage recapped (from subsection 3.5) in Figure 4 and assess their respective content and integrity.

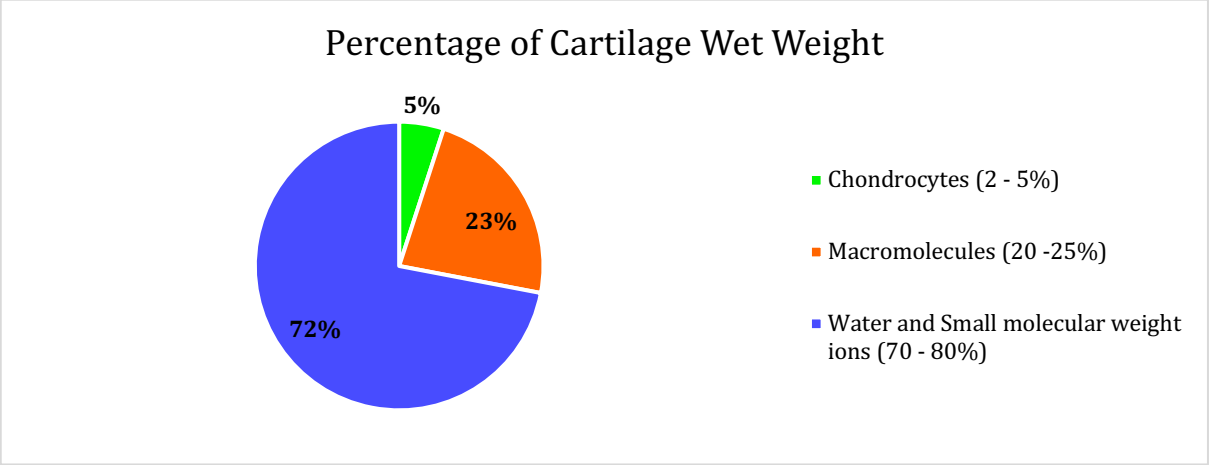

Figure 4 Components of articular, hyaline cartilage wet weight with the ECM accounting for 95-98% (encompassing 70-80% Water, 20-25% macromolecules and small molecular ions) and chondrocytes accounting for 2-5%. Figure adapted from (Gahunia et al., 2020)

Generally, quantitative compositional cartilage imaging techniques have been found to be more promising than morphological sequences when assessing injuries of acute nature (Klocke et al., 2013). The quantitative MRI techniques that have been employed to analyze cartilage composition are predominantly T2, T1rho and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), wherefore this subsection will concentrate on these techniques (Burstein et al., 2009).

T2 mapping

As an MR relaxation time that reflects interactions between water and local macromolecules as well as between water molecules and other water molecules, decreased T2 values depict increased levels of said interactions (Burstein et al., 2009). Primarily collagen content and hydration of cartilage has been found to correlate well with T2 mapping (Taylor et al., 2009). Furthermore, T2 is able to detect alterations in the extremely organized anisotropic arrangement of fibrils of collagen in the ECM (Mosher et al., 2001). Since the mobility of water within an extremely anisotropic cartilage ECM is restricted, comparatively low T2 values in the context of a hydrated tissue can be measured in healthy cartilage, namely 15-60 ms (Mosher & Dardzinski, 2004). Contrarily, elevated cartilage tissue hydration is affected by early-stage degenerative alterations in ECM due to an increase in water mobility as well as increase of water content by osmosis (Taylor et al., 2009). T2 maps of cartilage affected from osteoarthritic deterioration have been shown to be more heterogeneous

as well as frequently exhibit higher T2 values as a result of an elevated hydration (Burstein et al., 2009). While T2 values are strongly influenced by content of collagen and anisotropy of collagen fibrils, T2 mapping is insensitive to changes in PG loss, unlike T1rho and dGEMRIC (Mosher & Dardzinski, 2004).

T1rho

T1rho quantifies longitudinal relaxation in the rotating frame and is able to record interactions of extremely low frequency qualifying it as a marker for the exchange of spin-lattice energy between large molecules and water (Taylor et al., 2009). In the case of cartilage, T1rho as a measurement of relaxation is capable of investigating the exchange rate between water associated to macromolecules of the ECM of cartilage and free water protons and presents increased relaxation times if there is a disruption in ECM (mainly PG) (Duvvuri et al., 1997; Regatte et al., 2004). T1rho values have been demonstrated to be elevated in subjects suffering from OA and are particularly well suited to detect changes in PG content of cartilage (Akella et al., 2001; Wang & Regatte, 2014). T1rho has been of interest as an early marker for loss of PG, since PGs are an important component of cartilage due to their contribution to physical resilience and mechanical integrity of hyaline cartilage (compare subsection 3.5) (Taylor et al., 2009).

dGEMRIC

This technique is based on the intravenous injection of gadolinium-based contrast agent which is negatively charged and can be used as a marker indicating PG loss (Klocke et al., 2013; Williams et al., 2004). Such negatively charged contrast agents, like gadopentate (Gd-DTPA2) are capable of diffusing into hyaline cartilage and then being repulsed by equally negatively fixed density caused by PGs in the ECM (Burstein et al., 2001; Taylor et al., 2009). In other words, dGEMRIC is capable of measuring the fixed charge density caused by GAG chains of PG (Gray et al., 2008). Due to its sensitivity for PG content, dGEMRIC technique has been considered suited for the detection of early OA-related changes in cartilage (Taylor et al., 2009).

3.6.5 Semiquantitative cartilage assessment using WORMS

The whole-organ MRI scoring (WORMS) allows a semi-quantitative assessment of the knee's tissues including cartilage, ligaments, menisci, osteophytes, synovitis,

subarticular cysts and bone marrow edema pattern (BMEP)(Peterfy et al., 2004; C. Stehling et al., 2011). WORMS scoring has previously been validated as a tool to investigate existence and progression of pathological, morphologic alterations of knee tissues (Heilmeier et al., 2019b).

3.7 Treatment of Osteoarthritis

Currently no approved, effective pharmacotherapies are available to treat OA. Therefore, once irreversible damage to a joint has occurred, OA treatment focuses on the improvement of joint function, reduction of pain and surgical treatment as the maximum level of therapy escalation (Gui-Xing, 2010). Generally, treatment options for OA are split into the categories non-pharmacological, pharmacological, complementary, alternative or surgical (Sinusas, 2012). Ideally, treatment of OA starts with non-pharmacological options and involves programs to educate patients about exercises, postures and other beneficial, behavioural modifications, orthopaedic devices to modify unhealthy weight-bearing distribution and physiotherapy (Gui-Xing, 2010). As for the pharmacological treatment, both in hip and knee OA oral and topical Non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, tramadol as well as local corticosteroid injections are recommended based on the WHO pain ladder with a preference for topical NSAIDs in patients above 75 years of age (Taruc-Uy & Lynch, 2013; Vergne-Salle, 2016). Among the interventional approaches within the options to treat OA are cartilage repair treatments. These include (i) arthroscopic debridement which aims to accomplish relief of pain by removing mechanically disturbing tissue, inflamed tissue and dead tissue, (ii) tissue-engineered cartilage as a method to replace dysfunctional/destroyed cartilage tissue with an engineered tissue or (iii) transplantation of osteochondral grafts for defects affecting the full thickness of cartilage larger than 2 square centimeters (Grässel & Aszódi, 2017b, p. 3). Additionally, osteotomy has shown functional improvements, reduction in pain as well as a potential delay of total joint replacement of up to 5-10 years (Naudi et al., 1999). The final, most invasive escalation option of surgical treatment of OA is total joint replacement that proves most promising if not waited until preoperative, functional status of patients suffering from terminal OA has excessively declined (Hunter & Felson, 2006).

3.8 Type 2 Diabetes Mellitus in the context of Osteoarthritis

3.8.1 Definition

DM is a collective term for a heterogeneous group of metabolic disorders that are commonly characterized by a disruption of the insulin metabolism with elevated levels of blood sugar (Petersmann et al., 2020). DM mostly occurs when either the secretion of insulin by Beta-cells of the pancreas is impaired due to autoimmune destruction (type 1) or tissues with natural insulin sensitivity do not respond appropriately to the hormonal stimulus of insulin (type 2) (Galicia-Garcia et al., 2020; WHO, 2020b). The chronic subtype DM type 2 accounts for more than 90% of cases of DM and will be the focus of present work (Stumvoll et al., 2005). There is an association between chronic hyperglycemia and deterioration and dysfunction of numerous organs, especially kidneys, nerves, eyes and vessels (Association, 2014). Furthermore, hyperglycemia involves a chronic state of systemic inflammation that may also affect the tissues of joints (Alenazi et al., 2020; Atayde et al., 2012). Hyperglycemia can lead to a broad range of symptoms that encompass amongst others susceptibility to infections, polydipsia, polyuria and impaired vision (Association, 2014). The diagnostic criteria for DM are: either (1) a fasting plasma glucose value over 125 mg/dL or (2) a random plasma glucose value over 199 mg/dL or (3) 2h oral glucose tolerance test value over 199mg/dL in venous plasma or (4) a level of glycated hemoglobin over 6.4% (Petersmann et al., 2018).

3.8.2 Epidemiology

Being classified as one of the four priority noncommunicable diseases, it is estimated that globally DM affected approximately 422 million adults in 2014 (Petersmann et al., 2018). While affecting around 10.5% of the US population in 2018, a study published by the Robert Koch institute in the year 2016 estimates that 7.2% of Germans between 18 and 79 years were diagnosed with diabetes making it one of the most common systemic diseases in the western world (CDC, 2020a; Petersmann et al., 2018). In industrialized countries, the continuing trend of a rapid increase in prevalence of DM

remains unchanged (Thareja et al., 2012). In the period from 2012 to 2017 the economic costs of diabetes in the US have been estimated to have risen by 26% to over $237 bn with the annual health care expenditure being elevated by the factor 2.3 for diabetic subjects compared to healthy controls for the year 2017 (American Diabetes Association, 2018). The global economic burden of DM is projected to substantially rise by the year 2030 to at least $1.94 trillion annually (Bommer et al., 2018). Two meta-analyses have shown that type 2 diabetic subjects have a higher risk of suffering from OA than healthy controls with odds ratios (OR) ranging between 1.25 (95% confidence interval [CI] : 1.05 to 1.46) and 1.46 (95% CI: 1.21 to 1.65) (Louati et al., 2015; M. F. Williams et al., 2016).

3.8.3 Etiology and Pathophysiology of Type 2 Diabetes Mellitus

As with other non-communicable, chronic diseases the pathogenesis of DM is multifactorial. An intricate mix of metabolic and genetic factors as well as an environmental component are involved as risk factors for the onset and progression of DM (Galicia-Garcia et al., 2020). The specific risk factors include amongst others smoking, dietary factors like high intake of processed meat, sugar-sweetened beverages, physical inactivity and increased Body Mass Index (BMI) with many associations (like BMI, weight gain, waist circumference) being proxies of obesity (Bellou et al., 2018). Particularly visceral obesity measured by excessive amounts of intra-abdominal, visceral fat has been strongly associated with a decrease in peripheral insulin sensitivity (Gastaldelli et al., 2002). The loss of insulin sensitivity of peripheral cells in DM type 2 patients leads to relatively elevated levels of required insulin production. As the disease progresses, the metabolism of type 2 DM patients increasingly fails to provide the required levels of insulin production, which is leading to a disrupted glucose homeostasis (Galicia-Garcia et al., 2020). In adipose tissue, insulin resistance can lead to a restriction of glucose intake as well as limited suppression of lipolysis in adipocytes (Czech, 2020). Disproportionately enlarged adipocytes and generally elevated levels of adipose tissue mass are associated with hypoxia, fibroses, pathologic vascularization and an inflammation mediated by macrophages (Scherer, 2019). The inflamed and dysfunctional adipose tissue may lead to elevated levels of proinflammatory cytokines including IL-1Beta, IL-6 and TNF and thereby contribute to a chronic condition of system inflammation which is then called

"metabolic inflammation" (Galicia-Garcia et al., 2020; Roden & Shulman, 2019). Another highly relevant tissue affected by insulin resistance is skeletal muscle, where physiologically glycogen synthesis is stimulated by the uptake of glucose from blood plasma (Kahn & Flier, 2000). Skeletal muscle inflammation as a consequence of immune cell infiltration and production of proinflammatory molecules of the adipose tissue surrounding myocytes in obese subjects contributes to insulin resistance mediated by paracrine effects (Galicia-Garcia et al., 2020; H. Wu & Ballantyne, 2017). Furthermore, the liver constitutes an organ highly susceptible to insulin resistance. In a state of insulin resistance present in diabetic subjects, the liver is unable to detect the severe hyperglycaemia and therefore maintains a harmful level of lipolysis fueled by the intake of circulating fatty acids (Mohamed et al., 2016). These fatty acids accumulate and disturb Beta-oxidation in the mitochondria of the liver resulting in additional accumulation of fat in the liver (Roden & Shulman, 2019). The numerous other organ systems affected by DM go beyond the scope of present thesis.

