UNIVERSITEIT
GENT

Faculty of Medicine and Health Sciences

Department of Rehabilitation Sciences and Physiotherapy

Effects of *in vivo* exercise on cartilage deformation and functional adaptation

A fundamental approach toward clinical implementation in
the prevention and treatment of knee osteoarthritis

by

Ans Van Ginckel

Thesis submitted in fulfillment of the requirements for

the Degree of Doctor in Motor Rehabilitation and Physiotherapy

Ghent 2013

"In the middle of difficulty lies oppurtunity"

(Albert Einstein)

Promotor

Prof Dr Erik Witvrouw, Ghent, Belgium

Doctoral Guidance Committee

Prof Dr Erik Witvrouw, Ghent, Belgium

Prof Dr Philip Roosen, Ghent, Belgium

Prof Dr Koenraad Verstraete, Ghent, Belgium

Prof Dr Fredrik Almqvist, Ghent, Belgium

Doctoral Examination Committee

Prof Dr Jan Victor, Ghent, Belgium

Prof Dr May Arna Risberg, Oslo, Norway

Prof Dr Johan Bellemans, Louvain, Belgium

Prof Dr Dieter Van Assche, Louvain, Belgium

Prof Dr Nele Mahieu, Ghent, Belgium

Dr Annelies Maenhout, Ghent, Belgium

TABLE OF CONTENTS

LIST OF PUBLICATIONS

The work presented in this dissertation consists of the following manuscripts:

I **Van Ginckel A**, Almqvist F, Verstraete K, Roosen P, Witvrouw E. Human ankle cartilage deformation after different *in vivo* impact conditions. Knee Surg Sports Traumatol Arthrosc 2011;19(1):137-43. Epub 2010 May 20.

II **Van Ginckel A**, Baelde N, Almqvist KF, Roosen P, McNair P, Witvrouw E. Functional adaptation of knee cartilage in asymptomatic female novice runners compared to sedentary controls. A longitudinal analysis using delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC). Osteoarthritis Cartilage 2010;18(12):1564-9. Epub 2010 Oct 13.

III **Van Ginckel A**, Roosen P, Almqvist KF, Verstraete K, Witvrouw E. Effects of *in vivo* exercise on ankle cartilage deformation and recovery in healthy volunteers: an experimental study. Osteoarthritis Cartilage 2011;19(9):1123-31. Epub 2011 Jun 26.

IV **Van Ginckel A**, Verdonk P, Witvrouw E. Cartilage adaptations following anterior cruciate ligament injury and reconstruction: implications for clinical management and future research? A systematic review of longitudinal MRI studies. Osteoarthritis Cartilage 2013; doi:pii: S1063-4584(13)00787-5. 10.1016/j.joca.2013.04.015. Epub 2013 May 15.

V **Van Ginckel A**, Verdonk P, Victor J, Witvrouw E. Cartilage status in relation to return to sports after anterior cruciate ligament reconstruction. Am J Sports Med 2013; 41(3):550-559. Epub 2013 Feb 4.

VI **Van Ginckel A**, Witvrouw E. Acute cartilage loading responses after an *in vivo* squat exercise in doubtful or mild knee osteoarthritis. A case-control study. Phys Ther 2013; doi: 10.2522/ptj.20120491. Epub 2013 April 11.

LIST OF ABBREVIATIONS

(RMS)CV	(Root Means Square) Coefficient of Variation
2D IM TSE/ FSE	Two-dimensional Intermediate-weighted Turbo Spin-Echo/Fast Spin-Echo
3D DESS WE	Three-dimensional Dual Echo in the Steady State Water Excitation
3D FLASH WE	Three-dimensional Fast Low Angle Shot Water Excitation
3D GRE	Three-dimensional Gradient-Echo
3D SPGR WE	Three-dimensional Spoiled Gradient Water Excitation
3D	Three-dimensional
ACL	Anterior Cruciate Ligament
ADL	Activities Daily Living
AGE	Advanced Glycation End-products
BLOKS	Boston Leeds Osteoarthritis Knee Score
BMI	Body Mass Index
BML	Bone Marrow Lesion
BPTB	Bone-Pateller Tendon-Bone
COMP	Cartilage Oligomeric Matrix Protein
DEXA	Dual-energy X-Ray Absorptiometry
dGEMRIC	delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage
FA	Flip Angle
FL	Femur Lateral
FM	Femur Medial
FORSS	Factor Occupational Rating System Scale
FOV	Field Of View
FTSTS	Five Times Sit To Stand
GAG	Glycosaminoglycan
Gd-PTA^{2-}	Gadolineum-Diethylene Triamine Pentaacetic Acid
GLM	General Linear Model
IC/IM	Inter-Condylar/Inter-Malleolar
ICC	Intra-Class Correlation Coefficient
ICRS	International Cartilage Repair Society
IGF	Insuline-Growth Factor
IL	Interleukine
IR	Inversion Recovery
K/L grade	Kellgren-Lawrence grade
K/L	Kellgren/Lawrence
KOOS	Knee injury and Ostearthritis Outcome Score
KOSS	Knee Osteoarthritis Scoring System
LSI	Lower Symmetry Index
ME (T)SE or (F)SE	Multi-Echo (Turbo) Spin-Echo or (Fast) Spin-echo
ME GRE	Multi-Echo Gradient-Echo
MOAKS	MRI Osteoarthritis Knee Score

MRI	Magnetic Resonance Imaging
NS	Nonsignificant
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
OARSI-FDA	Osteoarthritis Research Society International – Food Drug Administration
PD	Proton-Density
PF	Patello-Femoral
PG	Proteoglycan
Sag	Sagittal
STL	Stereolithography
STR	Start To Run novice runner program
T	Tesla (e.g., 3T, 1.5T,...)
TA	Acquisition Time
TE	Echo Time
TI	Inversion Time
TL	Tibia Lateral
TM	Tibia Medial
TNF	Tumor Necrosis Factor
tpostt0-15-30-45	post-scans immediately after activity and according to 15-minute intervals
tpre	pre-scan prior to activity
TQS	Total Quality Score
TR	Repetition Time
TSL	Spin Lock Time
UTE	Ultra-short TE
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Universities Arthritis Index
WORMS	Whole Organ Magnetic Resonance Imaging Score
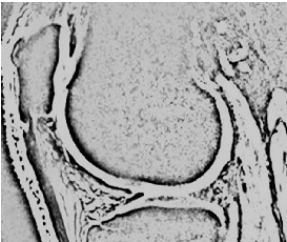
RATIONALE

"New knowledge of osteoarthrosis must be gained if the later years of our lengthening lives are not to be plagued by increasing pain and disability" —J.H. Kellgren (1961)[1]

Osteoarthritis (OA) is the most common form of arthritis and is depicted as a major cause of morbidity and disability as well as a burden on health-care resources, especially in the elderly.[2] While ankle and elbow are usually spared, frequently afflicted joints include hands, hip, lumbosacral spine and knee, the latter being addressed the most in epidemiological reports.[3, 4] A recent systematic review reported knee OA prevalence in epidemiological studies to range from 6.3% to 70.8 % with radiographic OA providing the highest estimates next to symptomatic or self-reported OA.[4] Due to the heterogeneity of the disease entity and the discordance between pathology and clinical presentation, stating a single definition of OA remains challenging.[2, 3, 5, 6] In 2011, the OARSI-FDA Initiative published the following operational definition formulated by consensus and based on the up-to-date research.[5]

> "*OA is usually a progressive disease of synovial joints that represents failed repair of joint damage that results from stresses that may be initiated by an abnormality in any of the synovial joint tissues, including articular cartilage, subchondral bone, ligaments, menisci (when present), peri-articular muscles, peripheral nerves, or synovium. This ultimately results in the breakdown of cartilage and bone, leading to symptoms of pain, stiffness and functional disability. Abnormal intra-articular stress and failure of repair may arise as a result of biomechanical, biochemical and/or genetic factors. This process may be localized to a single joint, a few joints, or generalized, and the factors that initiate OA likely vary depending on the joint site. The complexity and variability of OA etiology suggests the need for patient-specific, etiology-based treatment.*"[5]

Although OA should be considered a whole-organ disease, cartilage loss remains a hallmark of its progression.[3, 5, 7-10] Current research and clinical practice generally focus on the patient with established – and thus advanced or end-stage – disease, implementing mainly palliative care. Hence, a disease paradigm shift was proposed concentrating on those individuals at high risk of developing OA (i.e., obesity and joint injury such as ACL injury) or with early disease in which structural changes may be preventable or reversible.[11] In this respect, research should consider the effects of treatment on structural changes at the joint level separately from the effects on patient-reported symptoms or illness-level.[5]

Clinical guidelines on the management of knee OA prescribe exercise as a vital component of first-line treatment strategies. Both strengthening as well as aerobic exercise showed to alleviate symptoms (i.e., pain, stiffness) and improve physical function.[12-14] Despite these assets, however, effects of exercise on structural joint integrity remain elusive.[14-18] While physical therapy management in terms of exercise therapy may play a vital role in preservation of cartilage structural integrity in patients at risk for (accelerated) OA development, weight-bearing

exercise may be argued to further rather than decelerate OA progression.[14, 19] Therefore, more insight into the effects of *in vivo* exercise on cartilage status is critical.

The purpose of this dissertation was to investigate the effects of *in vivo* exercise on cartilage status in healthy volunteers, as well as in individuals at increased risk for disease development (i.e., anterior cruciate ligament (ACL) injury and reconstruction) and in those diagnosed with early radiographic OA (Kellgren-Lawrence (K/L) grade of maximum 2). To this end, this dissertation first provides a background to understand the current knowledge of MRI-measured exercise effects on human articular cartilage (General background). Secondly, based on the "Rationale" and "General Background" reasoning is provided for the specific research questions as pursued in this dissertation (Aims and outline). The results of this dissertation may facilitate appropriate exercise prescription or
implementation in the prevention and treatment of knee OA (Chapters 1 to 6, General Discussion).
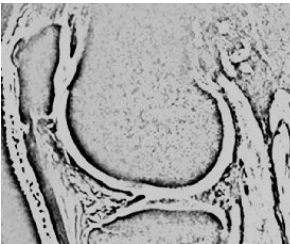
GENERAL BACKGROUND

1   Articular cartilage structure and function in health and disease

In diarthrodial joints, articular hyaline cartilage covers the subchondral bone and lines the joint surface. Its primary function consists of stress dissipation, providing a frictionless surface during joint articulation and improving joint surface congruence. To this end, the tissue presents as highly organized and complex exhibiting unique material properties that allow deformation to some extent in order to pursue its function.[20-23]

Within the tissue, a sparse population of cells ("**chondrocytes**") reside within - and synthesize - an extensive extra-cellular matrix comprising mainly **collagen** and **proteoglycan** (PG) macromolecules and **glycoproteins**. At this level, an organization into 4 zones is described which reflect the tissue's functional role (Figure 1): (1) the **superficial tangential zone** (i.e., 10-20% of cartilage thickness), (2) the   **middle or transitional zone** (i.e.,60% of cartilage thickness), (3) the **deep or radial zone** (i.e., 30% of cartilage thickness), and (4) the **calcified cartilage zone** representing the cartilage-bone interface.[20, 21, 23, 24]

Collagen fibers, predominantly type II, make up about 20% of cartilage wet weight and construct a 3D framework aiming to withstand tensile and shear stresses. The PG macromolecules, predominantly aggrecan, account for about 5% of the wet weight and are aggregates composing of sulphated **glycosaminoglycan** (GAG) (i.e., chondroitin sulphate, keratin sulphate) side chains attached onto a hyaluron acid core. The negatively charged GAGs are attached to the collagen fibers through linking glycoproteins (e.g., Anchorine CII, COMP) and serve as the **fixed charged density** of the matrix while attracting cations (i.e., Donnan Theory of Equilibrium[a])[24] and thus, water molecules. Consequently, within this porous-permeable structure, osmotic swelling pressures are created that, combined with intrinsic electrostatic repulsion (i.e., "chemical expansion stress"), enable cartilage to cushion or dissipate compression stress and maintain its degree of hydration. In this respect, the water component, **interstitial fluid**, is responsible for 70-80% of the tissue's wet weight of which 6% is reported to bind to collagen, 14% to bind to PG molecules and the remaining 80% to represent free, bulk water.[21-25]

In view of its complex tension-compression nonlinearity, anisotropy, spatial inhomogeneity, articular cartilage mechanical behavior remains challenging to comprehensively describe.[23]