3.8.4 Pathophysiological Link between Type 2 Diabetes and Osteoarthritis

While the exact (combination of) pathophysiological mechanisms are not fully understood at this point, it is well accepted that DM type 2 acts as a risk factor for the deterioration of cartilage (Bellou et al., 2018; Chanchek et al., 2018; Courties & Sellam, 2016; Eymard et al., 2015; Schett et al., 2013). A range of pathophysiological pathways will be considered in this section and especially in the discussion of the results of present thesis. All these pathophysiological pathways have in common that they involve systemic and local toxicity (Francis Berenbaum, 2012). Similar to the effect in numerous other organ systems, chronically elevated levels of blood sugar cause excessive production of pro-inflammatory cytokines as well as oxidative stress in the tissues of the whole joint (Courties & Sellam, 2016). In vitro studies suggest that high extracellular concentration of glucose could impair type II collagen synthesis by decreasing dyhdroascorbate transport into chondrocytes as well as increase the production of reactive oxygen species (ROS) which have been found to have a harmful impact on cartilage (Berenbaum, 2011; Henrotin et al., 2003; McNulty et al., 2005). An additional hypothesis that could contribute to the connection between OA and DM is an increased level of advanced glycation end products (AGE) in diabetic subjects

(Steenvoorden et al., 2006). Generally, human articular cartilage naturally accumulates AGEs with increasing age as the result of nonenzymatic glycation which involves the reaction of sugars with lysine and arginine residues in proteins (Nicole Verzijl et al., 2002). To this date, well-characterized AGEs that have been shown to increasingly accumulate in articular cartilage related with age include pentosidine, N-carboxy-methyllysine and N-carboxyethyllysine (Bank et al., 1998; N. Verzijl et al., 2000). Elevated levels of AGEs are associated with an impaired synthesis of ECM by chondrocytes as well as an increase in cartilage stiffness (Bank et al., 1998; DeGroot et al., 1999; Nicole Verzijl et al., 2002). However, it is not only age that may cause harmful levels of AGEs. An accumulation of AGEs has been observed in subjects suffering from DM as well, as AGE accumulation is accelerated by hyperglycemia (McCance et al., 1993; Steenvoorden et al., 2006). Furthermore, the diabetic neuropathy with its impairment of peripheral nerves could be another risk factor of OA by leading to muscle weakness and joint laxity (Berenbaum, 2012). Finally, one study found that the metabolic stress induced by hyperglycemia can disrupt mechanical properties of articular cartilage making it more permeable and softer than cartilage of healthy individuals (Athanasiou et al., 1999; Hardin et al., 2015). This contradicts the aforementioned observed increase in stiffness of articular cartilage under hyperglycemic stress and will be debated further in the discussion.

4 Material and Methods

4.1 OAI – Role and Database

This present study used data from the OAI. Launched in 2002, the OAI represents a longitudinal, prospective observational and multicenter study of OA of the knee (Lester, 2012). The database and study protocols are accessible via http://www.oai.ucsf.edu. Specifically, the OAI has gathered data from four recruitment centers across the US (Stehling et al., 2010): Memorial Hospital of Rhode Island, Pawtucket, RI; Ohio State University Columbus, OH; University of Maryland, School of Medicine, Baltimore, MD; University of Pittsburgh, Pittsburgh, PA (Kretzschmar et al., 2016). Almost 5,000 subjects with manifest early-stage OA or risk factor for the development of OA were enrolled in order to gather imaging data, biological specimen and clinical data for a period of 8 years (Lester, 2012). The ethnically diverse cohort of 4796 men and women aged 45-79 years enrolled in the OAI study protocol received annual MR imaging and radiographic examination of their knees as well as clinical checkups (Neumann et al., 2018; Peterfy et al., 2008). The purpose of the OAI is to promote the understanding of how non-modifiable as well as modifiable risk factors are associated with onset and worsening of OA in order to develop and improve strategies for diagnosis and treatment but also prevention of OA (Lester, 2012). The OAI is a public-private partnership sponsored by pharmaceutical companies and the US National Institutes of Health (NIH) with the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Center for Complementary and Alternative Medicine (NCCAM), National Institute on Aging (NIA), Office of Research on Women's Health (ORWH), National Center on Minority Health and Health Disparities (NCMHD), National Institute of Dental and Craniofacial Research (NIDCR), National Institute of Biomedical Imaging and Bioengineering (NIBIB) on the other hand (Peterfy et al., 2008). In summary, the OAI has evolved into a public resource of OA domain research for the scientific investigation of biomarkers as potential surrogate endpoints for development and worsening of OA (Felson & Nevitt, 2004). Informed consent forms were signed by all study participants ahead of enrollment.

4.2 Subject Selection

Figure 5 offers an illustration of our subject selection process as a whole. From the initial set of 4796 subjects included in the OAI study, 1104 individuals were excluded because of a KL grading higher than 2. Excluding individuals with a higher KL grade warranted an adequate presence of cartilage tissue for quantitative image (Jungmann et al., 2013). The resulting 3692 subjects were corrected for 206 OAI participants suffering from inflammatory arthropathies including but not limited to rheumatoid arthritis, which in turn led to 3486 remaining subjects. The final criterion to shape the pool of 3438 potential subjects to be included in our study was the availability of exhaustive T2 sequences at baseline.

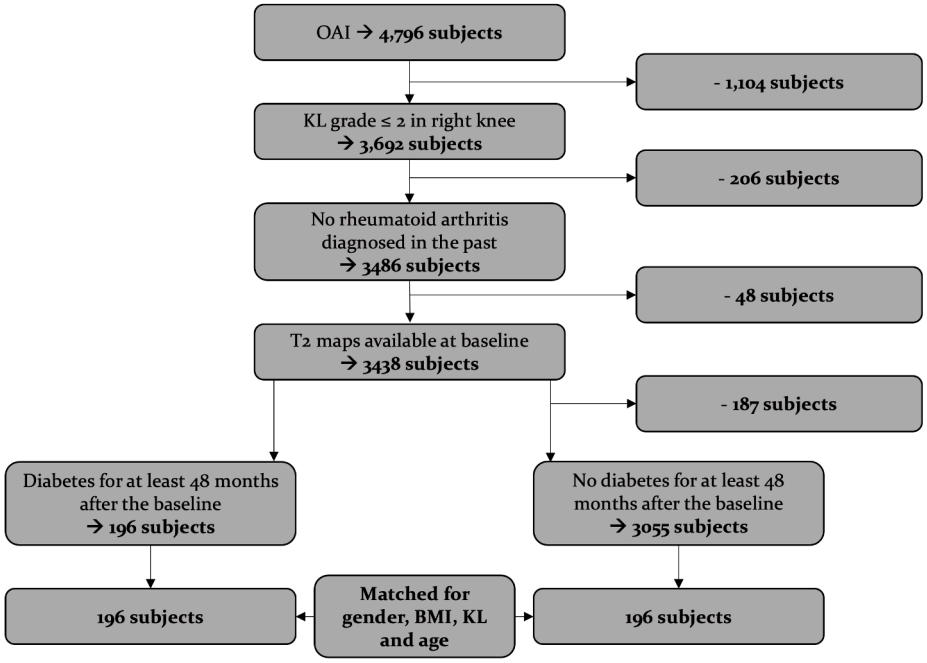

Figure 5 Subject selection from the OAI

The case subjects emerged from mentioned 3438 subjects by identifying the 196 subjects having a history of self-reported DM for at least 48 months after baseline. The status of diabetic disorder was determined with the help of a self-administered questionnaire included in the OAI study protocol. Similarly, the control cohort was corrected for individuals starting to suffer from DM within 48 months after enrollment. Thus, the corresponding control subjects were chosen from the 3055 out of 3438 subjects that did not have or develop diabetes for at least 48 months after

baseline. Specifically, the 196 subjects suffering from DM were matched in small sets for age, sex, BMI and KL grade to obtain 392 participants for this study. Participants of the OAI without documentation of all three demographic characteristics age, BMI and sex were not selected for this present study. The matching process created minor sets of 2 to 8 participants with matching categories created from the combination of subgroups of the matching variables age, gender, BMI and KL as shown in table 2.

Subgroup number	Subgroups for age	Subgroups for gender	Subgroups for BMI (in kg/m^2)	Subgroups for KL
1	45 to 49	female	17.5 to 19.9	0 to 1
2	50 to 54	male	20.0 to 22.4	2
3	55 to 59		22.5 to 24.9	
4	60 to 64		25.0 to 27.4	
5	65 to 69		27.5 to 29.9	
6	70 to 74		30.0 to 32.4	
7	75 to 79		32.5 to 34.9	
8			35.0 to 37.4	
9			37.5 to 39.9	
10			40.0 to 42.4	
11			42.5 to 44.9	
12			>45	

Table 1 Subgroups for the creation of categories for the 102 matching sets

In order to create 102 sets in total, the diabetic patients were arranged according to these subgroups displayed in table 2. For example a 52-year-old female with a KL of 1 and a BMI of 26 would be put into a set with following combination of subgroups: age = subgroup 2, gender = subgroup 1, BMI = subgroup 4 and KL = subgroup 1. Over the time period of interest, 31 healthy controls and 40 case subjects were lacking required data at the 2-year-follow up and therefore did drop out. Additionally, the quality of the

imaging data of 6 control subjects and 5 diabetic subjects was extremely impaired at the follow-up date, which led to an exclusion of these data points in the statistical analysis. In summary, imaging data of 159 controls and 151 diabetic subjects could be included in the analysis at the 2-year-follow-up.

Diabetes-related complications

The clinical checkups that are part of the OAI study scope include a questionnaire that not only includes self-reported information on DM status, but also complications related to DM. Table 2 offers a summary of the self-reported status of diabetic retinopathy and diabetic nephropathy of the 392 study subjects at baseline and the 310 study subjects at time-point of follow-up.

Diabetes-related complications	**Baseline (n=196)**	**24-months (n=151)**
Diabetic retinopathy	18 (9.2%)	14 (9.3%)
Diabetic nephropathy	5 (2.6%)	5 (3.3%)
Diabetic retinopathy and nephropathy	4 (2.0%)	2 (1.3%)
Absence of diabetic complications	169 (86.2%)	130 (86.1%)

Table 2 Diabetes-related complications

4.3 MR Imaging

4.3.1 MR Imaging protocol

This study was rendered possible owing to the large-scale MRI scans that constitute an important part of the OAI study protocol. In order to ensure reproducibility, the MRI scans of the included OAI subjects were exhaustively carried out using four identical 3.0T scanners at the four study sites (Siemens Magnetom Trio; Siemens Healthcare, Erlangen, Germany). Recapitulating from subsection 4.2, said 3.0 T scanners were based at the four OAI clinical sites University of Pittsburgh, Pittsburgh, Pennsylvania; Brown University, Pawtucket, Rhode Island / Memorial Hospital of Rhode Island; University of Maryland, Baltimore, Maryland; Ohio State University, Columbus, Ohio

(Kretzschmar et al., 2016). These scanners were equipped with quadrature transmit-receive coils manufactured by USA Instruments, Aurora, OH, USA.

T2 relaxation time measurements

The sequence employed for the measurements of T2 relaxation time was a 2D multi-echo spin-echo (MESE) sequence in a sagittal (SAG) image plane with specifications as displayed in table 3 adapted from Peterfy et al.: (Peterfy et al., 2008).

Parameter	**SAG 2D MESE**
Plane	Sagittal
Fat Suppression (FS)	No
Matrix (phase)	269
Matrix (frequency)	384
No. of slices	21
Field of View (FOV) (mm)	120
Slice thickness/gap (mm/mm)	3/0.5
Flip angle (°)	n/a
Echo time (TE)/repetition time (TR) (ms/ms)	10, 20, 30, 40, 50, 60, 70/2700 (7 echo times in total)
Bandwidth (Hz/pixel)	250
Chemical shift (pixels)	1.8
No. excitations averaged	1
ETL	1
Phase encode axis	A/P
Distance factor (%)	16
Phase oversampling	0
Slice oversampling	0
Phase resolution	70
Phase partial Fourier (8/8 = 1)	0.875
Readout partial fourier (8/8 = 1)	1
Slice partial Fourier (8/8 = 1)	0.75
X-resolution (mm)	0.313
Y-resolution (mm)	0.446

Table 3. Sequence parameters of the SAG 2D MESE sequence employed for T2 relaxation time measurements as published in (Peterfy et al., 2008)

According to table 3, the T2 relaxation time measurements were carried out employing the SAG 2D MESE sequence with a FOV of 120mm, an in-plane spatial resolution of 0.313x0.446mm², a TR of 2700ms, a FOV of 120mm, slices with a thickness of 3mm (with 0.5mm gap) and an overall seven echo times (see table 3) (Peterfy et al., 2008).

WORMS assessment

The morphologic sequences from the OAI protocol employed to assess knee pathologies semi-quantitatively are the SAG 2D intermediate-weighted FS fast spin-echo (FSE) (TE/TR 30/3200 ms), a SAG 3D dual-echo in steady state (DESS) that selectively excites water (TE/TR 4.7/16.3 ms, flip angle 25°) as well as a coronal (COR) IW 2D FSE sequence (TE/T2 29/3700 ms) (Heilmeier et al., 2019a).

4.3.2 Quantitative Image Analysis

For the quantitative image analysis, compartment-specific cartilage T2 measurements of both diabetic and non-diabetic study subjects were used based on mentioned SAG 2D MESE images. Computed on a pixel-by-pixel base with a three parameter fit that accounts for noise, these SAG 2D MESE images were used to generate T2 maps using a fitting function based on a monoexponential decay model (Heilmeier et al., 2019a; Marquardt, 1963). The mono-exponential decay model for the analysis of the T2 values is designed as a fitting function for the signal intensity using 6 echoes (TE 20-70 ms) subsequent to the exclusion of the first echo with the aim of minimizing errors and improving signal-to-noise ratio (Marquardt, 1963; Raya et al., 2010). For each compartment the T2 parameters were calculated and for the knee joint as a whole the mean T2 was computed by the use of all compartments. The step of segmentation was performed in order to be able to measure compartment-specific T2 times. The five compartments that the cartilage was segmented into were the following: medial tibia (MT), medial femur (MF), lateral tibia (LT), lateral femur (LF) and patella (PAT) as displayed in figure 6. The cartilage compartment of the femoral trochlea was not included in the analysis due to extreme artifacts caused by popliteal artery flow (Neumann et al., 2018).

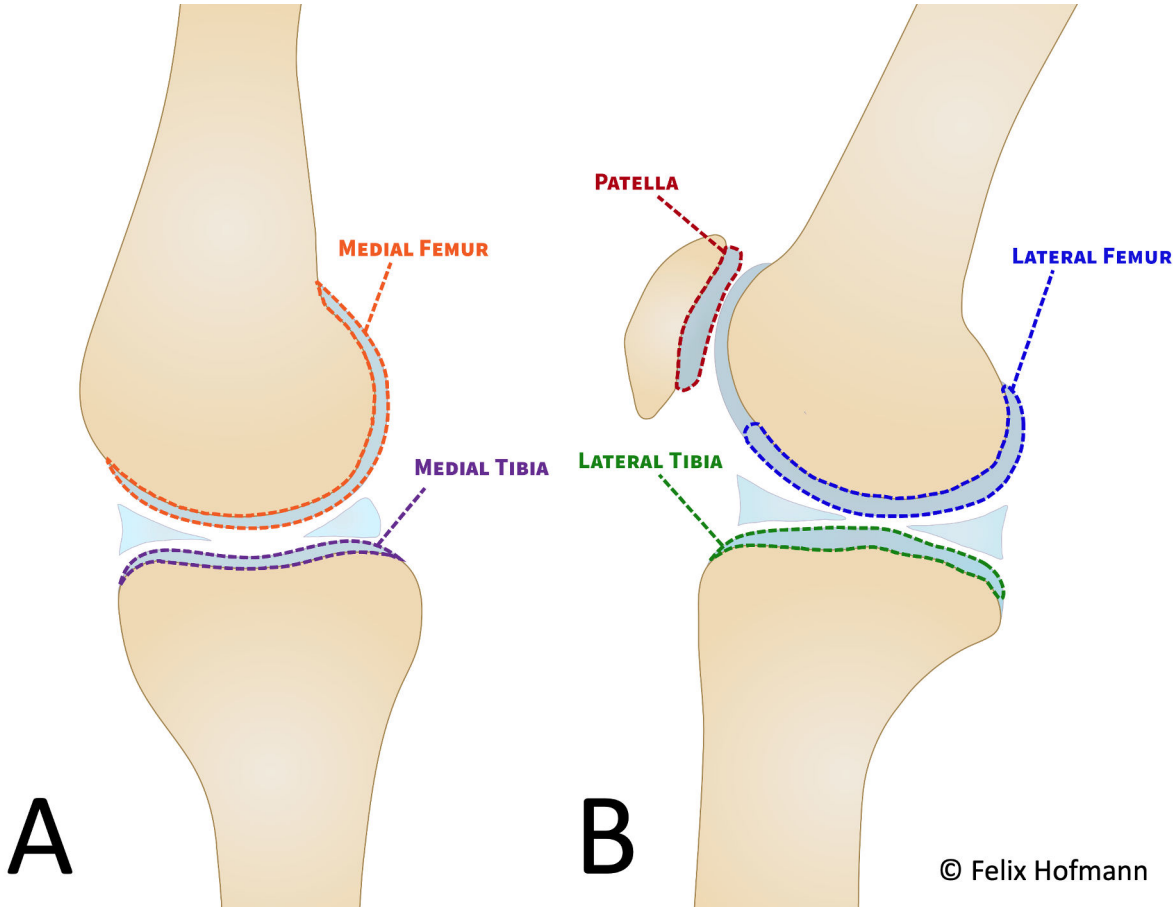

Figure 6 The five compartments included in the cartilage segmentation were in "A": MF (orange), MT (purple); and in "B": PAT (red), LF (blue) and LT (green) as displayed in this illustration

Supervised by an experienced radiologist (T.M.L.), two trained researchers (Felix Hofmann and Walid Ashmeik; refered to as F.H. and W.A.) segmented each compartment's cartilage autonomously. F.H. and W.A. were blinded to the status of DM as well as clinical information regarding the subjects. An algorithm written in MATLAB (Mathworks, Natick, Massachusetts) that was developed in-house and works based on splines was employed for the T2 analysis. This software enables researchers to semi-automatically segment all compartments and analyzing the T2 values in mentioned mono-exponential decay model. The procedure of semi-automatic segmentation is illustrated exemplarily for the medial knee compartments MF and MT in figure 7.

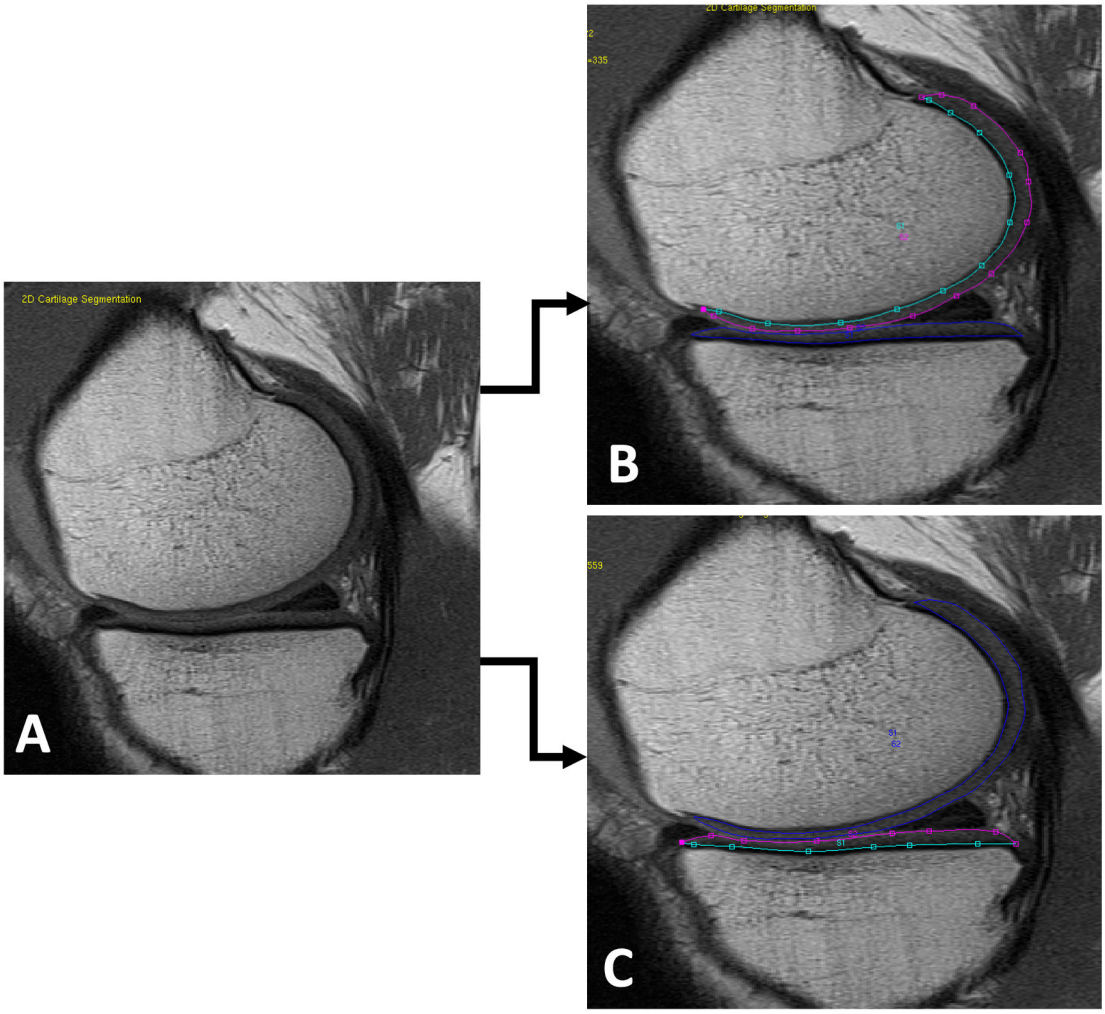

Figure 7 Illustration of segmentation of medial knee compartments MF and MT. "A" displays a raw slice that offers cartilage of appropriate quality for segmentation of MF ("B") and MT ("C").

The exact procedure illustrated in figure 7 is executed as follows: Initially, the chosen slice is retrieved and displayed within mentioned, proprietary MATLAB software (figure 7A). Then, dots are added manually along the bone layer (turquoise, dotted lines in 7B and 7C), at which it is important to exclude the black, chemical shift. Subsequently, the software tool can be triggered to calculate the associated articular layer (pink, dotted lines in 7B and 7C). After triggering the command to automatically generate the corresponding articular layer, the position of each point along the dotted lines needs to be adjusted as the program does not provide precision comparable to human segmentation.

Laminar Analysis

Based on the segmentations described above, knee cartilage can be partitioned into a deep layer which corresponds to the bone-cartilage interface and a superficial layer which corresponds to the articular surface (Carballido-Gamio et al., 2011). Both layers that emerge from this laminar analysis of T2 relaxation times have an approximately equal width (Hofmann et al., 2018).

GLCM Texture Analysis

The texture analysis of cartilage T2 maps was performed using GLCM texture analysis, which has previously been validated as a tool to investigate spatial distribution of T2 values (Heilmeier et al., 2019a). Based on the T2 values of the T2 maps which are portrayed by pixels colored/coded in a broad range of grey levels on the MR images, the grey level distributions of these cartilage T2 values can be used to compute GLCM parameters (Neumann et al., 2018). Specifically, the extent of heterogeneity of these T2 values over the cartilage matrix can be characterized with additional details than exclusive T2 values using the GLCM parameters entropy (a), contrast (b) and variance (c) (Hofmann et al., 2018; Joseph et al., 2011; T. J. Mosher et al., 2000). More precisely, entropy (a) refers to the disorder of the image by providing the probability of finding an additional couple of pixels with an identical value in the entire texture image (Neumann et al., 2018). This way, the grey level equation is expanded by entropy. Contrast (b) on the other hand refers to the level of inhomogeneity of neighboring pixel pairs in the matrix of the cartilage (Hofmann et al., 2018). More specifically, a high value of contrast corresponds to greater differences of grey levels in pixels that neighbor each other (Liebl et al., 2015). To compute contrast, each pixel is compared to its vertical or horizontal neighbor (Neumann et al., 2018). Thirdly, variance (c) provides an analysis of the distribution of pixels around the compartment average (Liu et al., 2019). This offers a quantitative value of how much pixels vary about the mean compartment grey level (Baum et al., 2013).

4.3.3 Semi-quantitative MR image analysis

The approach employed for semi-quantitative image analysis in this study was a modification of the original WORMS grading system that has been validated in recent studies and that includes exactly 5 knee compartments in comparison to the original

scoring system published by Peterfy et al (Gersing et al., 2017; Peterfy et al., 2004). These five compartments are chosen in accordance with the 5 compartments chosen for T2 relaxation time measurements and are LF, LT, MF, MT and PAT (Heilmeier et al., 2019a). Said modified WORMS grading system includes a scoring of cartilage defects from 0 to 6 and a scoring of BMEP from 0 to 3 in the same regions (LF, LT, MF, MT, PAT) (Gersing et al., 2017). Lesions of the medial and lateral meniscus were subclassified into posterior horn, body and anterior horn and subsequently graded based from zero to four (Neumann et al., 2019).

4.4 Statistical Analysis

As linear regression has previously proven itself for the estimation of adjusted average differences in T2 values between two cohorts, such linear regression models were used for the evaluation of these differences of the outcome measures of this study (Liebl et al., 2015). More precisely, both primary and secondary outcome measures were employed for the quantitative assessment of the biochemical composition of the knee cartilage. Primary outcome measures comprised change of mean T2 values over the longitudinal course for the compartments medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment. Secondary outcome measures for the compartments medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment encompassed texture analysis (entropy, variance, contrast), cross-sectional mean T2 analysis as well as laminar (superficial/bone layer) analysis following previous research endeavours (Baum, Stehling, et al., 2012; Gersing et al., 2017; Joseph et al., 2011; Jungmann et al., 2013).