To gain insight into the **material properties**, the ultra-structural organization of the tissue requires a **multi-phasic** approach considering the behavior of solid (i.e., mainly PG and collagen) and fluid phases (i.e., mainly water and dissolved electrolytes).[22, 23] In this respect, articular cartilage should be regarded, at minimum, as a 2-phase material, that is a porous-permeable fiber-reinforced solid phase and a freely flowing fluid phase.[22]

---

[a] Donnan Theory of Equilibrium: in pursuit of electro-neutrality the distribution of interstitial electrolytes is influenced by the fixed charged density.[24]
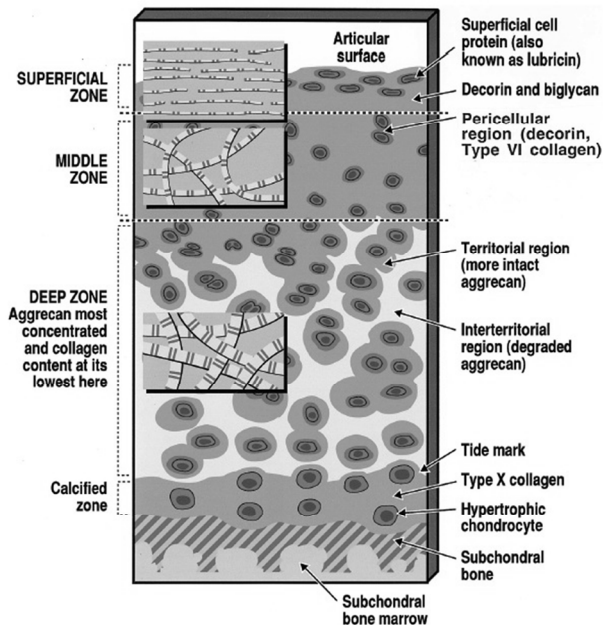
Figure 1. General structure of human adult articular displaying cartilage zones, regions, and relationship with subchondral bone. The insets show the relative diameters and organizations of collagen II macrofibrils in the different zones. Some special features of molecular content or properties also are indicated.[19]

Under loading conditions, cartilage displays **visco-elastic behavior** primarily due to flow of interstitial fluid that exerts frictional drag on the solid phase and depends upon the porosity and permeability of the extra-cellular matrix.[22] Consequently, the **linear biphasic theory** describes that loading induces instantaneous hydraulic pressurization within the tissue that is initially supported by the fluid phase (i.e., osmotic swelling pressure and chemical expansion stress). At this point, the tissue's compressive stiffness shields the solid phase from excessive strain. When loading continues, hydraulic pressures are gradually overwhelmed and fluids exude through the porous matrix, however, constrained by permeability. Deformation of the tissue will cease when equilibrium is reached and the load is entirely supported by the solid phase. Material properties of articular cartilage are (being) extensively studied in indentation, (un-)confined compression experiments that allow

quantification of compression stiffness moduli and permeability to fluid flow (e.g. Young's modulus, aggregate compression modulus, dynamic modulus, Poisson's ratio).[22, 26, 27] Measurements of knee cartilage compressive stiffness are prone to topographical variation and appear most likely related to PG or GAG content.[28]

Extra-cellular matrix homeostasis is maintained by an equilibrium between anabolic and catabolic pathways controlled by growth factors (e.g., Insuline-Growth Factor 1 (IGF-1), platelet-

derived growth factors) and cytokines (e.g., Interleukine (IL) 1, Tumor Necrosis Factor (TNF) α) respectively, synthesized by the chondrocytes and synovial lining cells.[3, 29] In **OA**, cartilage damage most likely presents first in the extra-cellular matrix. Despite the compensatory turn-over of matrix constituents by the chondrocytes (characterized by cell hypertrophy),[30-32] maintenance of homeostasis fails as degradation outweighs synthesis.[3, 31] The combination of a disrupted collagen network and/or decreasing PG content further increased matrix permeability that, in turn, brings about accumulation of bulk water and swelling of the tissue - a feature of early disease.[3] Increased permeability, and hence, decreased compressive stiffness upon a loading event, jeopardizes the protection of the solid matrix compounds evolving towards a negative vicious circle of matrix breakdown, chondrocyte apoptosis, and eventually cartilage volume loss.[3, 33-35] **Chondrocyte senescence and age-related changes in other synovial structures** are entangled with or predispose for OA development.[33] Changes in chondrocyte energy metabolism, growth factor stimulated cell signaling, and production of catabolic factors affect matrix structure and compressive stiffness (e.g., PG size, structure or sulphation, dehydration, collagen cleavage, increased deposition of advanced glycation endproducts (AGE) such as pentosidine, the latter reported to increase collagen crosslinking and decline anabolic processes). Combined with progressive cartilage thinning, concomitant meniscal and ligamentous degradation, bone (marrow) lesions (BML), sarcopenia, increased fat deposits, and changes in proprioception increase the ageing joint's vulnerability for OA development.[36-38]

The **relation of cartilage to (first) clinical signs in OA** is likely through secondary mechanisms such as (1) exposure of the subchondral bone nociceptors as would be the case in traumatic or osteoarthritic full-thickness defects, (2) alterations in cartilage deformational behavior in case of cartilage defects and swelling of the tissue leading to increased pressure onto the subchondral bone[39-41] with potential formation of BML, (3) vascular congestion of the underlying subchondral bone in case of BML leading to increased intra-osseous pressure and pain, (4) synovitis secondary to articular cartilage damage with activation of synovial membrane nociceptors.[42] Indeed, BML and synovitis and effusion are assigned important sources of pain in the OA knee.[43]   While clinical presentation and joint structural health do not strongly correlate in the early phases of OA,[5] technical investigation methods (e.g., MRI, biomarker monitoring in synovial fluid, urinary, or blood samples) could facilitate early diagnosis.

2   Importance of load for chondrocyte biosynthetic activity and cartilage viability: an *in vitro* perspective

Articular cartilage is an avascular, aneural and alymphatic tissue.[21, 44] Despite these depriving circumstances that account for limited repair capacity, chondrocytes are highly active cells that, while being few in number, need to maintain the structural integrity and quality of the entire

extra-cellular matrix.[44] To this end, mechanical stimulation or loading is of paramount importance to guarantee chondrocyte metabolism and cartilage viability.[45] Intermittent loading effects **an exchange of nutritional and waste products** between the extra-cellular matrix and the synovial fluid which is considered, next to the subchondral bone, the primary source for cartilage nutrition.[44]

Additionally, **mechanotransduction** processes are activated that translate the accompanying mechanical signals (i.e., cell and matrix deformation, hydrostatic pressure gradients, fluid flow with altering concentration of water molecules, ions, fixed charged density, etc.) into biochemical activity.[44, 45] During loading of the tissue, mechanical signals are recognized by the chondrocytes through dedicated receptors such as mechano-sensitive ion channels and integrins.[46] Additionally, mechanical loading stimulates chondrocytes to release anabolic and catabolic factors to bind and activate cell surface receptors that, together with mechanical stimuli, may work antagonistically, additively or synergistically.[46] Activation of receptors gives way to intra-cellular signaling cascades that, in turn, regulate the production of various molecules in order to maintain cartilage viability. *In vitro* experiments in healthy and osteoarthritic cartilage revealed that dynamic intermittent loading protocols - depending upon duration, frequency and magnitude - generally up-regulated matrix synthesis,[44, 45] while in contrast, static and injurious impacts tended to decrease the production of matrix compounds and to stimulate protease activity.[44]

3 Effects of *in vivo* exercise on cartilage status: an obscure relationship?

From a theoretical perspective, one may reason that – as intermittent dynamic loading is required for cartilage health – exercise and physical activity should be beneficial in view of structural longevity of the (knee) joint. However, several epidemiological studies investigated the relationship between physical activity and radiographic OA and reported conflicting results. While some studies established an increased risk for OA,[47, 48] others contrarily suggested no association or even a protection from degenerative disease.[49-52] The disparity in results may be due to different (self-reported) types of activity (e.g, moderate running vs cross-country ski), levels of participation (e.g., recreational vs elite), risk for concomitant joint injury, or person-related factors (e.g., age, gender, BMI, etc.).[53, 54] Foremost, those studies investigated joint structure using radiography that with high specificity, but low sensitivity, monitors OA-related bony changes only, hence, providing a limited view on the disease process.[54, 55] With the advent of Magnetic Resonance Imaging (MRI), exercise-related effects on all synovial structures, including cartilage, can be monitored both in the short- as well as in the long-term.

3.1 <u>MRI Investigation of cartilage responses to *in vivo* exercise</u>

MRI of articular cartilage has evolved into an important diagnostic tool in OA research.[56] As compared to other investigation methods such as radiography, arthroscopy, serum-, synovial fluid or urinary biomarkers, MRI proves advantageous in terms of location-specific visualization and direct evaluation of cartilage tissue with the potential for sub-surface or laminar analyses. As it is a fast evolving and innovating field, MRI techniques are now available to detect ultra-structural deterioration prior to overt macroscopic lesions or radiographic signs. Despite its cost and dependency on specialized post-processing algorithms, MRI markers show promise as endpoints in *in vivo* research on the management of OA, including the effect of treatment modalities such as exercise.[56]

An MRI cartilage evaluation might entail **semi-quantitative or quantitative techniques**. Whereas semi-quantitative evaluations concentrate on tissue **morphology**, quantitative techniques focus on either morphology or **biochemical composition**. Hence, semi-quantitative morphological scoring methods subjectively evaluate (sub-regional) depth and/or areal extent of potential cartilage lesions, while quantitative morphology uses computer-aided image processing techniques that allow extraction of (sub-regional) 3D metrics such as volume, thickness, or surface area.[57-60] Quantitative compositional imaging aims at targeting extra-cellular matrix ultra-structure mainly involving PG or GAG content and collagen fiber organization or concentration in interaction with the tissue's water content.[57, 61, 62 63]

Within this specific field of study, short-term effects that monitor acute cartilage loading responses immediately following a single *in vivo* exercise using quantitative imaging techniques are commonly referred to as "**deformation or deformational behavior**" and are considered an *in vivo* representation of cartilage function or tissue resiliency. Long-term effects or evaluations based on either semi-quantitative or quantitative techniques, are usually depicted as "**functional adaptation**".[64]

3.1.1 MR imaging techniques and post-processing methods

In clinical practice, **cartilage morphological assessment** with respect to the detection of focal (surface) lesions is most often performed implementing a fluid-sensitive 2D (fat-suppressed) intermediate (IM)-weighted Turbo Spin-Echo (i.e., 2D IM TSE) sequence. Next to proton density (PD)- or true T2-weighted imaging, 2D IM TSE sequences generate good contrast between the cartilage and subchondral bone or joint fluid within a reasonable scan time and appear less susceptible to magic angle effects[b].[61, 65, 66] While acquisition in 3 planes is required, however, TSE additionally suffers from anisotropic[c] voxels, thicker slices and inter-section gaps and is prone to partial volume averaging[d] limiting its capability to detect small defects.

3D gradient-echo (3D GRE) cartilage-dedicated sequences such as 3D Spoiled Gradient (SPGR) or Fast Low Angle Shot (FLASH) (fat-suppressed by means of water excitation (WE))

9

may overcome these limitations. Although 3D SPGR/FLASH may be less suitable for focal (surface) lesion detection and run with longer acquisition times when compared to 2D TSE, these sequences provide high intrinsic cartilage signal with high-resolution, near-isotropic acquisitions and thinner slices that avoid partial volume averaging.[61, 66] 3D Dual Echo in the steady State (DESS) WE sequences, another 3D GRE method, proved superior over 3D SPGR/FLASH WE in terms of time-efficient acquisitions, signal-to-noise ratio, cartilage-to-fluid contrasts and thinner sections.[67] Other emerging techniques in morphological imaging involve 3D TSE (e.g., SPACE), VIPR, bSSFP or DEFT imaging. However, these techniques remain to be consistently implemented in (large) clinical trials.[66, 68] In conclusion, semi-quantitative cartilage scoring preferably encompasses at least an IM-TSE sequence whereas cartilage-dedicated 3D GRE sequences are suitable for 3D quantitative assessment of cartilage morphology.[65, 66] (Figure 2)

---

[b] Magic angle effect/artifact: Increased signal on MR images acquired with short TE sequences in tissues with ordened fibrilar structure (e.g., tendon, hyaline cartilage). Artifact may present when fibres are oriented to the main magnetic field according to an angle equaling approximately 54.7356° (i.e., the magic angle) . The hyperintense signals may simulate pathologic features.