Based on these outcome measures, mentioned linear regression models were used to evaluate differences in the mean, the laminar analysis and the GLCM texture analysis of the T2 parameters of the cartilage. Aforementioned differences between diabetic and non-diabetic study subjects were assessed at baseline, at 2-years and regarding changes over 2-years. In order to account for the matching by sets described in 4.3, cluster robust standard errors were employed. With a level of confidence set to $p < 0.05$, beta values including their 95% confidence intervals (CIs) were computed and appended to the corresponding tabular overviews. As mentioned before, OA has been found to be associated with risk factors including amongst others increasing age,

female gender and obesity (Courties & Sellam, 2016; T. W. O'Neill et al., 2018; Palazzo et al., 2016; Panoutsopoulou & Zeggini, 2013; Spector & MacGregor, 2004; Valdes & Spector, 2011). Therefore, we adjusted for these risk factors using the variables sex, BMI and age and hereby intended to decrease confounding bias. In addition to that we adjusted our analyses for K/L scores, since it has been proven that differences in KL scores might induce deviations of T2 relaxation times (Dunn et al., 2004). The fulfillment of each statistical test's underlying assumptions was provided. Within this research project we faced the problem of cartilage compartments that had missing data because of wear and tear. We performed a sensitivity analysis to concern ourselves with this missing data. Multivariate normal imputation with 20 imputations was used to impute the T2 values that were missing due to wear and tear of the cartilage. These imputations were performed at the level of cartilage compartment based on T2 values in other compartments as well as age, sex, KL grade, BMI and race.

Recent research suggests that the composition of knee cartilage on a biochemical level differs between various ethnicities (Kretzschmar et al., 2016). Since there was a higher percentage of African Americans in the cohort of subjects with diabetes, we employed adjustments for race in our study as well. STATA software (Version 14, College Station, TX: StataCorp LP) as well as IBM SPSS Statistics Version 23 was used to perform statistical analysis. Chi-square tests for categorical variables and independent t-tests for continuous variables were employed to determine differences in the group characteristics.

4.4.1 Intra-/Interreader Reproducibility

With a calculation of mean T2 values on a percentage basis as the root mean square average, coefficients of variation (CV) were computed for the assessment of intra- and interreader reproducibilities (Stehling et al., 2011). These calculations of reproducibility did not comprise the secondary outcome measures texture and laminar analyses. Two readers assessed the images of 10 same study subjects for the interreader reproducibility and the computation of CVs was performed as aforementioned. To determine intrareader reproducibility, 10 image datasets chosen by chance were handed to both readers. Both readers were given the same images for them to read and reread at two different time points. The 10 images were read and reread with at least 4

weeks time interval between both readings. CVs had an overall average of 1.93% and varied from 1.59% in the lateral femur to 2.36% in the medial tibia for the interreader reproducibility. The CVs of the intrareader reproducibility had an overall average of 1.57% and varied from 0.64% in the lateral femur to 2.85% in the medial tibia. Our research group has previously reported reproducibilities that were similar to the ones in this research project (Gersing et al., 2017).

5 Results

5.1 Participant characteristics

A summary of subject characteristics at baseline can be found in Table 1. In present study the percentage of male subjects (46.2%) was slightly lower than the share of female participants. The mean age taken across the entire study population was 63.1 years (standard deviation [SD] ± 9.1). Regarding sex, BMI, age and KL a proper matching of the two subcohorts was achieved ($p>0.05$). Nevertheless, the portion of participants with African American ethnicity among patients without self-reported DM (14.8%) was lower compared to case subjects (36.2%). This resulted in a statistically significant difference between case and control group regarding racial composition ($p<0.001$). Additionally, there was no statistically significant difference ($p>0.05$) in the osteoarthritic risk factors family history of knee replacement surgery, history of knee surgery and history of knee injury. Out of the n=196 case subjects of present research project, 13.8% (27 participants) reported complications as previously displayed in table 2. The majority of 86.2% of case subjects with DM did not report complications related to their diabetic disease. More precisely, the 27 diabetic study subjects that reported diabetes-related complications were subdivided into 18 participants that complained ophthalmological complications, 5 that suffered from renal complications and four subjects that suffered from both ophthalmological complications and renal complications. Moreover, 17.9% of patients (n=35) with DM received treatment with insulin injections.

	Controls (n=196)	Diabetics (n=196)	
Attribute	**Mean +/- SD**	**Mean +/- SD**	**p-values ł**
Demographics at baseline			
Age (years)	63.31 ± 9.17	62.96 ± 8.99	0.701
Body mass index (kg/m²)	30.91 ± 4.50	31.20 ± 4.51	0.523
Height (m)	1.68 ± 0.09	1.69 ± 0.09	0.749
Females [n (%)] / Males [n (%)]	105 (53.6%) / 91 (46.4%)	106 (54.1%) / 90 (45.9%)	0.919
Physical Activity Score for the Elderly	154.19 ± 85.92	144.20 ± 78.99	0.232
Right knee Kellgren-Lawrence			0.965
Grade 0 [n (%)]	79 (40.3%)	79 (40.3%)	
Grade 1 [n (%)]	48 (24.5%)	50 (25.5%)	
Grade 2 [n (%)]	69 (35.2%)	67 (34.2%)	
Racial distribution at baseline			**<0.001**
Caucasian [n (%)]	162 (82.7%)	115 (58.7%)	
African American [n (%)]	29 (14.8%)	71 (36.2%)	
Asian [n (%)]	1 (0.5%)	4 (2.0%)	
Other Non-white [n (%)]	4 (2.0%)	6 (3.1%)	
OA risk factors at baseline			
History of knee injury [n (%)]	70 (35.7%)	73 (37.2%)	0.568
History of knee surgery [n (%)]	30 (15.3%)	36 (18.4%)	0.510
Family history of knee replacement surgery [n (%)]	26 (13.3%)	15 (7.7%)	0.233
24-months	**Controls (n=159)**	**Diabetics (n=151)**	**p-values ł**
* Continues data are expressed as mean ± SD. Categorical data are presented in numbers of participants with percentage in parentheses. ł p-values listed in the right column were calculated using either Pearson´s χ2-test or independent t-test as appropriate.			

Table 4. Participant characteristics at baseline

Over the course of the study not all study subjects were retained, yet the characteristics of the cohorts in total were not altered statistically significant (p>0.05). Table 5 offers At 24-months follow-up, the characteristics of study subjects did not statistically significant differ apart from ethnicity. Compared to the demographic data at baseline, the age of both controls (64.99 ± 8.96) and diabetics (65.40 ± 8.83) was elevated without a statistically significant difference between both groups. While there

were also no statistically significant differences between both groups regarding KL grade at time point of follow up, the share of KL 0 subjects slightly decreased to 35.8% for control subjects and 39.7% for diabetic subjects.

	Controls(n=159)	Diabetics (n=151)	
Attribute	**Mean +/- SD**	**Mean +/- SD**	**p-values ł**
Demographics at 24-months			
Age (years)	64.99 ± 8.96	65.40 ± 8.83	0.685
Body mass index (kg/m²)	30.48 ± 4.37	31.00 ± 4.48	0.303
Height (m)	1.68 ± 0.09	1.68 ± 0.09	0.798
Females [n (%)] / Males [n (%)]	84 (52.8%) / 75 (47.2%)	83 (55.0%) / 68 (45.0%)	0.706
Physical Activity Score for the Elderly	151.10 ± 84.13	141.45 ± 76.63	0.295
Right knee Kellgren-Lawrence			0.678
Grade 0 [n (%)]	57 (35.8%)	60 (39.7%)	
Grade 1 [n (%)]	41 (25.8%)	40 (26.5%)	
Grade 2 [n (%)]	61 (38.4%)	51 (33.8%)	
Racial distribution at 24-months			**<0.001**
Caucasian [n (%)]	133 (83.6%)	92 (60.9%)	
African American [n (%)]	23 (14.5%)	51 (33.8%)	
Asian [n (%)]	0 (0.0%)	3 (2.0%)	
Other Non-white [n (%)]	3 (1.9%)	5 (3.3%)	
OA risk factors at 24-months			
New history of knee injury [n (%)]	6 (3.8%)	6 (4.0%)	1.000
New history of knee surgery [n (%)]	5 (3.1%)	3 (2.0%)	0.520
* Continues data are expressed as mean ± SD. Categorical data are presented in numbers of participants with percentage in parentheses. ł p-values listed in the right column were calculated using either Pearson´s χ2-test or independent t-test as appropriate.			

Table 5 Participant characteristics at 24-months

5.2 T2 Relaxation Time Measurements

As described in previous sections, T2 measurements represent the biochemical composition of cartilage and have been used in present study as a surrogate for the molecular condition of the study participants' knee cartilage. Present section will portray the primary outcome measures of change of mean T2 values over the

longitudinal, 24-month course for the compartments medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment. Secondary outcome measures included in this section are for the compartments medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment encompassed cross-sectional mean T2 analysis for both baseline and 24-month follow-up as well as laminar (superficial/bone layer) analysis.

5.2.1 Cartilage mean T2 composition at Baseline

The results of the cross-sectional mean T2 analysis is displayed in table 6. Cross-sectional mean T2 analysis, a subset of secondary outcome measures as described in the Methods part, portrayed comparable global T2 values at baseline without statistically significant differences: 32.57 ms (participants with DM) vs. 32.47 ms (participants without DM). The only compartment that showed a statistically significant higher (p=0.003) mean T2 value for participants with DM was the region of the patella presenting T2 values of 32.61ms (participants with DM) vs. 31.70ms (participants without DM). Interestingly, the mean T2 value of the medial femur region showed an elevation in the control cohort reaching statistical significance (38.72 ms vs. 37.96 ms; p = 0.010). Additionally, laminar analysis of the superficial/articular layer showed inconsistent values with a global, statistically non-significant elevation of mean T2 value of control subjects compared to diabetic participants (35.71 ms vs. 35.43 ms; p=0.371). The region of the patella, however, stayed statistically significant elevated in the diabetic case group (35.90 ms vs. 34.76 ms; p= 0.004). Moreover, MT and LT were both higher for diabetic subjects in the articular layer without reaching statistical significance. Contrarily, both MF and LF showed higher mean T2 values for control subjects with LF even reaching statistical significance (37.64 ms vs. 36.85 ms; p = 0.02). Lastly, the laminar analysis of the bone/deep layer showed elevated values in diabetic subjects for all compartments, including global, except MF and LF. A statistical significance for diabetic subjects was reached in PAT and MT, with 29.50 ms vs. 28.61 ms (p = 0.001) for PAT and 27.35 ms vs. 26.92 ms (p = 0.04) for MT. Surprisingly, MF showed statistically significantly elevated mean T2 values for control subjects with 36.20 ms vs. 35.47 ms (p = 0.01).

	Diabetics (n=196)		Non-diabetics (n=196)		
Compartment	**Adjusted means**	**[95% CI]**	**Adjusted means**	**[95% CI]**	**p-value[1]**
	Cartilage Mean T2 at Baseline				
Global knee T2	32.57	[32.29,32.85]	32.47	[32.12,32.82]	0.669
PAT T2	32.61	[32.11,33.11]	31.70	[31.31,32.10]	**0.003**
MT T2	29.92	[29.60,30.24]	29.65	[29.19,30.11]	0.387
LT T2	27.82	[27.49,28.15]	27.55	[27.21,27.89]	0.314
MF T2	37.96	[37.61,38.30]	38.72	[38.26,39.17]	**0.011**
LF T2	34.71	[34.32,35.10]	35.17	[34.77,35.58]	0.111
	Superficial Layer T2 at Baseline				
Global knee T2	35.43	[35.01,35.86]	35.71	[35.36,36.06]	0.371
PAT T2	35.90	[35.26,36.55]	34.76	[34.24,35.27]	**0.004**
MT T2	33.40	[32.99,33.82]	32.71	[32.11,33.31]	0.085
LT T2	31.81	[31.36,32.27]	31.38	[30.87,31.89]	0.278
MF T2	40.62	[40.19,41.05]	41.14	[40.62,41.66]	0.130
LF T2	36.85	[36.41,37.30]	37.64	[37.17,38.12]	**0.023**
	Deep Layer T2 at Baseline				
Global knee T2	29.79	[29.56,30.02]	29.57	[29.30,29.85]	0.268
PAT T2	29.50	[29.05,29.95]	28.61	[28.28,28.94]	**0.001**
MT T2	27.35	[27.06,27.64]	26.92	[26.61,27.23]	**0.049**
LT T2	24.14	[23.88,24.40]	23.86	[23.60,24.11]	0.164
MF T2	35.47	[35.10,35.83]	36.20	[35.78,36.62]	**0.011**
LF T2	32.50	[32.14,32.86]	32.63	[32.27,32.99]	0.610

Data are given as adjusted means (in ms) and [95% confidence intervals]; P-value <0.05 are in bold with the statistically significant higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.

Table 6 Participants' T2 values in a cross-sectional analysis at baseline

5.2.2 Longitudinal Change in mean T2 relaxation

As mentioned before, longitudinal changes of mean T2 relaxation time measurements were considered primary outcome measures in present research project and are presented in Table 7. Compared to the control cohort without DM, diabetic participants expressed an increase of mean T2 values that was statistically significant more rapid than the control group. In comparison with the control subjects the global T2 values rose nearly twice the number in patients with DM (change in mean T2 of 1.77 ms vs. 0.98 ms; $p<0.001$). Apart from the patella, each cartilage compartment expressed a statistically significant higher pace of mean T2 value change ($p<0.05$). Interestingly, the patella was the only compartment to show slightly faster increase in the control cohort compared to diabetic cases without reaching statistical significance.