[c] isotropic voxels: Voxels cubical in shape (i.e., height=width=depth). (Near-) isotropic voxels facilitate multi-planar reformatting and reconstruction of accurate and smooth 3D reconstructions.

[d] Partial volume averaging/effects: Any artifact which is caused by the size of the image voxel. It occurs when multiple tissue types are encompassed within a single voxel (often in sections near structure margins or in orientations oblique to main magnetic field). Voxel signal intensity equals the weighted average of the quantity of multiple tissue present in the voxel (e.g water and fat). Partial volume effects may simulate abnormalities, decrease the visualization of low-contrast abnormalities, and blur or distort affected structures. Hence, these artifacts should be reduced in order to obtain accurate 3D reconstruction by reducing voxel size and section thickness (i.e., in-plane and through-plane resolution respectively)

Figure 2. Example of high-resolution (0.36x0.36) 3D DESS imaging with fat-supression by means of water-excitation improving intrinsic cartilage signal and cartilage-to-fluid or cartilage-to-bone contrast. (ID# 23 OA study K/L 1-2)

In the field, several **semi-quantitative scoring methods** are applied including the WORMS (Whole Organ Magnetic Resonance Imaging Score),[69] BLOKS (Boston Leeds Osteoarthritis Knee Score),[70] KOSS (Knee Osteoarthritis Scoring System),[71] MOAKS (MRI Osteoarthritis Knee Score),[72] ICRS or MRI-modified Outerbridge grades.[60] Whereas the MOAKS is developed to overcome limitations of WORMS and BLOKS rating systems, the latter two remain – to date – the most frequently used and widely disseminated.[58] As opposed to the ICRS or MRI-modified Outerbridge score, WORMS and BLOKS additionally include the areal extent of the cartilage lesion next to its depth (Figure 3) and also evaluate pathology of joint structures other than cartilage (i.e., bone atttrition, bone marrow lesion, effusion and synovitis, meniscus status and extrusion, ligaments, loose bodies, peri-articular fractures).[66] Both WORMS and BLOKS proved reliable and perform equally in evaluating prevalence and severity of cartilage loss both in cross-sectional and follow-up study designs.[73, 74]

Quantitative morphometry relies largely on computer-assisted post-processing algorithms and is therefore less observer-dependent than are semi-quantitative approaches.[58, 75] Due to varying contrast, structural complexity and inter-individual variability, reliable and accurate fully automated segmentation methods have not yet been universally implemented. However, next to plain manual segmentation, various semi-automatic interactive algorithms have been developed and validated such as "region growing", "edge detection", "LiveWire", or "B-spline snakes".[59, 76-78]
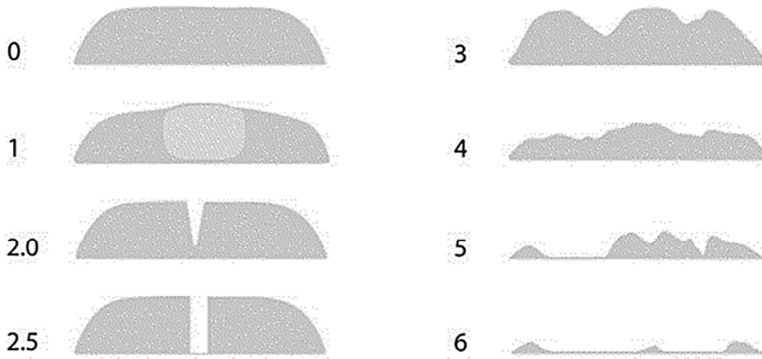
Figure 3. Cartilage WORMS grades as originally published by Peterfy et al.[69]

After segmentation, a 3D reconstruction is performed, most often implementing a modified marching-cubes algorithm[e], to generate a 3D surface polygon (or triangular) mesh[f] model. Subsequently, the voxels attributed to the segmentation and, thus, 3D reconstruction are summed up to compute cartilage 3D volumes.[59] Once the 3D volume is obtained, additional 3D metrics such as local thickness or surface area can be quantified by computational methods such as Euclidean Distance Transformation, normal vector or minimal distance methods and triangulation[g] respectively.[59] Figure 4 illustrates the main computer-assisted morphometric analysis methods as implemented in this dissertation.

[e+f] Marching cubes algorithm and 3D surface polygon or triangular mesh models: This computer-graphical algorithm renders 3D volumes in terms of a polygon mesh, out of a voxel surface. While considering 8 neighboring locations at the same time (i.e., marching cube), the algorithm proceeds through the segmented voxels and determines the polygon- or triangular shaped patches (i.e., mesh) that represent the outer surface that passes through the marching cube. Marching cube algorithms are often modified to suit MRI modalities. Hence, a surface polygon or triangular mesh 3D model is created.

[g] Triangulation: A method to compute surface areas from 3D surface triangular mesh models by integration of the triangular mesh.

When compared to established reference methods (i.e., anatomical sections, A-mode ultra-sound, CT-arthrography, stereophotogrammetry and water displacement of surgically removed tissue), these MRI-based and computer-assisted methods revealed high agreement for quantification of volume, thickness and surface area (r=0.80 to 0.99).[59] Intra-rater inter-scan variability (CV, Coefficient of Variation) in cartilage volume quantification is documented to range from 1.2% to 7.4% depending upon cartilage plate and scan orientation.[59] Whereas 3D SPGR/FLASH WE are assigned as golden standard sequences for quantitative morphological evaluation, 3D DESS-based morphometry performed equally in cross-validation and displays similar sensitivity to longitudinal change.[59, 62, 79]

The experimental work in this dissertation applied cartilage-WORMS scoring using a 2D IM-weighted TSE and a 3D DESS WE sequence. The 3D DESS WE sequence was also used to collect 3D morphological data in the cartilage deformation experiments where time-efficient and high-resolution acquisition is a prerequisite.

Quantitative compositional assessment may include T2 or Ultra-short TE (UTE) T2* mapping, T1rho, delayed Gadolinium- Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC), sodium and diffusion-weighted imaging. Of these techniques, T2, T1rho and dGEMRIC are most commonly applied.[57, 63, 66] Generally, these MRI techniques enable monitoring molecular compositional changes before pathology is detectable at a morphological level.

T2 mapping quantifies the T2 transverse relaxation time and reflects interactions among water molecules and between water and surrounding matrix macromolecules, such as the collagen fibers. Therefore, T2 mapping appears highly sensitive to changes in hydration (and nearly equivalently collagen concentration) and the anisotropic organization of the collagen fiber network.[66] Although a linear relationship between T2 values and OA severity remains controversial, damaged cartilage presents with increased T2 most likely due to disruption of the collagen network and accumulation of bulk water.[80-83] Typically, T2 relaxation times are derived from multi-echo(ME) (T)SE images acquired with varying echo-times (TE). Whereas for T2 quantification signal intensity from each pixel as input to a mono-exponential signal decay model (1) is considered appropriate in clinical imaging, T2 relaxation may reveal a multi-exponential - instead of a mono-exponential - signal decay (caused by (fragmented) macromolecules, free or trapped water)) (Figure 5).[63, 84] Hence, the traditional two-parameter curve-fit methods (i.e., linear least-squares regression and nonlinear fit to an exponential) employing a mono-exponential decay model may overestimate T2 near the cartilage bone-interface and fail to detect early disease.[85]
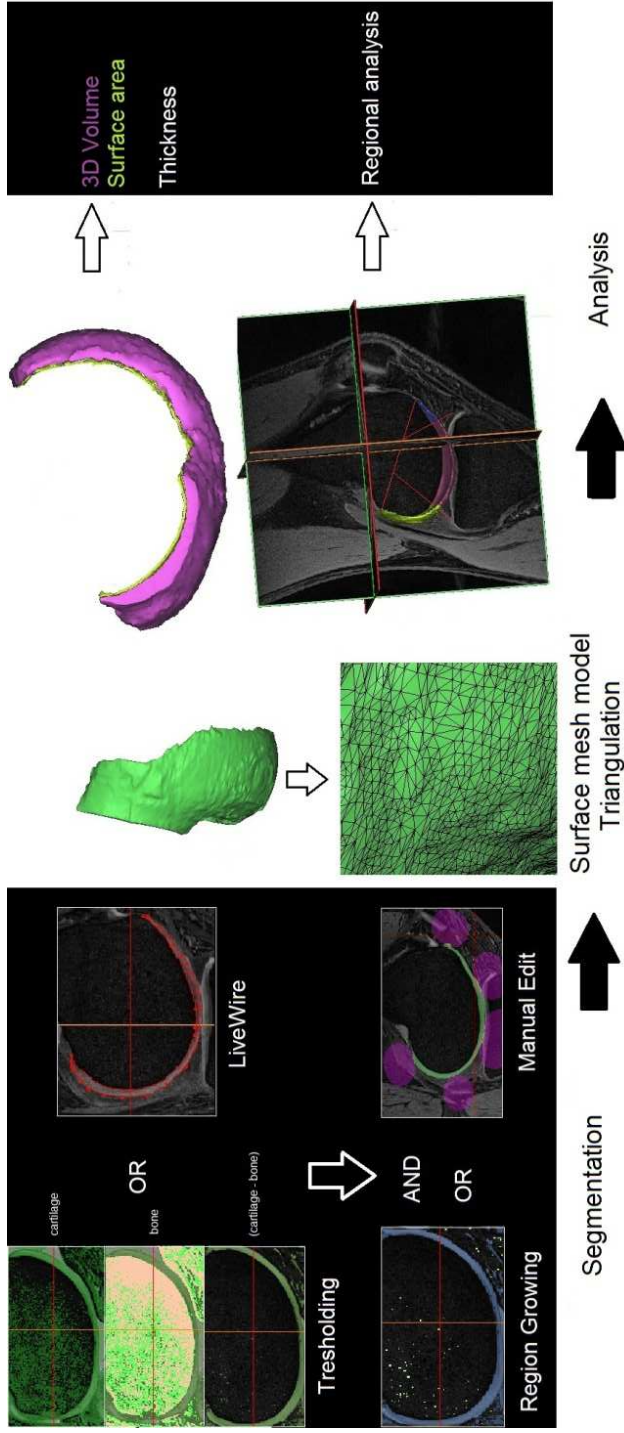
Figure 4. Illustration of the computer-assisted analyses techniques in quantitative morphometry as implemented in this dissertation. *Segmentation* was performed using either gray-value oriented tresholding or active LiveWire contours. Cartilage layers were delineated by colored masks corrected by means of region growing algorithms (that connect voxels within a similar signal intensity range allowing abundant voxels to be excluded from the mask) and/or manual editing. Subsequently, *3D surface mesh models* were reconstructed by the software package covered with a triangular mesh. Based upon the voxel count (i.e.; summation of individual voxel volumes) and triangulation (i.e., integrating surface areas of individual triangles), 3D volumes and surface areas are computed that allow subsequent *analyses* such as thickness measurements or regional analysis.

14

With regard to the latter, traditional ME SE imaging methods are typically designed with TE longer than 10-12 msec which may be adequate for monitoring the signal decays of long T2 components (i.e., free water) but inadequate for short T2 components (i.e., trapped water, bound to macromolecules such as PG or collagen fibers) (Figure 5).[25, 84] Short T2 signals typically arise from tissues such as the bone, tendons, menisci, radial and calcified cartilage. UTE or T2* imaging, the latter commonly acquired by means of a ME GRE sequence, apply shorter TE-times (<10 msec), are more able to detect fastly decaying signals and, hence, provide with improved, hyper-intense signal near the osteochondral junction.[86] Additionally, T2* imaging has the potential for fast, high-resolution image acquisition. Hence, despite its drawbacks such as sensitivity to scanner imperfections and susceptibility artifacts, Ultra-short TE or T2* is a promising MRI-marker with potential for improved sensitivity to subtle matrix change when compared to standard T2 mapping.[87] Both standard T2 and T2* are validated in histologic analyses of cartilage explants.[25, 80, 87-89] Whereas standard T2 tends to increase with matrix degeneration, T2* tends to decrease.[82, 87] As opposed to T2*, standard T2 mapping is a widespread technique applied in numerous (multi-)centre trials such as the Osteoarthritis Initiative. Intra-rater variability (CV%) for T2 and T2* estimation is documented to range from 1-9% and 0.2%-14.6% respectively.[84, 90]

$$S(TE_i) \propto S_0\, e^{(-TE_i/T2)}$$

<div align="right">(1)</div>
<div align="right">S: signal intensity</div>
<div align="right">S_0: noise-free signal intensity at TE=0</div>

T1rho relaxation times describe the duration of spin-lattice relaxation in the rotating frame based upon varying spin lock times (TSL). T1rho is suggested being sensitive to the interaction between trapped water molecules and their macromolecular environment such as GAG or collagen fibers. Next to PG depletion, changes to collagen organization/concentration or other macro-molecules may also effect an increase in T1rho.[66, 92] Despite it's nonspecificity, T1rho is suggested to detect early deterioration with increased sensitivity over standard T2 mapping.[66, 93] Although T1rho quantification appears less orientation-dependent at certain spin-lock frequencies, both T2 and T1rho relaxation times may (erroneously) increase due to magic angle artifacts.[92, 94, 95] Similar to T2, T1rho estimation is often addressed using a mono-exponential signal model (2) regardless of the suggested (orientation-and/or frequency dependent) multi-component signal decaying behavior of hyaline cartilage.[95] T1rho imaging underwent validation using osteoarthritic cartilage specimen whereas intra-rater variability (CV%) is documented to range from 3.3%-8.5%.[89, 96]
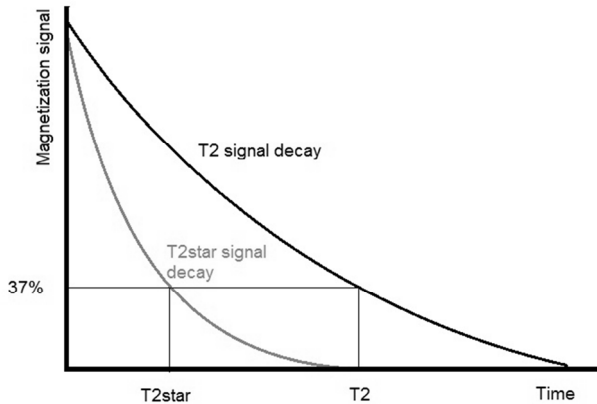
Figure 5. Schematic display of a T2 signal decay versus a T2star signal decay. The *T2, or transverse relaxation time*, characterizes the rate at which the magnetization vector decays after being tipped into the transverse plane. T2 is defined as the time (in ms) it takes for the transverse signal to reach 37% of its initial value[91]. Due to differences in biochemical and biophysical microenvironment surrounding spins in the compartments of extracellular matrix in cartilage such as water/fluid, proteoglycans, and collagen fibers, articular cartilage presents with a *multi-component exponential decay*. In this respect, the signal decays of free water are longer than those of trapped or bound water. As such, quantification of T2* relaxation times may be more suited to detect change related to water molecules bound to PG or collagen that are reflected by T2 signal that decay more rapidly.[84, 87]

$$S(\text{TSL}_i) \propto S_0 \, e^{(-\text{TSL}_i/\text{T1rho})}$$

(2)

S: signal intensity
S_0: noise-free signal intensity at TSL=0

Although in the field of OA research T1rho quantification is gaining interest as a noninvasive tool to detect PG depletion, the most commonly used technique to estimate relative PG or GAG concentration remains **delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC)**, a contrast-MRI technique based on the "Donnan theory of Equilibrium".[24] Via gadolinium-based contrast administration (i.e., gadopentetate dimeglumine), the negatively charged mobile ion "Gd-PTA^{2-}" is introduced into the cartilage tissue and will distribute along the GAG side chains when given sufficient time. The contrast agent will distribute in higher concentrations where GAG concentration is low and *vice versa*.[24, 97] T1 longitudinal relaxation times, commonly acquired by means of 2D or 3D inversion recovery (T)SE or GRE images with varying inversion times (TI), are inversely related to the gadolinium concentration according to a dose-response relationship.[98-100] Hence, T1 quantification after full-penetration of the contrast-agent (T1Gd, or dGEMRIC index) is put forward as an index of the contrast agent's concentration and is proportional to the GAG content of the tissue.[97, 101] A low GAG level is associated with a high concentration of gadolinium and results in low T1Gd values or dGEMRIC indices.[97] To facilitate transport of the charged contrast agent into the tissue, a short loading event (e.g., 10 min of walking, cycling, taking stairs) is introduced immediately after contrast

administration.[98] Following contrast administration (i.e., double or triple dose via the antecubital vein) a delay of 90-120 min is required for optimal distribution in the cartilage tissue.[98] T1 relaxation times are commonly calculated through signal intensity inputs into a three-parameter fit exponential signal decay model (3).[98]

$$S(TI_i) \propto S_0 (1-2Ae^{-(TI_i/T1)}+e^{-(TR/T1)})$$

(3)
S: signal intensity
S_0: noise-free signal intensity at TI=0
A: inversion efficiency

Although the dGEMRIC technique was subjected to extensive validation in both *in vitro* and *in vivo* experiments, *in vivo* (long-term) interpretation, warrants circumspection.[24, 102, 103] The distribution of gadolinium within the cartilage tissue does not only depend upon the concentration of sulphated GAG side chains, but also relies on the pharmacokinetics of GdPTA^{2-} and the rate of its supply into- and removal from the joint.[30] While the Donnan theory assumes a closed system (i.e., a steady state of contrast influx and elimination), within the standardized delay time frames, body composition (i.e., transport via interstitial fluids in lean and adipose tissue), joint circulation (i.e., inflammation, synovitis) and matrix integrity and permeability may affect contrast supply and distribution.[30, 104] In this respect, BMI corrections for the T1 values showed to correct for body composition and are advised to be implemented when dealing with overweight and/or obese individuals.[104] Additionally, the dGEMRIC technique appears to only detect disease when the compensating GAG turn-over fails and the cartilage is probably fibrillated already.[30] Intra-rater variability (CV%) for T1Gd quantification is reported to range from 5.4%-15.2%.[100, 105]

Biochemical maps display zonal and/or laminar variation due to the natural (topographical) variation in GAG, collagen and water contents. (Figure 6)

In the experimental work of this dissertation, both in vivo estimation of GAG content, water or collagen concentration/organization (and their interaction) is addressed by means of dGEMRIC, standard T2 and T2* mapping.
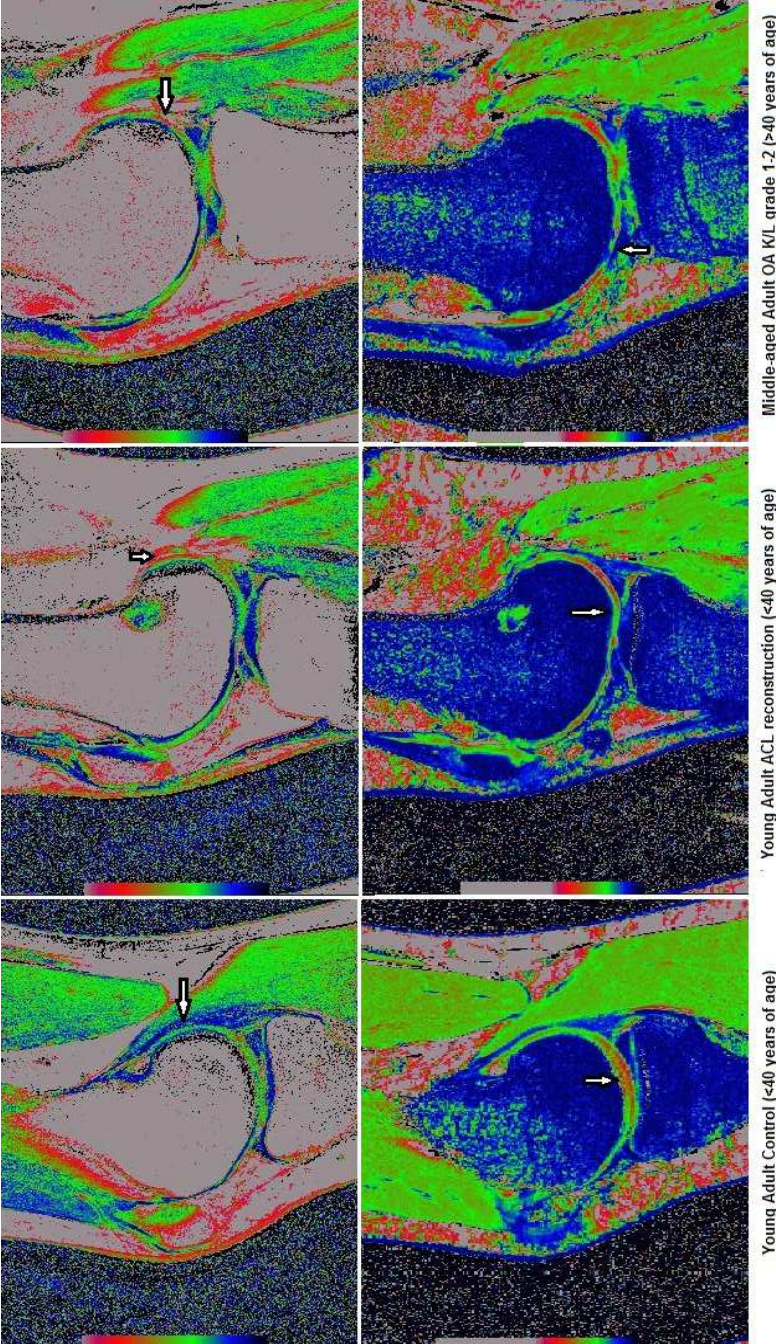
Figure 6. Example of color-coded biochemical T2 (top row) and T2star (bottom row) maps (without overlay on original anatomical images) in a young adult control (ID #9 ACL study control) vs ACL-reconstruction (ID #16 ACL study) and doubtful-to-mild radiographic OA (ID #20, OA study K/L 1-2). Note the gradual increase in T2 in the posterior femoral condyles and the gradual decrease in T2star in the weight-bearing femoral condyles. (MapIt, Siemens Medical Solutions, Erlangen, Germany)

Experience and research over the past years have demonstrated that MR imaging is a biologically safe imaging modality. Specifically, no convincing evidence exists on any long-term or irreversible biologic effects associated with the radiation and magnetic fields used in MR imaging. However, safety precautions taking into account absolute and relative contra-indications for this imaging technique need to be considered to avoid severe injuries or even death. As also applied in all experimental procedures in this dissertation, more specifically subjects or patients need to be checked or questioned for foreign ferromagnetic (metal) objects, pregnancy, claustrophobia. Whereas the dGEMRIC technique should better not be applied in patients with renal insufficiencies, caution is advised when dealing with patients with asthma or allergies or a history of allergic reactions to contrast agents.[106, 107]

3.2 Short-term and long-term effects of *in vivo* exercise on human articular cartilage: an overview of the MRI literature

3.2.1 *In vivo exercise and short-term effects including cartilage deformation in healthy volunteers and patient populations*

While studies in patient populations are emerging, a large majority of reports documents on short-term load or exercise effects in (young) healthy adults (i.e., no clinical or radiographic OA disease) focusing on knee cartilage. These reports describe MRI-measured cartilaginous responses following a variety of *in vivo* activities such as full weight-bearing stance and gait, single-leg lunges, static and dynamic knee bending, cycling, drop landings and running, all summarized herein.