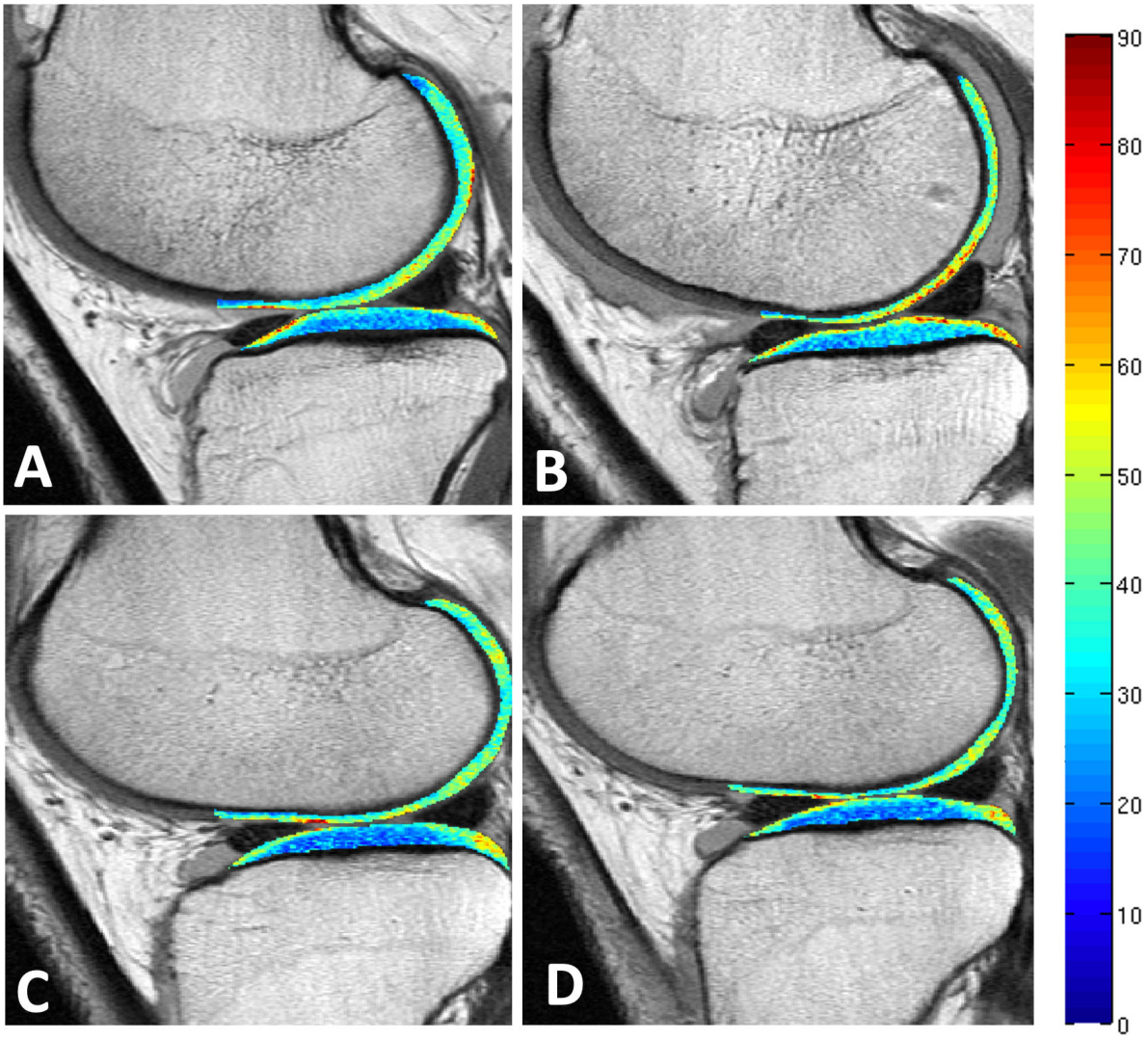

Figure 8 Longitudinal change of T2 color maps of both a diabetic subject ([A] at baseline and [B] at 24-month-followup) and a healthy control ([C] in baseline and [D] at 24-month-followup). Lower values are coded in blue and green whereas high values are coded in orange and red. The compartments displayed for both diabetic and non-diabetic patient are LF and LT. While for the diabetic subject the slices of baseline [A] and 24-month followup [B] do not exactly match, this specific patient showed an increase in overall LF from 36.24ms to 39.50ms and in overall LT from 28.72ms to 31.12ms. In terms of change in mean T2 the corresponding control showed a slower increase in overall LF from 33.71ms to 36.03ms and in overall LT from 27.99ms to 29.05ms (from [C] to [D]).

The results of the aforementioned primary outcome measures did not statistically significantly change when performing sensitivity analysis to address the problem of unsuccessful patient retention. Aforesaid sensitivity analysis was performed by employing multiple imputation with effect sizes for the individual compartments with imputation of missing-data as compared to analysis without imputation of missing-

data with the following results: medial femur 0.55 [0.05,1.06] (p=0.031) vs. 0.56 [0.07,1.05] (p=0.026); lateral femur 0.66 [0.20,1.11] (p=0.005) vs. 0.56 [0.09,1.03] (p=0.020); medial tibia 0.60 [0.08,1.11] (p=0.025) vs. 0.58 [0.05,1.11] (p=0.033); lateral tibia 0.59 [0.05,1.12] (p=0.031) vs. 0.56 [0.04,1.07] (p=0.034); patella 0.03 [-0.72,0.77] (p=0.944) vs. -0.05 [-0.79,0.68] (p=0.885).

Regarding laminar analysis of the superficial/articular layer, the surge of mean T2 was faster for diabetic subjects in all compartments, including global knee , except for the patella. Statistical significance was reached for LT (2.27 ms vs. 1.62 ms; p = 0.049) and LF (2.25 ms vs. 1.46 ms; p = 0.008).

In relation to the bone/deep layer, T2 value change was statistically significantly higher in diabetic subjects in the global knee (1.51 ms vs. 0.98 ms; p = 0.006) and the MF (1.56 ms vs. 0.81 ms; p = 0.004). While mean T2 change was faster in LF and LT without reaching statistical significance, PAT and MT showed both an equal increase in both cohorts which amounted 1.21 (p = 0.991) for PAT and 1.19 for MT (p = 0.984).

	Diabetics (n=151)		Non-diabetics (n=159)		
Compartment	**Adjusted means**	**[95% CI]**	**Adjusted means**	**[95% CI]**	**p-value**
	Longitudinal change in mean T2 values over 24 months				
Global knee T2	1.77	[1.51,2.03]	0.98	[0.68,1.28]	**<0.001**
PAT T2	1.33	[0.82,1.84]	1.38	[0.87,1.89]	0.885
MT T2	1.85	[1.50,2.20]	1.27	[0.90,1.64]	**0.033**
LT T2	1.92	[1.59,2.25]	1.36	[0.99,1.74]	**0.034**
MF T2	1.08	[0.76,1.40]	0.52	[0.14,0.91]	**0.026**
LF T2	2.03	[1.69,2.36]	1.47	[1.12,1.82]	**0.020**
	Longitudinal change in laminar (superficial) values over 2 years				
Global knee T2	1.82	[1.51,2.13]	1.37	[1.02,1.71]	0.061
PAT T2	1.41	[0.81,2.00]	1.65	[0.96,2.34]	0.592
MT T2	2.25	[1.76,2.73]	1.72	[1.25,2.20]	0.159
LT T2	2.27	[1.85,2.69]	1.62	[1.14,2.10]	**0.049**
MF T2	0.89	[0.48,1.29]	0.59	[0.16,1.03]	0.325
LF T2	2.25	[1.85,2.65]	1.46	[1.05,1.88]	**0.008**
	Longitudinal change in laminar (bone) values over 2 years				
Global knee T2	1.51	[1.28,1.75]	0.98	[0.71,1.25]	**0.006**
PAT T2	1.21	[0.73,1.69]	1.21	[0.78,1.64]	0.991
MT T2	1.19	[0.84,1.55]	1.19	[0.85,1.53]	0.984
LT T2	1.31	[0.97,1.65]	1.06	[0.73,1.38]	0.292
MF T2	1.56	[1.26,1.87]	0.81	[0.43,1.18]	**0.004**
LF T2	1.91	[1.57,2.24]	1.52	[1.16,1.87]	0.107

Data are given as adjusted means (in ms) and [95% confidence intervals] and computed as the overall change between the 24 months and the baseline time point; P-value <0.05 are in bold with the statistically significantly higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.

Table 7 Longitudinal change in T2 values over 24 months

Severe Diabetes

Moreover, we performed sensitivity analysis focusing on diabetics with severe illness determined by necessity of insulin treatment and/or comorbidity with ophthalmological and/or renal complications associated with DM. The results are presented in table 8. While this subgroup of diabetic patients also expressed a higher pace of mean T2 value increase in comparison to control subjects without DM, these results were not associated with a statistical significance.

	Severe-Diabetics (n=38)		**Non-diabetics (n=159)**			
Compartment	Adjusted means	[95% CI]	Adjusted means	[95% CI]	Effect size [95% CI]*	p-value
Absolute change in mean T2 values over 24 months – severe diabetes						
Global knee T2	1.29	[0.76,1.83]	1.07	[0.80,1.35]	0.22 [-0.40,0.83]	0.482
PAT T2	1.55	[0.55,2.54]	1.34	[0.84,1.84]	0.21 [-0.82,1.24]	0.682
MT T2	1.29	[0.48,2.09]	1.26	[0.90,1.63]	0.03 [-0.90,0.95]	0.960
LT T2	1.52	[0.86,2.17]	1.33	[0.96,1.70]	0.19 [-0.64,1.01]	0.652
MF T2	0.71	[0.01,1.41]	0.46	[0.09,0.83]	0.25 [-0.54,1.04]	0.536
LF T2	1.43	[0.61,2.25]	1.47	[1.13,1.81]	-0.04 [-0.94,0.85]	0.920

Data are given as adjusted means, corrected for race, age, sex, baseline BMI, baseline KL score, with [95% confidence intervals] and computed as the absolute change between the 24-months and the baseline; * = Effect size for difference in T2 and texture parameters between groups; P-values <0.05 are in bold. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.

Table 8 Change in subjects with severe diabetes

5.2.3 Cartilage mean T2 Composition at 24-months

With regard to the cross-sectional differences in mean T2 relaxation times at the time point of 24-month follow-up, there were various cartilage compartments showing statistically significantly higher T2 values in participants with DM due to the higher pace of increase of mean T2 relaxation times in diabetic subjects. The results are depicted in table 9. At the time point of 24-month follow-up, the medial tibia (31.72 ms

vs. 31.05 ms; p = 0.02), the lateral tibia (29.81 ms vs. 28.96 ms; p=0.006), the patella (34.10 ms vs. 33.19 ms; p=0.012) and the global knee compartment (34.26 ms vs. 33.71 ms; p=0.009) showed statistically significantly elevated T2 values. Interestingly, the remaining compartments MF and LF showed an elevation in non-diabetic study participants, without statistical significance.

The laminar analysis of the superficial, articular layer provided statistically significantly higher mean T2 values for diabetic subjects as well, with the exception of MF and LT. Again, the MF region depicted a higher mean T2 for the control cohort without statistical significance. The laminar analysis of the deep, bone layer provided statistically significantly higher T2 mean values in the diabetic cohort for the global knee (31.21 ms vs. 30.74 ms; p = 0.01), the patella region (30.76 ms vs. 29.78 ms; p = 0.002) and the lateral tibia region (25.51 ms vs. 24.94 ms; p = 0.02). Without portraying statistical significance, MT and LF demonstrated higher mean T2 values for diabetic subjects as well. Contrarily, MF indicated a slightly higher mean T2 for the control cohort without reaching statistical significance.

	Diabetics (n=151)		**Non-diabetics (n=159)**		
Compartment	**Adjusted means**	**[95% CI]**	**Adjusted means**	**[95% CI]**	**p-value**
	Cartilage mean T2 Composition at 24-months				
Global knee T2	34.26	[33.99,34.54]	33.71	[33.42,34.00]	**0.009**
PAT T2	34.10	[33.61,34.59]	33.19	[32.64,33.74]	**0.012**
MT T2	31.72	[31.35,32.08]	31.05	[30.67,31.43]	**0.025**
LT T2	29.81	[29.37,30.25]	28.96	[28.62,29.30]	**0.006**
MF T2	39.10	[38.70,39.50]	39.24	[38.81,39.66]	0.639
LF T2	36.84	[36.47,37.20]	36.88	[36.50,37.25]	0.878
	24-months (Superficial Layer)				
Global knee T2	37.63	[37.29,37.96]	36.98	[36.61,37.35]	**0.016**
PAT T2	37.61	[37.09,38.12]	36.71	[35.98,37.44]	**0.045**
MT T2	35.64	[35.16,36.12]	34.60	[34.02,35.19]	**0.014**
LT T2	34.20	[33.63,34.76]	33.01	[32.57,33.45]	**0.003**
MF T2	41.62	[41.13,42.11]	41.90	[41.40,42.41]	0.426

LF T2	39.28	[38.79,39.76]	39.31	[38.85,39.77]	0.926
	24-months (Bone Layer)				
Global knee T2	31.21	[30.95,31.47]	30.74	[30.48,30.99]	**0.014**
PAT T2	30.76	[30.32,31.20]	29.78	[29.33,30.23]	**0.002**
MT T2	28.42	[28.06,28.79]	28.23	[27.94,28.53]	0.470
LT T2	25.51	[25.12,25.90]	24.94	[24.65,25.23]	**0.028**
MF T2	37.02	[36.57,37.47]	37.05	[36.64,37.46]	0.920
LF T2	34.54	[34.19,34.88]	34.32	[33.99,34.65]	0.343
Data are given as adjusted means (in ms) and [95% confidence intervals]; P-value <0.05 are in bold with the statistically significantly higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.					