Loading of the knee during **full weight-bearing (static) stance** and treadmill **gait** showed to effect a sharp immediate increase in contact-deformation in the overlapping and thicker tibiofemoral cartilage layers.[108, 109] Contact areas tended to be larger in the medial compartment when compared to the lateral resulting in increased contact deformation in overlapping layers laterally or in single cartilage plates laterally and medially.[108-110] The degree of knee flexion at heel strike was associated with the rotational antero-posterior location (i.e., location at degree relative to the long axis of the femur) of the thickest cartilage in the medial femoral condyle which may support that local variations in cartilage thickness are in part mediated by frequent loading cycles during walking.[111] Next to tibiofemoral compartments, patellar cartilage was also reported to endure deformation - though to a relatively small extent - during walking.[110] Although not in truly upright position, in situ *in vivo* static compression using an axial MRI-dedicated loading device, effected T2 and dGEMRIC index decreases in central and peripheral femoral and/or tibial cartilage. These detected changes are presumably the result of net loss or expulsion of free interstitial water.[112, 113] Similar to full-weight-bearing stance, **single-leg lunges** caused greater medial contact areas, combined with larger medial contact-deformations that attained a maximum at deep 120-degree knee flexion.[114]

Bilateral knee bend exercises of at least 30 repetitions were reported to cause pronounced deformational changes in patellar cartilage mainly accommodated by the superior lateral and medial patellar facets. With regard to the extent of deformation after the exercise, patellar cartilage appeared to deform the most followed by the lateral tibial plates and femoral condyles.[64, 110, 115-117] No substantial difference in patellar cartilage deformational outcomes could be disclosed after sets of 50 or 100 knee bends, nor after 15 minute interval-repeated sets of 50 repetitions. Recovery time after 100 knee bends correlated with the initial degree of patellar cartilage deformation and progressed approximately linear in time requiring at least 90 minutes for restitution of baseline quantitative morphology. Extended recovery and the limit in deformational outcome supports the role of the fluid phase and permeability of the superficial cartilage layers in cushioning compression stresses.[116] In this respect, after 60 knee bends T2 values of patellar cartilage increased by 2.6% at 45 minutes of rest supporting the role of water displacement in *in vivo* cartilage deformation.[118] In asymptomatic older aged adults (i.e., 50-78 years of age), after comparison to young adults (i.e., 20-30 years of age), decreased deformation was established in the patellar plates following 30 knee bends. Age-related effects on cartilage thickness, matrix water content, AGE deposition such as pentosidine, or altered movement strategies reducing joint load may have led to this observation.[119] Mainly recorded in the central aspects of the lateral patellar facets, **static bilateral knee bends** led to higher patellar cartilage thickness changes when compared to dynamic exercise.[115] Similarly, deformational outcomes in tibiofemoral compartments were of greater magnitude after **static unilateral stance** when compared to unilateral dynamic knee bend exercise.[108, 110, 114] In this regard, statically applied loads are suggested to allow deformation without large pressure surges within the matrix accounting for the larger morphological changes.[115, 116]

High impact **drop landings** were mainly absorbed by the tibial plates followed by the patella whereas **cycling** showed to induce similar degrees of patellar cartilage deformation when compared to knee bend exercises or running.[110, 120]

Running exercise for a distance of 200 m or duration up to a 30 minute- or 1 hour run generally unveiled considerable deformation in the patellar plates followed by the tibial and femoral layers in terms of degree of deformation.[110, 120-125] Whereas more deformation was recorded in tibial when compared to femoral plates, the direction of medio-lateral distribution was not uniform amongst studies. Divergence in outcomes might be due to inter-individual differences in lower limb kinematics. More specifically, during foot roll-over, the medio-lateral displacement and alignment of the ground reaction force vector relative to the knee joint center may have differed influencing knee adduction moments, and hence, medio-lateral load and compression distribution.[121] Whereas the load is suggested to be primarily transferred through the central weight-bearing regions of the cartilage plates, compression mostly induced greater anisotropy in the superficial layers.[123-125] Although baseline physical activity level may affect deformational outcomes (i.e., the lower activity level, the higher the cartilage change), older age (<46 vs 46-64 years of age), or gender did not seem to influence the degree of deformation after running.[123-125]

Running distances up to a **marathon** brought about substantial quantitative morphological decreases, T2 or T1rho increases in all cartilage plates, however, predominantly in the patella-femoral and medial tibiofemoral compartments.[126-128] Semi-quantitative evaluations of synovial structures after a marathon race reported no-to-mild changes including more or larger cartilage abnormalities and meniscal signal intensity changes.[129-132] Interestingly, as opposed to tibial cartilage changes, running a distance of 20 km caused larger volume changes in the menisci that required slower recovery.[127] Following a 3-month recovery after a marathon, post-marathon increases in meniscal T2 were normalized, however T1rho relaxation times remained elevated.[133] Similarly, post-marathon increases in cartilage T2 were normalized for all plates except the medial femoral condyles whereas cartilage T1rho remained elevated after the 3-month recovery time span.[128] Although MRI markers for meniscal biochemical composition require further validation, these results suggest that persisting alterations in meniscal extra-cellular matrix composition (i.e., increased water and decreased collagen content)[83, 134] maintain the vulnerability of the opposing cartilage. In this respect, long-distance running in experienced runners is suggested not to enhance long-term macro-morphological deleterious effects on cartilage unless meniscal quality remains preserved.[135, 136] Table 1 presents a systematic overview of the extent and location of significant cartilage deformation measured immediately after or during a variety of *in vivo* activities.

In general, short-term effects of exercise and more specifically deformational patterns are likely to respond to range of motion, load-cycle frequency and intensity of particular activities and depend upon the material properties of each cartilage plate.[110, 120] With regard to the latter, patellar cartilage is likely to deform to a greater extent than the opposing femoral cartilage under a similar load because of its higher proportion of water relative to PG. The resulting lower compressive stiffness moduli (i.e. aggregate compressive modulus), higher permeability to fluid flow and increased thickness of the patellar plate is suggested to render the tissue vulnerable to deformational or shear stress.[137] Within the tibiofemoral compartment, femoral cartilage exhibits increased compressive stiffness moduli (i.e., Young's and dynamic modulus) compared to tibial cartilage.[28]

During the past 2 years, the short-term effects of exercise have been investigated in patient populations as well including radiographic OA, ACL deficiency and reconstruction, or patella-femoral (PF) pain. **In situ *in vivo* static compression of OA joints (i.e., K/L grade 2-4)** using an axial MRI-dedicated loading device showed - with increasing K/L grade - increasing and medially translating contact-areas, predominantly monitored in medial over lateral compartments. Additionally, tendencies towards increased deformation were documented when compared to subjects categorized as "healthy" (i.e., K/L 0 and/or cartilage WORMS grade 0) with the medial compartments driving the largest changes.[138-140] During a **full weight-bearing single-leg lunge**, **ACL-deficient and reconstructed knees** exhibited shifts in cartilage-cartilage contact points towards regions of thinner cartilage on the tibial plateaus accompanied by increased contact-deformation when compared to the contra-lateral knee.[141, 142] Interestingly, while increased contact-deformation may influence load transferal within the ACL-deficient or reconstructed knee, a semi-quantitative evaluation of ACL-reconstructed knees before and after a **half marathon race** revealed, apart from a trend towards greater incidence of BML, no changes in cartilage defects in the reconstructed knees when compared to the contra-lateral joint.[143] In **PF pain patients** (i.e., 20-40 years of age), patellar cartilage thickness and deformation following 50 knee bends was reduced when compared to matched controls.[40]

Next to increased stresses within the cartilage matrix suggested to predispose for future OA, incompliant cartilage deformational responses may subject the subchondral bone to higher impact stresses eliciting pain complaints.[40] Increased deformational responses as noted in the radiographic OA and ACL patients are most likely a result from disruption of the collagen network and/or PG loss resulting into increased tissue permeability, bulk water accumulation and decreased compressive stiffness.[33-35, 140]

Table 1. Systematic overview of the extent of significant cartilage deformations after a variety of *in vivo* activities in healthy adults

In vivo activity	Authors	Parameter	Cartilage plate	%change (S.D.) or range
Full weight-bearing stance	Hosseini 2010[108]	Peak contact deformation	Medial compartment	10.5 (0.8)%
			Lateral compartment	12.6 (3.4)%
Gait	Liu 2010[109]	Peak contact deformation	Medial compartment	23 (6)%
			Lateral compartment	16 (4)%
Walking	Eckstein 2005[110]	Volume	Patella	-2.8 (0.8)%
Unilateral static stance	Eckstein 2005[110]	Volume	Medial tibia	-3.1 (4.5)%
			Lateral femur	-3.3 (6.2)%
Axial static compression	Mayerhoefer 2010[112]	T1Gd	Medial femur subzones	NR
	Nag 2004[113]	T2	Medial tibia subzones	NR
			Mediolateral femur subzones	NR
			Mediolateral tibia subzones	NR
Single-leg lunges	Bingham 2008[114]	Peak contact deformation	Medial compartment	30(13)%
			Lateral compartment	30(10)%
Bilateral knee bend exercises (repetitions 30-100)	Eckstein 2005[110]	Volume	Patella	-5.9 (2.1)%
			Lateral tibia	-2.8 (4.0)%
	Eckstein 2000[115]	Volume	Patella	-5.9 (2.1)%
		Thickness	Patella	-4.9 (1.4)%
	Eckstein 1999[116]	Volume	Patella	-5 to 6% (2.4%-8.6%)
	Eckstein 1998[117]	Volume	Patella	-6.0%
	Hudelmaier 2001[119]	Thickness	Patella	-2.2 to -2.6 (1.7)% (elderly)
				-4.5 to -6.2 (1.3,2.1)% (young)
Static bilateral knee bend exercise	Eckstein 2000[115]	Volume	Patella	-4.7 (1.6)%
		Thickness	Patella	-4.9 (1.4)%
Drop landing	Eckstein 2005[110]	Volume	Lateral tibia	-6.1 (3.5)%
			Medial tibia	-7.2 (4.2)%
	Niehoff 2011[120]	Volume	Patella	-2.8%
			Medial tibia	-2.5%
			Lateral tibia	-1.6%
		Thickness	Patella	-1.9%
			Medial tibia	-2.2%
			Lateral tibia	-1.8%
Cycling	Eckstein 2005[110]	Volume	Patella	-4.5 (1.6)%
Running (200m, 30 min to 1 hr)	Eckstein 2005[110]	Volume	Patella	-5.0 (1.3)%
	Niehoff 2011[120]	Volume	Patella	-3.5%
			Medial tibia	-2.7%
			Medial femur	-3.8%
			Lateral tibia	-6.1%
		Thickness	Patella	-3.1%
			Medial tibia	-2.2%

Table 1 continued

Study	Measurement	Region	Value
Boococke 2009[121]	Volume	Medial femur	-2.6%
		Lateral tibia	-5.8%
		Medial femur	-5.3%
		Lateral femur	-4.0%
Kersting 2005[122]	Volume	Femur total	-5.7%
		Lateral tibia	NR
Mosher 2005[124]	T2	Weight-bearing femur superficial	NR
	Thickness	Tibia	-40%
Mosher 2010[123]	T2	Weight-bearing femur middle, superficial	NR
		superficial	NR (young, elderly)
		Weight-bearing tibia superficial	NR (young, elderly)
		Medial femur superficial	NR (pooled)
		Lateral femur superficial	NR (pooled)
		Medial tibia superficial	NR (pooled)
		Lateral tibial superficial	NR (pooled)
	Thickness	Femur	NR (young)
		Tibia	NR (young)
Subburaj 2010[125]	T1rho	Patella	-11.7%
		Femoral trochlea	-13.5%
		Medial tibia	-14.3%
Running **(5-10-20 km)**			
Kessler 2006[126]	Volume	Patella	-6.6(2.5)% to -8.1 (4.8)%
		Tibia	-3.6(2.5)% to -6.1(3.4)%
Kessler 2008[127]	Volume	Patella	-7.0%
		Tibia	-5.1%

SD.: Standard Deviation. NR.: Not Reported.

24

3.2.2 In vivo exercise and cartilage functional adaptation in healthy volunteers and patient populations

Unloading of the knee during 6 to 8 weeks **immobilization**, non-weight-bearing after surgical interventions or 6 degree head-down tilt bed rest led to decreases in thickness, serum COMP levels, and increases in T2 and T1rho.[144-147] General remobilization or whole-body vibration training effected an attempt to thickness and T1rho recovery.[145-147] As cartilage thickness in spinal cord injured patients showed to gradually decrease following injury, repetitive *in vivo* loading cycles appear necessary for articular cartilage to maintain its ultra-structure and gross morphology over time.[148, 149] However, *in vivo* research presents conflicting results and cartilage does not appear to functionally adapt to exercise in the same way – or according to the same rate – as do muscle or bone.[145, 150] Herein, results regarding the long-term effects of physical activity or exercise on knee cartilage are summarized, stratified according to age-category (i.e., children, young adults, adults, middle-aged adults, older aged adults) and baseline radiographic status (if reported) as – to date – the latter remains the most common form of disease status classification.[151] In this way, it was attempted to account for the potential influence of ageing and disease on cartilage adaptive capacity.

In terms of cartilage volume accrual, most **children** gain articular cartilage at the tibial sites during growth, with younger children, boys, and those undertaking more vigorous sports exhibiting substantially higher accrual rates.[152] In **young adult (i.e., 20-30 years of age)** professional athletes, joint surface areas showed to be larger, however, patellar, femoral or tibial cartilage plates were not convincingly thicker when compared to untrained volunteers.[110, 150, 153, 154] At an ultra-structural level, comparison between the sedentary and recreational or elite runners revealed increasing dGEMRIC indices according to increasing activity, suggesting adaptive capacity of medial and lateral femoral knee cartilage to some extent.[155] Although those with severe internal knee damage ceased running activity, a 10-year follow-up study supported that long-distance runners - in case of no damage at baseline - do not seem to sustain considerable permanent lesions to the internal knee structures in the longer-term.[136] In **adults (i.e., 26-62 years of age) without clinical OA disease, however with potential underlying radiographic signs of OA,** occupational activities that require frequent knee bending, squatting, heavy lifting, stair climbing or walking were associated with an increased risk for patellar cartilage defects or decreased patellar cartilage volume in females.[156] Although modest in magnitude, a 2 year-longitudinal study revealed that strenuous exercise, however, was associated with a decreased risk of progressing lateral tibiofemoral cartilage defects. Additionally, changes in physical work capacity and muscle strength were respectively negatively or positively associated with lateral and total (i.e., lateral and medial tibia) tibial plateau area and lateral tibial or total (lateral, medial tibia and patella) cartilage volumes. Whereas baseline muscle strength was suggested protective against total cartilage volume loss

or tibial area reduction, in females, increased physical work capacity displayed a deleterious relationship with either lateral tibial and total cartilage volume (i.e., loss) or lateral tibial plateau area (i.e., reduction).[157] In **adults at risk for OA development** (i.e., 3-5 years after a partial meniscectomy procedure), a four-month structured exercise program encompassing neuromotor control, strength and aerobic exercise was suggested to induce a chondroprotective effect in terms of dGEMRIC index changes in medial femoral cartilage.[19] In **middle-aged adults (i.e., approximately 45-55 years of age) without clinical or radiographic OA disease (i.e., K/L grade ≤1)**, exercise level (i.e., sedentary, light, moderate to strenuous) did not influence tibiofemoral and/or patellar cartilage T2 in subjects without risk factors for knee OA. In those at risk for radiographic OA progression (e.g., previous knee injury or surgery, family history of total knee replacement, Heberden's nodes, and occasional knee symptoms), light exercise was associated with lower T2 suggesting a beneficial effect. Moderate-to-strenuous exercise, especially in women, and frequent knee bending were associated with higher tibiofemoral and/or patellar cartilage T2 values and/or a higher prevalence and grade of knee abnormalities (e.g., total cartilage and meniscal lesions).[158-160] Despite potential effects of exercise at an ultra-structural level, in women subjected to a longitudinal 3-month strength or endurance exercise program, cartilage quantitative morphometry failed to disclose significant change in all knee cartilage plates.[161] In **middle-aged women without clinical OA disease, however with uncertain status of radiographic OA,** participation in fortnightly exercise (causing tachypnea and increased pulse rates for at least 20 minutes) was positively associated with tibial or patellar cartilage volume or reduced rates of volume loss and did not associate with the presence of cartilage defects.[162, 163] In **older aged adults (i.e., 50-80 years of age) without clinical OA disease, however with uncertain status of radiographic OA,** a 2-year follow-up study described that participation in vigorous physical activity (i.e., jogging, swimming, cycling, singles tennis, aerobic dance, skiing or other similar activities) was associated with reduced rates of tibial or patellar cartilage volume loss and with a trend towards decreased risks for worsening cartilage defects. In case of no baseline cartilage defects, next to reduced rates of volume loss, a trend was apparent for fewer newly developed defects.[164, 165] In this respect, report of regular walking was associated with a reduced risk of bone marrow lesion development.[164] Follow-up until 3 years, however, documented that persistent participation in vigorous activity was associated with worsened medial tibiofemoral cartilage defects and trends towards decreased cartilage volumes, especially in case of baseline BML presence.[166] **In older aged adults with clinical and radiographic signs of OA disease (K/L grade 2-4)**, a therapeutic exercise program entailing aerobic, strengthening and flexibility exercises only or added with agility and perturbation revealed variable but overall small changes in medial or lateral tibiofemoral cartilage volume, predominant in the central medial femur. The patients that progressed towards cartilage loss were younger, presenting with higher body mass indices, higher K/L grades in the medial compartment and increased progression of knee varus alignment during the 1-year follow-up.[18] **In older aged adults with potential clinical and**

radiographic signs of OA disease, a 3-year follow-up study assigned physical activity, expressed as step count per day, as protective against medial or lateral tibiofemoral cartilage volume loss in those with more baseline volume, however, as accelerative in those with less baseline volume. Additionally, excessive physical activity (i.e., ≥10.000 steps/day) increased the risk for BML development, worsening of meniscal pathology scores especially in case of baseline meniscal involvement, and for tibiofemoral cartilage defects in those with at baseline diagnosed BML. Consequently, more than 10.000 steps/day aggravated knee structural deterioration especially in case of pre-existing internal knee abnormalities.[55]

In general, the body of literature does not depict a straight forward view on human articular cartilage functional adaptive capacity to in vivo exercise. Depending upon age, type or level of exercise and baseline joint status, however, potential for protective effect against MRI-detected cartilage deterioration is suggested. In young healthy adults, exercise appears to beneficially influence cartilage ultra-structure. With increasing age, protective effects (i.e., braking progressive cartilage deterioration) persist in case of light-to-moderate exercise in those individuals without radiographic signs of OA or at risk for progressive radiographic OA. One needs to stress that in case of pre-existing internal knee derangements (i.e., cartilage defects, meniscal pathology, BML presence), prolonged and excessive physical activity is suggested to accelerate – instead of brake – cartilage deterioration.

Nonetheless, **circumspection is warranted when attempted to draw generalized conclusions**. (1) One should consider the effect of chondrocyte senescence, matrix composition and age-related changes in other synovial structures when evaluating functional adaptive capacity in different age categories. (2) Various definitions for "health" and "disease" and, hence, baseline joint status are being used. (3) Often, mixed cohorts are being investigated in view of gender distribution and presence of OA risk factors. (4) A considerable number of studies applied cross-sectional - instead of longitudinal - study designs. (5) Various definitions for exercise or physical activity level and exercise regimen are being implemented which may exert increased strain on particular cartilage plates more than on others. (6) Various methods are being used to evaluate cartilage change, that is (semi-)quantitative morphology next to biochemical compositional markers, with different sensitivity in terms of rate of detectable change. With regard to the latter, MRI techniques targeting ultra-structural changes are more likely successful in detecting exercise-related change than are quantitative morphometric markers, especially in longitudinal designs. Additionally, as early OA progression is associated, next to (regional) thinning, with swelling of the tissue and (regional) increases in cartilage volume or thickness,[167] caution is warranted to attribute positive associations between physical activity level and cartilage volume to beneficial effects without supportive ultra-structural data. In fact, increases in thickness beyond a certain threshold (i.e., "optimal"

thickness) are suggested functionally disadvantageous in terms of impaired in- and outflux of nutritional or waste products and/or impaired hydraulic pressurization upon loading incidents.[150]

4 Take-home messages

- In OA research and clinical management, a paradigm shift is proposed focusing on those individuals at increased risk for developing OA (e.g., ACL injured) or diagnosed with early disease as in these cases accelerated structural change may be preventable.
- In view of the discordance between structural joint status and clinical presentation, especially in the early stages of OA development, research should separate treatment effects into symptomatic versus structural outcomes.
- Although from an *in vitro* perspective cartilage load is necessary for chondrocyte viability and matrix structural maintenance, the relationship between *in vivo* physical activity and long-term development of radiographic OA remains inconclusive.
- One important drawback of radiography as an investigation tool to assess disease development is its low sensitivity for early change and its insensitivity to detect change in synovial structures other than bone.
- Although OA should be considered as a whole-organ disease, cartilage loss remains a hallmark for its progression. MRI techniques are able to investigate cartilage tissue in a direct and location-specific fashion with the possibility for sub-surface and ultra-structural assessment.
- MRI techniques can evaluate macroscopic morphology (i.e., semi-quantitative and quantitative morphology) and ultra-structural or biochemical composition (e.g., T2 and UTE or UTE T2* imaging, T1rho, dGEMRIC). With regard to the latter, UTE or UTE T2* and T1rho imaging may be more sensitive in detecting early matrix deterioration over standard T2 mapping.
- To date, short-term effects of exercise including deformational behavior are most often evaluated in knee joints of healthy adults. Deformational changes are detectable after a variety of *in vivo* activities by MRI-measured morpohological or ultra-structural outcomes and mainly rely on range of motion, intensity and load-cycle frequency of the activity and the material properties of the respective cartilage plates. In patients at risk for or diagnosed with radiographic OA, shifts in cartilage-cartilage contact points are observed combined with altered deformational responses.
- The extent, or manner, of human cartilage functional adaptation to *in vivo* exercise appears not straight forward and is suggested to depend on age and/or type or level of activity and/or baseline joint or cartilage status. Overall, light-to-moderate exercise

appears protective against progressive cartilage deterioration in those individuals without radiographic OA or at risk for OA. With regard to individuals sustained with pre-existing internal knee abnormalities, prolonged excessive physical activity risks worsening cartilage degeneration. However, many factors need to be considered when drawing generalized conclusions pertaining to the adaptive capacity in ageing joints, the use of different definitions, cross-sectional vs longitudinal study designs, and the sensitivity of evaluation methods.

- Despite the fact that exercise (therapy) is granted a vital role in first-line treatment guidelines of OA, the diverse short- and long-term outcomes in healthy individuals and the few reports on patient populations hamper drawing sound conclusions regarding its feasibility for implementation in knee OA prevention and/or treatment strategies.
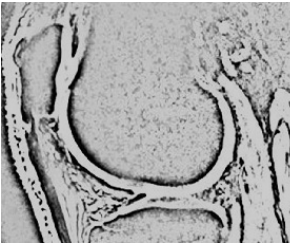
**AIMS AND OUTLINE
OF THIS DISSERTATION**

The **two-fold goal of this dissertation** is to contribute to the understanding of how exercise may assist in chondroprotection in view of OA prevention in a first part, and, in a second part, how exercise may be implemented in those individuals at risk for radiographic OA development or accelerated OA progression.

Part 1 – Exercise and chondroprotection: a fundamental approach

In the literature, the majority of studies focus on knee joint cartilage, an evident choice as the knee is one of the weight-bearing joints most vulnerable to OA affliction and presents with the thickest cartilage compared to hip or ankle.[168, 169]

In this dissertation, however, we decided to approach this issue from a different angle. Instead of only focusing on the vulnerable knee joint, we addressed the upper ankle as well which– despite transferal of the highest forces per square centimeter in the lower limb – rarely sustains idiopathic OA and presents with less full-thickness cartilage defects.[170, 171] Based on human cadaveric ipsilateral knee-ankle pairs, several metabolic, biochemical, and biomechanical differences between knee and ankle were put forward in favor of an inherent protection of ankle cartilage.[169] Most important features include a decreased response of ankle chondrocytes to catabolic factors, and potential for greater repair capacity. In this regard, ankle chondrocytes appear to synthesize PG at a higher rate in response to damage. Furthermore, ankle cartilage contains a higher concentration of PG and lower water content which, combined with an increased PG turn-over and synthesis, results into increased *in vitro* compressive stiffness and reduced permeability to interstitial fluid flow. The latter features may protect the cartilage matrix from potential deleterious high impact or compressive forces. A comparison of ankle and knee chondrocyte biosynthetic activity in their native matrix versus a new matrix in alginate or agarose showed that the native matrix is important to discern the material properties and PG content or synthesis between joints.[169, 171-176] Whereas load is important for chondrocyte activity and matrix structure, insight into ankle cartilage responses to *in vivo* load and typical load transfer may assist in identifying those features exercise needs to encompass in order to preserve cartilage structural integrity.

To this end, 2 main research questions were addressed: (1) When compared to knee joints, to what extent deforms ankle cartilage when subjected to several impact conditions? (**Chapter 1**), and (2) How long does it take for intial volumes to recover following *in vivo* load? (**Chapter 2**). Within these chapters, possible influencing factors for deformational outcomes were discussed.

At the time the outline for this PhD project was drafted, a few studies suggested that cartilage might functionally adapt to exercise at an ultra-structural level rather than at a macro-

morphological level.[150] In this regard, while the knee joints of experienced long-distance runners (with no cartilage damage at baseline) did not present with permanent internal knee damage,[136] running exercise was suggested to beneficially influence the dGEMRIC index – a surrogate marker for cartilage quality.[155] No longitudinal study existed, however, to corroborate this suggestion. From a preventative point of view, we opted to evaluate the effect of a moderate-running regimen, a Start To Run novice runner program, on knee cartilage ultra-structure in sedentary pre-menopausal women.