Table 9 Participants' T2 values in a cross-sectional analysis at 24-months follow-up

5.2.4 KL Comparison

Additionally, we performed a sensitivity analysis comparing diabetic subjects with KL score 2 (mild radiographic OA) with diabetics that had no radiographic OA (KL score 0 and 1). While not finding statistically significant differences between these two groups, there was a marginally higher increase of average T2 values in diabetics with mild radiographic OA.

5.3 Texture Analysis

As previously clarified, present study additionally included texture analysis (GLCM parameters contrast, variance and entropy) as extra secondary outcome measures for the compartments medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment.

5.3.1 Texture Composition at Baseline

Generally, the cross-sectional comparison of diabetic and non-diabetic subjects yielded a more inordinate and heterogeneous composition of cartilage at baseline. GLCM Texture parameters at baseline are potrayed in table 10. At baseline, GLCM contrast was statistically significantly higher in the diabetic cohort for the global knee (312.68 vs. 297.80; p = 0.021), PAT (284.12 vs. 261.72; p = 0.010), MT (361.93 vs. 319.44; p =

<0.001) and LT (209.50 vs. 187.54; <0.001). For MF and LT, the GLCM contrast parameter was higher in the control cohort without statistical significance.

In regards to GLCM variance, a statistical significance was reached with an elevation in the diabetic cohort for the compartments global knee (224.90 vs. 215.09; p = 0.029), PAT (214.76 vs. 197.50; p = 0.009), MT (244.86 vs. 220.07; p < 0.001) and LT (165.63 vs. 149.55; p < 0.001). Again, for the compartments MF and LF an elevation in the control cohort was observed that did not reach statistical significance.

For entropy on the other hand, no compartment demonstrated an elevation in the non-diabetic study participants. While LF provided equal values for diabetic and non-diabetic group (6.63 vs. 6.63; p = 0.965), all other compartments, including global knee, were elevated in the case cohort. Statistical significance was reached for global knee (6.29 vs. 6.20; p = 0.001), PAT (6.08 vs. 5.98; p = 0.009) and MT (6.03 vs. 5.87; p < 0.001).

	Diabetics (n=196)		**Non-diabetics (n=196)**		
Compartment	**Mean**	**[95% CI]**	**Mean**	**[95% CI]**	**p-value**
	Texture Contrast at Baseline				
Global knee T2	312.68	[303.50,321.86]	297.80	[288.91,306.70]	**0.021**
PAT T2	284.12	[271.26,296.99]	261.72	[249.67,273.76]	**0.010**
MT T2	361.93	[346.13,377.72]	319.44	[306.88,331.99]	**<0.001**
LT T2	209.50	[200.83,218.17]	187.54	[180.69,194.40]	**<0.001**
MF T2	431.66	[416.93,446.40]	449.28	[433.83,464.73]	0.111
LF T2	265.563	[257.75,273.37]	272.56	[264.33,280.79]	0.223
	Texture Variance at Baseline				
Global knee T2	224.90	[218.63,231.16]	215.09	[209.02,221.17]	**0.029**
PAT T2	214.76	[204.54,224.99]	197.50	[188.58,206.42]	**0.009**
MT T2	244.86	[235.46,254.26]	220.07	[211.80,228.34]	**<0.001**
LT T2	165.63	[159.56,171.70]	149.55	[144.89,154.21]	**<0.001**
MF T2	300.72	[291.25,310.19]	307.09	[297.51,316.67]	0.360
LF T2	193.76	[188.08,199.43]	201.75	[196.09,207.40]	0.069
	Texture Entropy at Baseline				

Global knee T2	6.29	[6.26,6.32]	6.20	[6.17,6.24]	**0.001**
PAT T2	6.08	[6.02,6.14]	5.98	[5.93,6.02]	**0.009**
MT T2	6.03	[5.98,6.07]	5.87	[5.81,5.93]	**<0.001**
LT T2	5.80	[5.74,5.85]	5.73	[5.68,5.78]	0.144
MF T2	6.93	[6.89,6.97]	6.90	[6.87,6.93]	0.165
LF T2	6.63	[6.60,6.67]	6.63	[6.60,6.67]	0.965
Data are given as adjusted means and [95% confidence intervals]; P-value <0.05 are in bold with the statistically significant higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.					

Table 10 GLMC Texture parameters at baseline

5.3.2 Longitudinal Change in Texture Parameters

Longitudinal changes in GLCM Parameters are shown in table 11. The longitudinal changes in GLCM texture parameters over time were significantly faster for subjects with diabetes in the GLCM parameters Contrast global knee (93.75 vs. 56.08; p=0.009), Contrast MF (155.36 vs. 68.29; p < 0.001), Contrast LF (85.69 vs. 39.44; p <0.001), Variance MF (70.08 vs. 42.83; p = 0.021) and Variance LF (44.45 vs. 24.75; p = 0.002).

Surprisingly, Entropy MT (0.05 vs. 0.15, p < 0.001) and Entropy LF (-0.03 vs. 0.04; p = 0.002) showed a statistically significant faster progress in the control cohort.

T2 entropy of the patella and the medial femur in both cohorts and lateral femur in the diabetic cohort were the only texture parameters that didn't show an increase regartding their longitudinal changes. Thus, they demonstrated a reduction in T2 entropy values. The explanation for this decrease might be the higher level of thickness of the cartilage compartment of the patella compared to other cartilage compartments. More precisely, a thicker cartilage as found in the compartment of the patella might provide an altered chance of encountering equivalent pixel pairs and hence express a decreased level of order of the cartilage composition.

	Diabetics (n=151)		Non-diabetics (n=159)		
Compartment	Adjusted means	[95% CI]	Adjusted means	[95% CI]	p-value
	Contrast - Longitudinal change over 2 years				
Global knee T2	93.75	[73.01,114.48]	56.08	[38.09,74.08]	**0.009**
PAT T2	78.25	[59.30,97.20]	53.89	[33.11,74.66]	0.112
MT T2	83.80	[52.75,114.86]	59.36	[38.74,79.98]	0.228
LT T2	44.72	[28.02,61.41]	32.42	[20.37,44.46]	0.256
MF T2	155.36	[120.33,190.39]	68.29	[39.96,96.62]	**<0.001**
LF T2	85.69	[68.53,102.84]	39.44	[23.17,55.72]	**<0.001**
	Variance - Longitudinal change over 2 years				
Global knee T2	44.50	[34.35,54.65]	35.18	[25.81,44.55]	0.190
PAT T2	35.21	[24.12,46.30]	33.88	[22.28,45.48]	0.872
MT T2	39.69	[24.50,54.88]	38.79	[27.57,50.00]	0.932
LT T2	24.70	[15.39,34.01]	25.70	[17.74,33.66]	0.870
MF T2	70.08	[53.60,86.55]	42.83	[27.84,57.81]	**0.021**
LF T2	44.45	[35.77,53.14]	24.75	[15.97,33.53]	**0.002**
	Entropy - Longitudinal change over 2 years				
Global knee T2	0.01	[-0.02,0.04]	0.04	[0.01,0.07]	0.255
PAT T2	-0.04	[-0.09,0.01]	-0.08	[-0.13,-0.03]	0.260
MT T2	0.05	[0.01,0.09]	0.15	[0.10,0.20]	**<0.001**
LT T2	0.12	[0.07,0.17]	0.12	[0.07,0.18]	0.954
MF T2	-0.04	[-0.07,-0.01]	-0.01	[-0.03,0.02]	0.078
LF T2	-0.03	[-0.06,0.01]	0.04	[0.01,0.06]	**0.002**

Data are given as adjusted means and [95% confidence intervals] and computed as the overall change between the 24 months and the baseline time point; P-value <0.05 are in bold with the significant higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.

Table 11 GLCM Parameters - Longitudinal change over 2 years

5.3.3 Texture Composition at 24-months

Similarly to baseline, the cross-sectional comparison of diabetic and non-diabetic subjects yielded a more inordinate and heterogeneous composition of cartilage at 24-months-followup. At the time point of 2-year follow up, GLCM Contrast depicted a statistically significant elevation for the diabetic cohort in the knee in its in enterity. GLCM Variance on the other hand was elevated in all compartments of the diabetic study participants as well, while statistical significance was reached for the global knee (267.96 vs. 249.17; p = 0.007), PAT (248.80 vs. 228.22; p = 0.009), MT (281.02 vs. 255.48; p = 0.004) and LT (187.77 vs. 175.29; p = 0.041). GLCM entropy was increased in all compartments of the knee of diabetic participants, except for LF at 24-months follow-up. Two values of GLCM entropy at 24-months follow-up were statistically significant: On the one hand, GLCM entropy for PAT was elevated in diabetics (6.03 vs. 5.88; p = 0.002). On the other hand, GLCM entropy for LF was elevated in the control cohort (6.67 vs. 6.61; p = 0.047).

	Diabetics (n=151)		Non-diabetics (n=159)		
Compartment	**Mean**	**[95% CI]**	**Mean**	**[95% CI]**	**p-value**
	Texture Contrast at 24-months				
Global knee T2	402.68	[382.18,423.17]	351.92	[334.79,369.06]	**<0.001**
PAT T2	355.31	[333.74,376.89]	304.74	[285.63,323.84]	**0.001**
MT T2	444.63	[418.32,470.94]	374.25	[356.05,392.45]	**<0.001**
LT T2	248.87	[233.71,264.06]	218.49	[206.76,230.22]	**0.004**
MF T2	586.29	[550.51,622.08]	521.23	[492.62,549.84]	**0.011**
LF T2	349.00	[331.80,366.20]	312.27	[298.06,326.49]	**0.002**
	Texture Variance at 24-months				
Global knee T2	267.96	[258.40,277.52]	249.17	[240.31,258.03]	**0.007**
PAT T2	248.80	[237.04,260.56]	228.22	[216.56,239.88]	**0.009**
MT T2	281.02	[268.42,293.62]	255.48	[245.54,265.42]	**0.004**
LT T2	187.77	[178.85,196.68]	175.29	[167.71,182.86]	**0.041**
MF T2	371.60	[354.90,388.30]	353.01	[339.20,366.82]	0.119
LF T2	237.31	[228.12,246.50]	228.05	[220.20,235.90]	0.139
	Texture Entropy at 24-months				
Global knee T2	6.29	[6.26,6.32]	6.25	[6.22,6.28]	0.058
PAT T2	6.03	[5.96,6.09]	5.88	[5.82,5.95]	**0.002**
MT T2	6.06	[6.03,6.10]	6.03	[5.99,6.07]	0.203
LT T2	5.89	[5.84,5.95]	5.84	[5.79,5.88]	0.121
MF T2	6.90	[6.86,6.94]	6.89	[6.86,6.92]	0.720
LF T2	6.61	[6.56,6.65]	6.67	[6.63,6.71]	**0.047**

Data are given as adjusted means and [95% confidence intervals]; P-value <0.05 are in bold with the statistically significant higher mean T2 value in underlined characters. PAT = patella, MT = medial tibia, LT = lateral tibia, MF = medial femur, LF = lateral femur.

Table 12 GLMC Texture parameters at 24-months

6 Discussion

6.1 Overview

In this study we investigated how DM affects the status and composition of the knee cartilage matrix over a study period of 2 years. The surrogate biomarkers used to examine the integrity and potential loss of homogeneity of knee cartilage matrix over time were change of mean T2 values over the longitudinal course as primary outcome measures as well as GLCM texture analysis (contrast, variance, entropy), cross-sectional mean T2 analysis and laminar analysis (superficial/deep layer) as secondary outcome measures. The analysis was performed compartment-specific and comprised medial femur, lateral femur, medial tibia, lateral tibia, patella as well as global knee compartment. The increase in mean T2 values was statistically significantly higher in the diabetic cohort which resulted in a distinct elevation of T2 values after two years. Additionally, the diabetic cohort demonstrated a statistically significantly more inhomogeneous composition of cartilage ECM at baseline and followup measured using GLCM texture parameters. The results of this study suggest that the cartilage ECM of subjects suffering from diabetes deteriorates faster over time, as the more rapid rise in T2 values as well as the more heterogeneous texture parameters signify an increased destruction of collagen fiber architecture and general loss in collagen content (Blumenkrantz et al., 2004; Haralick et al., 1973; Joseph et al., 2011; A. Williams et al., 2017).

Various studies have studied and proven the general association of DM and OA so far (Atayde et al., 2012; King & Rosenthal, 2015; Steenvoorden et al., 2006; Nicole Verzijl et al., 2002, 2003). A recent cross-sectional study published in 2016 focused on the composition of both tendon and cartilage tissue composition measured with quantitative in vivo 7 T sodium MRI in patients suffering from type 1 DM (Marik et al., 2016). In said study, that included a total of 17 subjects (8 case subjects and 9 controls), Marik et al. found a statistically significant elevation of Sodium-normalized mean signal intensities (NMSIs) in the non-weight-bearing femoral cartilage and patellar

tendon of patients suffering from type 1 DM in comparison to healthy controls (Marik et al., 2016). As sodium MRI has demonstrated its ability to detect changes in cartilage and tendon GAG content, Marik et al. hypothesize that their results signify early alterations in the biochemical composition prior to potential morphological changes in the future (Juras et al., 2013; Wang et al., 2009).

The clinical relevance of our findings are clarified by previous research that found a link between higher levels of pain and an elevation of cartilage T2 values (Baum, Joseph, et al., 2012). Besides, early T2 elevation has been confirmed to predict the onset of definite knee OA (Liebl et al., 2015). Potential adverse effects of DM on hyaline cartilage homeostasis and reparation have been described and encompass a range of pathways to be considered to explain present study's results (Courties & Sellam, 2016; Eymard et al., 2015; King & Rosenthal, 2015; Kirkman, 2015; Nicole Verzijl et al., 2003).

6.2 Molecular Mechanisms

It has been shown in an in vitro model that chondrocytes increase their reactive oxygen species (ROS) production when exposed to excessive amounts of glucose and that unlike other cells in the body, chondrocytes cannot easily downregulate uptake of glucose via glucose transporter-1 (GLUT-1) during hyperglycemia (Rosa et al., 2009). Rosa et al. hypothesize that said inability to downregulate GLUT-1 leading to increased levels of ROS may be one of the mechanisms by which DM could potentially harm chondrocytes and eventually promote the onset or progression of OA (Rosa et al., 2009). Specifically, ROS released by chondrocytes include nitric oxide (NO) and superoxide anion (O2-) which in turn lead to derivative radicals like hydrogen peroxide and peroxynitrite (ONOO-) (Henrotin et al., 2003; Hiran et al., 1997; Moulton et al., 1997). When the antioxidant capacity of chondrocytes is exhausted and can no longer render ROS harmless, the resulting oxidative stress can damage extracellular components including PG and collagens (Henrotin et al., 2003). In addition to the increased level of ROS caused by hyperglycemia, hyperglycemia has also been demonstrated to promote the production of detrimental MMPs that are involved in

cartilage destruction as described in subsection 3.5 (Flik et al., 2007; Shimura et al., 2013).

Moreover, hyperglycemia may result in excessive concentrations of AGEs in hyaline cartilage that have been shown to cause additional cartilage decomposition (Brownlee, 2001; Steenvoorden et al., 2006). Increased production of AGEs can harm cartilage in multiple ways. AGEs can lead to pathological cross-linking between collagen fibers, which can additionally impede physiological turnover of ECM by limiting availability of proteolytic sites (DeGroot et al., 2001; King & Rosenthal, 2015; Reddy, 2004). Additionally, AGEs themselves have been found to enhance the expression and enzymatic activity of both ADAMTS and MMPs (which have been found to have harmful effects on cartilage ECM) in a porcine chondrocyte model (Huang et al., 2011; Suzuki et al., 2020).

AGEs do also possess the capacity to alter the biomechanical properties of hyaline cartilage and to increase tensile stiffness as shown in a study using bovine cartilage samples (Chen et al., 2002).

In addition to their pro-oxidant effects on chondrocytes via increased ROS production as mentioned above, AGEs do also exert an inflammatory stimulus on chondrocytes by binding to the receptor for AGEs (RAGE) which prompts a pathophysiological signal transduction (Suzuki et al., 2020). This signal transduction involves signaling molecules like NF- κB as well as proinflammatory cytokines like amongst others IL-1, TNF-α and adipokines that favor inflammation (King & Rosenthal, 2015). Inflammation has been shown to be a crucial agonist in the destruction of cartilage ECM with chondrocytes responding to inflammatory stimulus (mediated by amongst others IL-1β and TNF-α as mentioned above) with the expression of ECM degrading proteins including MMPs and ADAMTS (Maldonado & Nam, 2013).

Interestingly, the cartilage of the patella was the only compartment that did not demonstrate a statistically significantly faster increase of mean T2 values in the diabetic cohort compared to the healthy controls, while the cross-sectional elevation of the patellar cartilage was distinct at baseline. This suggests that pre-morphologic damage to the patellar cartilage was already too pronounced to show further increased deterioration of the ECM over time. One potential explanation for this are the

biochemical alterations of the patellar tendon in diabetics that Marik et al. have found using quantitative sodium MR at 7T and that could exert inflammatory/deleterious stimulus per continuitatem at an earlier stage compared to other compartments (Marik et al., 2016).

However, apart from the patellar compartment, all compartments including the global knee mean T2 have presented a statistically significantly faster increase in mean T2 which indicates a whole joint response to DM. Such global abnormalities of the cartilage matrix might occur due to an impaired homeostasis of the cartilage linked to a stress response of chondrocytes with resulting production of ECM degeneration products (Houard et al., 2013).

6.3 Discussion of Laminar and Texture Analysis

Moreover, the speed of deterioration (change in mean T2 over 24-months) was distinctively, statistically significantly faster in diabetics for the global knee in the laminar bone sub-analysis. This statistically significant elevation for the global knee T2 was not observed in the laminar articular sub-analysis, even though generally almost all compartments did show a faster increase in both sub-analyses. This distinction between articular and bone laminar sub-analysis could potentially indicate a faster deterioration of ECM in the bone layer. A potential explanation are the increased numbers of chondrocytes producing cartilage degrading collagenase-3 in the deeper layers of arthritic cartilage which have been found to respond to IL-1β stimulus in human cartilage specimen (Moldovan et al., 1997). Generally, the deeper layer of hyaline cartilage yields lower mean T2 values when compared to the articular layer (which also applies for this present study) due to the tidemark as well as the calcified layer which contain less (T2 increasing) water (Mosher & Dardzinski, 2004). While the regular OA development of cartilage degeneration (e.g. trauma-induced) in osteoarthritis begins in the superficial layer, our results (which differ from this rule) support an additional inflammatory pathway of OA pathogenesis in diabetic subjects which disproportionally affects the deeper layer as described above (Grässel & Aszódi, 2017a; Timothy J. Mosher & Dardzinski, 2004).

Remarkably, GLCM Contrast was the texture parameter that allowed the clearest distinction between diabetics and healthy controls both at baseline and at 24-follow-

up with a distinctively statistically significant elevation in diabetic subjects. This finding was further corroborated by a statistically significantly faster increase of GLCM contrast for the global knee contrast over the study period. GLCM contrast measures the differences in neighboring pixel values and high contrast consequently signifies a high amount of neighboring pixels with varying values. Increased values of GLCM contrast have previously been associated with mild OA and osteoarthritic cartilage (Carballido-Gamio et al., 2009). Since GLCM contrast and several GLCM entropy and GLCM variance parameters were elevated in diabetic subjects prior to mean T2 values, GLCM Texture analysis might be more sensitive for early cartilage ECM degradation that is diabetes-induced. An advantage of quantitative MRI techniques are the numerical results yielded in these analyses with GLCM texture parameters potentially offering a way to quantify early diabetes-induced cartilage degradation prior to traditional T2 mean values (Blumenkrantz et al., 2008).

6.4 Chronological Sequence of Biochemical Changes

Interestingly, the compartments medial femur and lateral femur demonstrated similar values for the non-diabetic and diabetic cohort in both the baseline and 24-month-followup cross-sectional comparison even though the change in mean T2 was faster in diabetic subjects. Mean T2 for the medial femur was even statistically significantly elevated in the non-diabetic cohort at baseline. Equally, texture parameters of medial femur and lateral femur demonstrate similar values in the baseline comparison while an especially steep increase over 24 months occurs in GLCM Contrast for these two compartments that results in statistically significantly elevated GLCM Contrast parameters in diabetic subjects in the 24-month cross-sectional follow-up. Possibly there are at least two opposed effects on cartilage ECM in diabetic subjects that examined individually would lead to either increase or decrease in T2 values. When looking at the ensemble of diabetic influences on articular cartilage that might yield such paradox results as we can observe in this present study. Appropriately, one recent study by Foreman et al. has shown that the initial stages of cartilage degradation might be associated with an increased mineralization of the deep cartilage layer which resulted in lower ultra-short echo time (UTE) T2* values (Foreman et al., 2019). Additionally, one histologic study in cartilage of racing horses suggests that

endochondral ossification of cartilage might help protect articular cartilage in the short-term without being able to halt the mechanisms that eventually lead to regular cartilage degradation (P. Muir et al., 2006). Such increased mineralization/ossification as a short-term protective mechanism in cartilage of diabetic subjects would be consistent with our partially non-elevated T2 values in diabetics at baseline that equal contained water content as observed in compensatory mineralized/ossified tissue. Over time, an accelerating decompensation is reflected in our statistically significantly faster T2 increase in the diabetic cohort due to pathological cartilage hydration and molecular collagen structure breakdown as observed in a pre-morphological stage of OA (Blumenkrantz et al., 2004; Joseph et al., 2011). Besides, our results are further confirmed by recent findings that show both the possibility of diabetic status without elevation of T2 values as well as biochemical alteration of knee cartilage ECM measured with quantitative in vivo sodium MRI that occurs prior to T2 elevation (Marik et al., 2016). This does not contradict diabetes-induced ECM damage in a prestructural stage measured by increased T2 values that has previously been demonstrated in a cross-sectional comparison between subjects with metabolic risk factors including DM, hypertension, high abdominal circumference and fat consumption.

Ultimately, our findings in the context of the latest research of biochemical changes in diabetic subjects measured using quantitative MRI techniques suggest that a complex chronological sequence of biochemical changes require different quantitative techniques to assess the level of damage occurred.

6.5 Limitations

This study has a few limitations. The share of African American study subjects was higher in our diabetic case group than in our healthy control group. In that context it is of particular importance to mention that racial differences have been proven when using T2 cartilage relaxation times to assess ECM composition (Kretzschmar et al., 2016; Yu et al., 2015). While there is evidence for a higher share of African American individuals among subjects suffering from DM, we addressed this imbalance by

consequently adjusting our analysis for race (Mokdad et al., 2000). Another limitation refers to the predictor variable chosen for this present study. While the OAI allows assessment of numerous study subjects and therefore renders large study cohorts possible, the large-scale clinical checkups included in the OAI are to a large portion limited to self-administered questionnaires (Lester, 2012). Equally, both study subjects suffering from DM and healthy subjects have been chosen based on self-reported diabetic status. The diagnosis of DM requires either measurement of fasting plasma glucose level, measurement of random plasma glucose, performance of a 2h oral glucose tolerance test or evaluation of the level of glycated hemoglobin (Petersmann et al., 2018). Clearly, self-reported diabetes status is prone to yield both false-positives and false-negatives in both cohorts as no appropriate diagnostic testing has been performed. Nevertheless, this present study compensates this qualitative assessment of diabetic status with large cohorts and robust study design. Besides, the self-reported diabetes status provided in the OAI clinical check-ups does not distinguish between type 1 and type 2 DM. Generally, the vast majority of DM cases, namely approximately 90%, can be attributed to DM type 2 (CDC, 2020a). Hence, our diabetic study cohort most likely encompasses mainly DM type 2 subjects.

Also, it has to be mentioned that we did not determine intra- and interreader reproducibility for the secondary outcome measures texture and laminar analyses. Our calculations of CVs were only performed for mean T2 values with the intuition that no further reproducibility calculations are needed as all parameters are based on the same cartilage segmentations/ROIs. This was handled equally in previous research projects with similar methodology (Gersing et al., 2017).

Moreover, there have well been contradictory results on T2 values in subjects suffering from diabetes in the past. While Jungmann et al. demonstrated that metabolic risk factors including hypertension, high fat consumption, DM and high abdominal circumference are linked to elevated T2 values in a cross-sectional comparison, Marik et al. did not find a statistically significant difference between diabetic subjects and healthy controls regarding cross-sectional mean T2 values (Jungmann et al., 2013; Marik et al., 2016). In that context it has to be noted that Marik et al. did not investigate T2 texture parameters that could have shown a difference between diabetics and controls. Happily, this present study's results might allow an explanation

of this contradiction as we have observed similar T2 values in the cross-sectional comparison at baseline and increasingly elevated T2 values in diabetic subjects at 24-month-follow-up. Thus, mean T2 values mainly depend on the point of time in the pathologic evolution of cartilage in diabetic subjects. Marik et al. suggest that very early biochemical changes might not even occur on T2 maps and further quantitative assessment is required to identify such damage using for example quantitative in vivo sodium MRI (Marik et al., 2016). Further research is needed to evaluate whether T2, sodium MRI, dGEMRIC, T1rho or sparsely investigated techniques like GAG Chemical Exchange Saturation Transfer (gagCEST) imaging that focuses on GAGs are best suited to detect early-stage diabetes-induced changes (Brinkhof et al., 2018). A combination of various techniques appears most promising.

6.6 Conclusion

Finally, this study provides distinct confirmation of the association between DM and OA. The diabetic study cohort of this study demonstrated a statistically significantly faster increase of mean T2 in the hyaline cartilage of the knee as well as more inhomogeneous GLCM Texture parameters at the cross-sectional comparison at baseline and at 24-month-follow-up.

Ideally, future research endeavours will further elucidate pathophysiological pathways that connect DM and OA. Regarding non-invasive, MRI-based evaluation, long-term studies are suggested that simultaneously perform various quantitative MRI measurements in the same cohorts over a preferably long study duration with frequent measurements. It is recommended to connect imaging methods with serological verification of diabetes as well as the investigation of inflammatory markers. Further longterm assessment on protective effects of pharmaceutical diabetes treatment could be investigated in the future.