Hence, in **Chapter 3**, we addressed the following research question: How does knee cartilage ultra-structure (i.e., dGEMRIC index) changes after completion of a 10 week moderate-running Start To Run program in sedentary female volunteers compared to sedentary controls?

Part 2 – Exercise and chondroprotection: clinical implementation in individuals at increased risk for - or diagnosed with early radiographic OA

To comply with the second aim and in line with the proposed paradigm shift, 2 patient groups were considered: (1) patients who sustained an ACL injury and underwent arthroscopic ACL surgery, and (2) patients who presented with doubtful-to-mild radiographic OA signs up to a K/L grade 2 at maximum. While the latter group is at higher risk for further radiographic OA development,[7] the first group served as an at-risk model for post-traumatic OA.[11]

After the traumatic insult and impact of an ACL injury, cartilage deterioration is furthered by activated inflammatory pathways upon hemarthrosis and surgical trauma potentially maintained and/or aggravated by altered biomechanical loading patterns and concomitant injuries.[93, 177-180] Although ACL reconstruction aims at restoring knee stability in order to prevent subsequent meniscal and chondral damage,[181] epidemiological reports suggest that reconstruction may not prevent premature OA development.[182, 183] One may argue that recent meta-analyses showed that radiographic OA development after ACL injury and/or reconstruction is not as high as initially perceived in case the menisci are preserved.[182] However, if radiographic OA is established, post-ACL injury OA closely mimics degenerative OA[184] and patients are usually younger than in case of OA development due to natural ageing. Post-traumatic OA development involves a complex array of potential risk factors including physical activity and return to sports.[185] In fact, emerging MRI investigations that monitor joint and/or cartilage integrity during the first years following injury or surgery suggested that ACL-reconstructed knees may benefit from longer recovery periods.[177] As return to sports takes place at on average 6 months after surgery,[186, 187] exercise implementation in terms of return-to-sports approvals is suggested insufficiently tailored to the morphological sequellae the ACL-injured knee seems to endure in the early recovery phase.

Hence, we addressed the following research questions: (1) How does cartilage status evolves over time following ACL injury and reconstruction? (2) Which factors might influence rate of change? (3) Does the course of cartilage adaptation have consequences for clinical management including return to play decisions or future research directions? (**Chapter 4**) (4) In view of return to play decisions, what is the status of cartilage quality and function (i.e., quantitative morphology, biochemical composition, *in vivo* function or deformational behavior) compared to matched healthy counterparts at 6 months after surgery? (5) How does cartilage status relate to timing of return to play and surgical delay? (**Chapter 5**)

In line with the proposal put forward by the OARSI-FDA Initiative to separate treatment effects at an "illness-level" (symptom-related) from those at the "joint-level" (related to structural integrity),[5] we considered the potential implementation of "general loading" exercise in the group of patients with doubtful-to-mild radiographic signs of OA. In theory, these exercises should induce generalized joint load with the ultimate goal to maintain PG synthesis and turn-over and to postpone or slow down the process of matrix homeostasis failure.[45] In a population at risk for OA development, an exercise regimen addressing neuro-muscular control and strength, showed positive effects in terms of PG turn-over, next to reducing pain and function.[19, 188] As these exercises are preferably weight-bearing in nature (e.g., squat exercise),[19] optimal chondrocyte metabolism requires optimal balances of charge and pH, possibly disturbed in prolonged deformation, and dehydration.[189] As individuals presenting with early radiographic signs of OA are at increased risk for accelerated progression,[7] repetition of load may be suggested to consider the potentially altered deformational responses of cartilage.[138-140] To this end, we addressed the following research questions: (1) How does tibiofemoral knee cartilage respond to a dynamic 30-repetition squat exercise in terms of deformation in individuals with signs of doubtful-to-mild radiographic OA? (2) How long does it take for the respective cartilage plates to recover to baseline quantitative morphological state? (**Chapter 6**)

REFERENCES

1. Kellgren JH. Osteoarthrosis in patients and populations. B M J. 1961;2(5243):1-6.
2. Creamer P, Hochberg MC. Osteoarthritis. Lancet. 1997;350(9076):503-508.
3. Felson DT. An update on the pathogenesis and epidemiology of osteoarthritis. Radiol Clin North Am. 2004;42(1):1-9, v.
4. Pereira D, Peleteiro B, Araujo J, Branco J, Santos RA, Ramos E. The effect of osteoarthritis definition on prevalence and incidence estimates: a systematic review. Osteoarthritis Cartilage. 2011;19(11):1270-1285.
5. Lane NE, Brandt K, Hawker G, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarthritis Cartilage. 2011;19(5):478-482.
6. Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol. 2000;27(6):1513-1517.
7. Cibere J, Sayre EC, Guermazi A, et al. Natural history of cartilage damage and osteoarthritis progression on magnetic resonance imaging in a population-based cohort with knee pain. Osteoarthritis Cartilage. 2011;19(6):683-688.
8. Eckstein F, Maschek S, Wirth W, et al. One year change of knee cartilage morphology in the first release of participants from the Osteoarthritis Initiative progression subcohort: association with sex, body mass index, symptoms and radiographic osteoarthritis status. Ann Rheum Dis. 2009;68(5):674-679.
9. Hunter DJ, Niu J, Zhang Y, et al. Change in cartilage morphometry: a sample of the progression cohort of the Osteoarthritis Initiative. Ann Rheum Dis. 2009;68(3):349-356.
10. Wluka AE, Forbes A, Wang Y, Hanna F, Jones G, Cicuttini FM. Knee cartilage loss in symptomatic knee osteoarthritis over 4.5 years. Arthritis Res Ther. 2006;8(4):R90.
11. Hunter DJ. Lower extremity osteoarthritis management needs a paradigm shift. Br J Sports Med. 2011;45(4):283-288.
12. Bennell KL, Hinman RS. A review of the clinical evidence for exercise in osteoarthritis of the hip and knee. J Sci Med Sport. 2011;14(1):4-9.
13. Fransen M, McConnell S. Exercise for osteoarthritis of the knee. Cochrane Database Syst Rev. 2008(4):CD004376.
14. Helmark IC, Petersen MC, Christensen HE, Kjaer M, Langberg H. Moderate loading of the human osteoarthritic knee joint leads to lowering of intraarticular cartilage oligomeric matrix protein. Rheumatol Int. 2012;32(4):1009-1014.
15. Chua SD, Jr., Messier SP, Legault C, Lenz ME, Thonar EJ, Loeser RF. Effect of an exercise and dietary intervention on serum biomarkers in overweight and obese adults with osteoarthritis of the knee. Osteoarthritis Cartilage. 2008;16(9):1047-1053.
16. Petersen SG, Saxne T, Heinegard D, et al. Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training. Osteoarthritis Cartilage. 2010;18(1):34-40.
17. Bautch JC, Clayton MK, Chu Q, Johnson KA. Synovial fluid chondroitin sulphate epitopes 3B3 and 7D4, and glycosaminoglycan in human knee osteoarthritis after exercise. Ann Rheum Dis. 2000;59(11):887-891.
18. Woollard JD, Gil AB, Sparto P, et al. Change in knee cartilage volume in individuals completing a therapeutic exercise program for knee osteoarthritis. J Orthop Sports Phys Ther. 2011;41(10):708-722.
19. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum. 2005;52(11):3507-3514.
20. Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res. 2001(391 Suppl):S26-33.
21. James CB, Uhl TL. A review of articular cartilage pathology and the use of glucosamine sulfate. J Athl Training. 2001;36(4):413-419.
22. Cohen NP, Foster RJ, Mow VC. Composition and dynamics of articular cartilage: Structure, function, and maintaining healthy state. J Orthop Sports Phys Ther. 1998;28(4):203-215.
23. Eckstein F, Reiser M, englmeier KH, Putz R. In vivo morphometry and functional analysis of human articular cartilage with quantitative magnetic resonance imaging-from image to data. From data to theory. Anat Embryol (Berl). 2001;203:147-173.
24. Gray ML, Burstein D, Kim YJ, Maroudas A. 2007 Elizabeth Winston Lanier Award Winner. Magnetic resonance imaging of cartilage glycosaminoglycan: basic principles, imaging technique, and clinical applications. J Orthop Res. 2008;26(3):281-291.
25. Pauli C, Bae WC, Lee M, et al. Ultrashort-Echo Time MR Imaging of the Patella with Bicomponent Analysis: Correlation with Histopathologic and Polarized Light Microscopic Findings. Radiology. 2012;264(2):484-493.
26. Eckstein F, Reiser M, Englmeier KH, Putz R. In vivo morphometry and functional analysis of human articular cartilage with quantitative magnetic resonance imaging--from image to data, from data to theory. Anat Embryol (Berl). 2001;203(3):147-173.
27. Knecht S, Vanwanseele B, Stussi E. A review on the mechanical quality of articular cartilage - implications for the diagnosis of osteoarthritis. Clin Biomech (Bristol, Avon). 2006;21(10):999-1012.
28. Kurkijarvi JE, Nissi MJ, Kiviranta I, Jurvelin JS, Nieminen MT. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) and T2 characteristics of human knee articular cartilage: topographical variation and relationships to mechanical properties. Magn Reson Med. 2004;52(1):41-46.
29. Loeser RF. Molecular mechanisms of cartilage destruction: mechanics, inflammatory mediators, and aging collide. Arthritis Rheum. 2006;54(5):1357-1360.
30. Stubendorff JJ, Lammentausta E, Struglics A, Lindberg L, Heinegard D, Dahlberg LE. Is cartilage sGAG content related to early changes in cartilage disease? Implications for interpretation of dGEMRIC. Osteoarthritis Cartilage. 2012;20(5):396-404.
31. van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? Osteoarthr Cartilage. 2012;20(3):223-232.
32. Hosseininia S, Lindberg LR, Dahlberg LE. Cartilage collagen damage in hip osteoarthritis similar to that seen in knee osteoarthritis; a case-control study of relationship between collagen, glycosaminoglycan and cartilage swelling. BMC Musculoskelet Disord. 2013;14:18.

33. Loeser RF, Jr. Aging and the etiopathogenesis and treatment of osteoarthritis. Rheum Dis Clin North Am. 2000;26(3):547-567.

34. Bank RA, Soudry M, Maroudas A, Mizrahi J, TeKoppele JM. The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation. Arthritis Rheum. 2000;43(10):2202-2210.

35. Silver FH, Bradica G, Tria A. Viscoelastic behavior of osteoarthritic cartilage. Connect Tissue Res. 2001;42(3):223-233.

36. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum. 2012;64(6):1697-1707.

37. Loeser RF. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clin Geriatr Med. 2010;26(3):371-386.

38. Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis Cartilage. 2009;17(8):971-979.

39. Baum T, Joseph GB, Arulanandan A, et al. Association of MRI-based knee cartilage T2 measurements and focal knee lesions with knee pain - data from the Osteoarthritis Initiative. Arthritis Care Res (Hoboken). 2011.

40. Farrokhi S, Colletti PM, Powers CM. Differences in Patellar Cartilage Thickness, Transverse Relaxation Time, and Deformational Behavior A Comparison of Young Women With and Without Patellofemoral Pain. Am J Sports Med. 2011;39(2):384-391.

41. Hunter DJ, March L, Sambrook PN. The association of cartilage volume with knee pain. Osteoarthritis Cartilage. 2003;11(10):725-729.

42. Hunter DJ. Imaging insights on the epidemiology and pathophysiology of osteoarthritis. Rheum Dis Clin North Am. 2009;35(3):447-463.

43. Yusuf E, Kortekaas MC, Watt I, Huizinga TW, Kloppenburg M. Do knee abnormalities visualised on MRI explain knee pain in knee osteoarthritis? A systematic review. Ann Rheum Dis. 2011;70(1):60-67.

44. Ramage L, Nuki G, Salter DM. Signalling cascades in mechanotransduction: cell-matrix interactions and mechanical loading. Scand J Med Sci Sports. 2009;19(4):457-469.

45. Jeon J, Schrobback K, Hutmacher D, Klein T. Dynamic compression improves biosynthesis of human zonal chondrocytes from osteoarthritis patients. J Tissue Eng Regen Med. 2012;6:70-70.

46. Loeser RF. Chondrocyte integrin expression and function. Biorheology. 2000;37(1-2):109-116.

47. Felson DT, Zhang YQ, Hannan MT, et al. Risk factors for incident radiographic knee osteoarthritis in the elderly - The Framingham Study. Arthritis Rheum. 1997;40(4):728-733.