7 Abstract/Zusammenfassung

7.1 Abstract in English

Objective

The aim was to explore the longitudinal impact of diabetes mellitus (DM) on the rate of degeneration of cartilage extracellular matrix (ECM) over a time period of 2 years. Another aim was to investigate the pathophysiological link between DM and osteoarthritis (OA) by assessing the composition of articular knee cartilage using quantitative/advanced cartilage imaging technique.

Methods

The study population of the Osteoarthritis Initiative (OAI), a prospective, multicenter observational study served as the data basis for this study. 196 subjects suffering from DM and 196 healthy controls from the Osteoarthritis Initiative (OAI) were matched in small sets for age, sex, BMI and KL grade using self-reported diabetes status as predictor variable. MR-based quantitative compositional knee cartilage imaging was performed using the sagittal 2D multi-echo spin-echo sequence (SAG 2D MESE) included in the OAI protocol. T2 maps were created from the SAG 2D MESE images to generate compartment-specific T2 parameters for the five compartments medial tibia (MT), medial femur (MF), lateral tibia (LT), lateral femur (LF) and patella (PAT). Semi-automatic segmentation to yield these compartments occurred under supervision of experienced radiologists. The resulting T2 values including laminar and grey-level co-occurrence matrix (GLCM) texture sub-analysis were used to assess cross-sectional differences as well as longitudinal changes in the composition of cartilage ECM. Cross-sectional differences of T2 values at baseline and follow-up as well as changes over the period of interest of 24-months were assessed using linear regression models.

Results

The two study cohorts were comparable regarding gender (46.4% male in the control group vs. 45.9% in the diabetic group; p = 0.919), age (63.31 ± 9.17 in the control group vs. 62.96 ± 8.99 in the diabetic group; p = 0.701), KL grade distribution (p=0.965) and Body Mass Index (BMI) (30.91 ± 4.50 kg/m² in the control group vs. 31.20 ± 4.51 kg/m²

in the diabetic group; p = 0.523). Over the follow-up interval of 24 months, all compartments except for the patella showed a statistically significantly higher increase in mean T2 values that amounted 1.77 ms (CI: 1.51, 2.03) for diabetics vs. 0.98 ms (CI: 0.68, 1.28) in the controls for the global knee cartilage compartment (p<0.001). Similarly, the laminar bone layer cartilage sub-analysis displayed a statistically significantly faster change in mean T2 values of the global knee compartment with 1.51 ms (CI: 1.28, 1.75) for diabetics vs. 0.98 ms (CI: 0.71, 1.25) in the controls for the global knee compartment (p=0.006). GLCM Texture parameters were statistically significantly elevated (p<0.05) in diabetic subjects compared to non-diabetic controls for the majority of compartments at both cross-sectional time points baseline and 24-month follow-up with a statistically significant elevation of GLCM Contrast in all compartments at 24-month follow-up.

Conclusion

This present dissertation shed light on the association between DM and OA. The articular knee cartilage of subjects suffering from DM experiences a statistically significantly higher increase in mean T2 values as well as a more inhomogeneous texture measured using GLCM texture parameters. Thus, our results suggest a more severe deterioration of the cartilage ECM over time in subjects suffering from diabetes compared to healthy controls. Further research on different quantitative MRI techniques will have to assess the chronologic sequence of ECM deterioration in diabetic patients. To sum it up, our study confirms DM as a risk factor in the early, pre-morphologic development of OA.

7.2 Zusammenfassung auf Deutsch

Zielsetzung

Ziel dieser Studie war den longitudinalen Einfluss von Diabetes mellitus (DM) auf die Geschwindigkeit der Degeneration der extrazellulären Matrix (EZM) von Knorpelgewebe über einen Zeitraum von zwei Jahren zu untersuchen. Ein weiteres Ziel war es, die pathophysiologische Verbindung zwischen DM und Arthrose zu erforschen, indem die Zusammensetzung von Knorpelgewebe des Kniegelenks mittels quantitativer/fortschrittlicher Techniken der Knorpelbildgebung analysiert wurde.

Methodik

Die Studienpopulation der Osteoarthritis Initiative (OAI), einer prospektiven, multizentrischen Beobachtungsstudie, diente als Grundlage der Datenerhebung. 196 Studienteilnehmer, die an DM leiden, sowie 196 gesunde Studienteilnehmer jeweils aus der OAI wurden gegenübergestellt, wobei eine Aufteilung in Gruppen mit jeweils ähnlichem Alter, Geschlecht, BMI, sowie Kellgren & Lawrence (KL)-Grad erfolgte. Als Prädiktorvariable diente das durch Selbstauskunft ermittelte Leiden an Diabetes. MRT-basierte, quantitative Knorpelbildgebung wurde eingesetzt, um die Zusammensetzung der EZM zu analysieren. Hierbei wurde die sagittale 2D multi-echo spin-echo Sequenz (SAG 2D MESE) aus dem OAI Studienprotokoll verwendet.

T2 maps wurden mithilfe der SAG 2D MESE Aufnahmen erstellt, um mit deren Hilfe die Kompartiment-spezifischen T2-Parameter für die fünf Kompartimente mediale Tibia (MT), mediales Femur (MF), laterale Tibia (LT), laterales Femur (LF) und Patella (PAT) zu generieren. Halbautomatische Segmentierung, die eingesetzt wurde, um diese Kompartimente zu erhalten, erfolgte unter der Aufsicht erfahrener Radiologen. Die hieraus gewonnenen T2-Werte, einschließlich der laminaren Subanalyse, sowie der Grey-Level Co-occurrence Matrix (GLCM)-basierten Textur-Subanalyse wurden eingesetzt, um Unterschiede der beiden Gruppen im Querschnitt zu Studienbeginn und Studienende zu analysieren, sowie Veränderungen der Zusammensetzung der EZM des Knieknorpels im longitudinalen Verlauf zu vergleichen. Hierbei wurden die T2-Werte im Querschnitt zu Studienbeginn und -ende, sowie Veränderungen über die betrachtete Studiendauer von 24 Monaten mithilfe von linearen Regressionsmodellen untersucht.

Ergebnisse

Die beiden Studienkohorten waren vergleichbar hinsichtlich Geschlecht (46,4% männliche Teilnehmer in der Kontrollgruppe versus 45,9% in der diabetischen Fallgruppe; $p = 0{,}919$), Alter (63,31 ± 9,17 in der Kontrollgruppe versus 62,96 ± 8,99 in der diabetischen Fallgruppe; $p = 0{,}701$), KL-Grad-Verteilung ($p = 0{,}965$) und Verteilung des Body Mass Index (BMI) (30,91 ± 4,50 kg/m² in der Kontrollgruppe versus 31,20 ± 4,51 kg/m² in der diabetischen Fallgruppe; $p = 0{,}523$). Über den Zeitraum der Studiendauer zeigten alle Kompartimente außer der Patella einen statistisch

signifikant stärkeren Anstieg im mittleren T2-Wert der sich auf 1,77ms (Konfidenziuntervall [KI]: 1,51; 2,03) für die diabetische Fallgruppe versus 0,98ms (KI: 0,68; 1,28) für die Kontrollgruppe belief, bezogen auf das globale Knieknorpel-Kompartiment (p<0,001). Gleichermaßen zeigte die laminare Subanalyse, dass die dem Knochen aufliegende Knorpelschicht einen statistisch signifikant schnelleren Anstieg des mittleren T2-Wertes für das globale Knieknorpel-Kompartiment für die diabetische Fallgruppe im Vergleich zur Kontrollgruppe zeigte mit 1,51 ms (KI: 1,28; 1,75) für die diabetische Fallgruppe versus 0,98 ms (KI: 0,71; 1,25) in der Kontrollgruppe für das globale Knieknorpel-Kompartiment (p=0,006). GLCM-Texturparameter waren statistisch signifikant erhöht (p<0.05) in der diabetischen Fallgruppe im Vergleich zur Kontrollgruppe für die Mehrheit der Kompartimente zu den beiden Querschnitts-Zeitpunkten Studienbeginn und Verlaufskontrolle nach 24 Monaten. Hierbei war der GLCM Texturparameter Contrast zum Zeitpunkt der 24 Monate Verlaufskontrolle statistisch signifikant erhöht für alle Kompartimente.

Schlussfolgerungen

Diese Dissertation setzt sich mit dem Zusammenhang zwischen DM und Arthrose auseinander. Der Gelenkknorpel des Knies von Studienteilnehmern, die an DM leiden, erfährt einen statistisch signifikant schnelleren Anstieg des mittleren T2-Wertes und stellt sich mit einer inhomogeneren Textur gemessen mithilfe der GLCM Texturparameter dar. Die Ergebnisse der Dissertation suggerieren eine stärkere Abnutzung der EZM des Knorpels im zeitlichen Verlauf bei Subjekten, die an Diabetes leiden im Vergleich zu gesunden Kontrollen. Weitere Forschung zu verschiedenen quantitativen MRT-Techniken wird benötigt, um die chronologische Abfolge der Degeneration der EZM des Knorpels zu untersuchen. Zusammengefasst bestätigt die Dissertation, dass DM als Risikofaktor wirkt für die Entwicklung einer frühen, prämorphologischen Form der Arthrose.

8 List of figures

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10 References

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11 Publications

Journal papers

3. Neumann J, **Hofmann FC**, Heilmeier U, Ashmeik W, Tang K, Gersing AS, Schwaiger BJ, Nevitt MC, Joseph GB, Lane NE, McCulloch CE, Link TM. Type 2 diabetes patients have accelerated cartilage matrix degeneration compared to diabetes free controls: data from the Osteoarthritis Initiative. Osteoarthritis Cartilage. 2018 Jun;26(6):751-761. doi: 10.1016/j.joca.2018.03.010. Epub 2018 Mar 29. PMID: 29605381; PMCID: PMC5962437.
4. Chanchek N, Gersing AS, Schwaiger BJ, Nevitt MC, Neumann J, Joseph GB, Lane NE, Zarnowski J, **Hofmann FC**, Heilmeier U, McCulloch CE, Link TM. Association of diabetes mellitus and biochemical knee cartilage composition assessed by T_2 relaxation time measurements: Data from the osteoarthritis initiative. J Magn Reson Imaging. 2018 Feb;47(2):380-390. doi: 10.1002/jmri.25766. Epub 2017 May 26. PMID: 28556419; PMCID: PMC5702599.
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Conference paper

1. Neumann, J., Heilmeier, U., Joseph, G. B., **Hofmann, F. C.**, Ashmeik, W., Gersing, A. S., Chanchek, N., Schwaiger, B. J., Nevitt, M. C., McCulloch, C. E., Lane, N. E., Liu, F., Lynch, J. A., & Link, T. M. (2017). Texture analysis of T2 maps of the cartilage indicates differences in knee cartilage matrix in subjects with type 2 diabetes: data from the osteoarthritis initiative. Osteoarthritis and Cartilage, 25, S73–S74. https://doi.org/10.1016/j.joca.2017.02.130
2. **Hofmann, F.**, Heilmeier, U., Mbapte Wamba, J., Joseph, G., Darakananda, K., Callan, J., Neumann, J., Kretzschmar, M., Nevitt, M., McCulloch, C., Liu, F., Lynch, J., & Link, T. (2017). MRT-basierte, semi-quantitative Analyse des Kniegelenks eignet sich zur Vorhersage der Implantation von Knie-Totalendoprothesen. RöFo - Fortschritte auf dem Gebiet der Röntgenstrahlen und der bildgebenden Verfahren, 189(S 01), S1–S124. https://doi.org/10.1055/s-0037-1600368
3. Bayat A., Sekuboyina A., **Hofmann F.**, Husseini M.E., Kirschke J.S., Menze B.H. (2020) Vertebral Labelling in Radiographs: Learning a Coordinate Corrector to Enforce Spinal Shape. In: Cai Y., Wang L., Audette M., Zheng G., Li S. (eds) Computational Methods and Clinical Applications for Spine Imaging. CSI 2019. Lecture Notes in Computer Science, vol 11963. Springer, Cham

Conference posters

2. **Hofmann, F. C.**, Neumann, J., Heilmeier, U., Joseph, G. B., Ashmeik, W., Nevitt, M. C., McCulloch, C. E., Lane, N. E., Liu, F., Lynch, J. A., & Link, T. M. (2017). MRI-Based T2 Knee Cartilage Texture Analysis Can Differentiate Subjects With and Without History of Knee Injury Despite Similar Morphological Scores: Data from the Osteoarthritis Initiative. Osteoarthritis and Cartilage, 25, S239–S240. https://doi.org/10.1016/j.joca.2017.02.406
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5. Heilmeier, U., Wamba, J. M., Joseph, G. B., Darakananda, K., Callan, J., Neumann, J., Hofmann, F., Kretzschmar, M., Nevitt, M. C., McCulloch, C. E., Lane, N. E., Liu, F., Lynch, J. A., & Link, T. M. (2017). MR-Based Whole-Organ Magnetic Resonance Imaging Score (Worms) Parameters are Predictors of Incident Total knee Arthroplasty 4 to 7 Years Later. Osteoarthritis and Cartilage, 25, S236. https://doi.org/10.1016/j.joca.2017.02.402

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