48. Spector TD, Harris PA, Hart DJ, et al. Risk of osteoarthritis associated with long-term weight-bearing sports: a radiologic survey of the hips and knees in female ex-athletes and population controls. Arthritis Rheum. 1996;39(6):988-995.

49. Felson DT, Niu J, Clancy M, Sack B, Aliabadi P, Zhang Y. Effect of recreational physical activities on the development of knee osteoarthritis in older adults of different weights: the Framingham Study. Arthritis Rheum. 2007;57(1):6-12.

50. Hannan MT, Felson DT, Anderson JJ, Naimark A. Habitual physical activity is not associated with knee osteoarthritis: the Framingham Study. J Rheumatol. 1993;20(4):704-709.

51. Lane NE, Michel B, Bjorkengren A, et al. The risk of osteoarthritis with running and aging: a 5-year longitudinal study. J Rheumatol. 1993;20(3):461-468.

52. Lane NE, Oehlert JW, Bloch DA, Fries JF. The relationship of running to osteoarthritis of the knee and hip and bone mineral density of the lumbar spine: a 9 year longitudinal study. J Rheumatol. 1998;25(2):334-341.

53. Urquhart DM, Tobing JFL, Hanna FS, et al. What Is the Effect of Physical Activity on the Knee Joint? A Systematic Review. Med Sci Sport Exerc. 2011;43(3):432-442.

54. Hunter DJ, Eckstein F. Exercise and osteoarthritis. J Anat. 2009;214(2):197-207.

55. Doré DA, Winzenberg T, Ding C, et al. The association between objectively measured physical activity and knee structural change using MRI. Ann Rheum Dis. 2012.

56. Eckstein F, Ateshian G, Burgkart R, et al. Proposal for a nomenclature for magnetic resonance imaging based measures of articular cartilage in osteoarthritis. Osteoarthritis Cartilage. 2006;14(10):974-983.

57. Roemer FW, Crema MD, Trattnig S, Guermazi A. Advances in imaging of osteoarthritis and cartilage. Radiology. 2011;260(2):332-354.

58. Hunter DJ, Zaim S, Mosher TJ. What semi-quantitative scoring instrument for knee OA MRI should you use? Osteoarthritis Cartilage. 2010;18(11):1363-1364.

59. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage. 2006;14 Suppl A:A46-75.

60. Guermazi A, Roemer FW, Haugen IK, Crema MD, Hayashi D. MRI-based semiquantitative scoring of joint pathology in osteoarthritis. Nat Rev Rheumatol. 2013.

61. Crema MD, Roemer FW, Marra MD, et al. Articular cartilage in the knee: current MR imaging techniques and applications in clinical practice and research. Radiographics. 2011;31(1):37-61.

62. Eckstein F, Hudelmaier M, Wirth W, et al. Double echo steady state magnetic resonance imaging of knee articular cartilage at 3 Tesla: a pilot study for the Osteoarthritis Initiative. Ann Rheum Dis. 2006;65(4):433-441.

63. Burstein D, Gray M, Mosher T, Dardzinski B. Measures of molecular composition and structure in osteoarthritis. Radiol Clin North Am. 2009;47(4):675-686.

64. Eckstein F, Hudelmaier M, Putz R. The effects of exercise on human articular cartilage. J Anat. 2006;208(4):491-512.

65. Link TM. MR imaging in osteoarthritis: hardware, coils, and sequences. Radiol Clin North Am. 2009;47(4):617-632.

66. Crema MD, Roemer FW, Guermazi A. Magnetic resonance imaging in knee osteoarthritis research: semiquantitative and compositional assessment. Magn Reson Imaging Clin N Am. 2011;19(2):295-321.

67. Guermazi A, Roemer FW, Hayashi D. Imaging of osteoarthritis: update from a radiological perspective. Curr Opin Rheumatol. 2011;23(5):484-491.

68. Crema MD, Roemer FW, Marra MD, et al. Articular cartilage in the knee: current MR imaging techniques and applications in clinical practice and research. Radiographics. 2011;31(1):37-61.

69. Peterfy CG, Guermazi A, Zaim S, et al. Whole-organ magnetic resonance imaging score (WORMS) of the knee in osteoarthritis. Osteoarthritis Cartilage. 2004;12(3):14.

70. Hunter DJ, Lo GH, Gale D, Grainger AJ, Guermazi A, Conaghan PG. The reliability of a new scoring system for knee osteoarthritis MRI and the validity of bone marrow lesion assessment: BLOKS (Boston Leeds Osteoarthritis Knee Score). Ann Rheum Dis. 2008;67(2):206-211.

71.   Kornaat PR, Ceulemans RY, Kroon HM, et al. MRI assessment of knee osteoarthritis: Knee Osteoarthritis Scoring System (KOSS)--inter-observer and intra-observer reproducibility of a compartment-based scoring system. Skeletal Radiol. 2005;34(2):95-102.

72.   Hunter DJ, Guermazi A, Lo GH, et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). Osteoarthritis Cartilage. 2011;19(8):990-1002.

73.   Lynch JA, Roemer FW, Nevitt MC, et al. Comparison of BLOKS and WORMS scoring systems part I. Cross sectional comparison of methods to assess cartilage morphology, meniscal damage and bone marrow lesions on knee MRI: data from the osteoarthritis initiative. Osteoarthritis Cartilage. 2010;18(11):1393-1401.

74.   Felson DT, Lynch J, Guermazi A, et al. Comparison of BLOKS and WORMS scoring systems part II. Longitudinal assessment of knee MRIs for osteoarthritis and suggested approach based on their performance: data from the Osteoarthritis Initiative. Osteoarthritis Cartilage. 2010;18(11):1402-1407.

75.   Hunter DJ, Zhang W, Conaghan PG, et al. Responsiveness and reliability of MRI in knee osteoarthritis: a meta-analysis of published evidence. Osteoarthritis Cartilage. 2011;19(5):589-605.

76.   Stammberger T, Eckstein F, Michaelis M, Englmeier KH, Reiser M. Interobserver reproducibility of quantitative cartilage measurements: comparison of B-spline snakes and manual segmentation. Magn Reson Imaging. 1999;17(7):1033-1042.

77.   Bowers ME, Trinh N, Tung GA, Crisco JJ, Kimia BB, Fleming BC. Quantitative MR imaging using "LiveWire" to measure tibiofemoral articular cartilage thickness. Osteoarthritis Cartilage. 2008;16(10):1167-1173.

78.   Tamez-Pena JG, Farber J, Gonzalez PC, Schreyer E, Schneider E, Totterman S. Unsupervised segmentation and quantification of anatomical knee features: data from the Osteoarthritis Initiative. IEEE transactions on bio-medical engineering. 2012;59(4):1177-1186.

79.   Wirth W, Nevitt M, Hellio Le Graverand MP, et al. Sensitivity to change of cartilage morphometry using coronal FLASH, sagittal DESS, and coronal MPR DESS protocols--comparative data from the Osteoarthritis Initiative (OAI). Osteoarthritis Cartilage. 2010;18(4):547-554.

80.   Dunn TC, Lu Y, Jin H, Ries MD, Majumdar S. T2 relaxation time of cartilage at MR imaging: Comparison with severity of knee osteoarthritis. Radiology. 2004;232(2):592-598.

81.   Link TM, Steinbach LS, Ghosh S, et al. Osteoarthritis: MR imaging findings in different stages of disease and correlation with clinical findings. Radiology. 2003;226(2):373-381.

82.   Stahl R, Blumenkrantz G, Carballido-Gamio J, et al. MRI-derived T2 relaxation times and cartilage morphometry of the tibio-femoral joint in subjects with and without osteoarthritis during a 1-year follow-up. Osteoarthritis Cartilage. 2007;15(11):1225-1234.

83.   Zarins ZA, Bolbos RI, Pialat JB, et al. Cartilage and meniscus assessment using T1rho and T2 measurements in healthy subjects and patients with osteoarthritis. Osteoarthritis Cartilage. 2010;18(11):1408-1416.

84.   Qian Y, Williams AA, Chu CR, Boada FE. Repeatability of ultrashort echo time-based two-component T(2) (*) measurements on cartilages in human knee at 3 T. Magn Reson Med. 2012.

85.   Raya JG, Dietrich O, Horng A, Weber J, Reiser MF, Glaser C. T(2) Measurement in Articular Cartilage: Impact of the Fitting Method on Accuracy and Precision at Low SNR. Magn Reson Med. 2010;63(1):181-193.

86.   Goto H, Fujii M, Iwama Y, Aoyama N, Ohno Y, Sugimura K. Magnetic resonance imaging (MRI) of articular cartilage of the knee using ultrashort echo time (uTE) sequences with spiral acquisition. J Med Imag Radiat Oncol. 2012;56(3):318-323.

87.   Bittersohl B, Miese FR, Hosalkar HS, et al. T2* mapping of hip joint cartilage in various histological grades of degeneration. Osteoarthritis Cartilage. 2012;20(7):653-660.

88.   Chou MC, Tsai PH, Huang GS, et al. Correlation between the MR T2 value at 4.7 T and relative water content in articular cartilage in experimental osteoarthritis induced by ACL transection. Osteoarthritis Cartilage. 2009;17(4):441-447.

89.   Li X, Cheng J, Lin K, et al. Quantitative MRI using T1rho and T2 in human osteoarthritic cartilage specimens: correlation with biochemical measurements and histology. Magn Reson Imaging. 2011;29(3):324-334.

90.   Joseph GB, Baum T, Alizai H, et al. Baseline mean and heterogeneity of MR cartilage T2 are associated with morphologic degeneration of cartilage, meniscus, and bone marrow over 3 years--data from the Osteoarthritis Initiative. Osteoarthritis Cartilage. 2012;20(7):727-735.

91.   Currie S, Hoggard N, Craven IJ, Hadjivassiliou M, Wilkinson ID. Understanding MRI: basic MR physics for physicians. Postgrad Med J. 2012.

92.   Li X, Benjamin Ma C, Link TM, et al. In vivo T(1rho) and T(2) mapping of articular cartilage in osteoarthritis of the knee using 3 T MRI. Osteoarthritis Cartilage. 2007;15(7):789-797.

93.   Li X, Kuo D, Theologis A, et al. Cartilage in anterior cruciate ligament-reconstructed knees: MR imaging T1{rho} and T2--initial experience with 1-year follow-up. Radiology. 2011;258(2):505-514.

94.   Li X, Pai A, Blumenkrantz G, et al. Spatial distribution and relationship of T1rho and T2 relaxation times in knee cartilage with osteoarthritis. Magn Reson Medicine. 2009;61(6):1310-1318.

95.   Wang N, Xia Y. Dependencies of multi-component T2 and T1rho relaxation on the anisotropy of collagen fibrils in bovine nasal cartilage. J Magn Reson. 2011;212(1):124-132.

96.   Pakin SK, Schweitzer ME, Regatte RR. 3D-T1rho quantitation of patellar cartilage at 3.0T. J Magn Reson Imaging. 2006;24(6):1357-1363.

97.   Williams A, Gillis A, McKenzie C, et al. Glycosaminoglycan distribution in cartilage as determined by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): potential clinical applications. Am J Roentgenol. 2004;182(1):167-172.

98.   Burstein D, Velyvis J, Scott KT, et al. Protocol issues for delayed Gd(DTPA)(2-)-enhanced MRI (dGEMRIC) for clinical evaluation of articular cartilage. Magn Reson Med. 2001;45(1):36-41.

99.   McKenzie CA, Williams A, Prasad PV, Burstein D. Three-dimensional delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) at 1.5T and 3.0T. J Magn Reson Imaging. 2006;24(4):928-933.

100.   Siversson C, Tiderius CJ, Neuman P, Dahlberg L, Svensson J. Repeatability of T1-quantification in dGEMRIC for three different acquisition techniques: two-dimensional inversion recovery, three-dimensional look locker, and three-dimensional variable flip angle. J Magn Reson Imaging. 2010;31(5):1203-1209.

101.   Williams A, Mikulis B, Krishnan N, Gray M, McKenzie C, Burstein D. Suitability of T(1Gd) as the dGEMRIC index at 1.5T and 3.0T. Magn Reson Med. 2007;58(4):830-834.

102.   Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed Gd(DTPA)(2-)-enhanced MR imaging. Radiology. 1997;205(2):551-558.

103.   Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med. 1999;41(5):857-865.

104. Tiderius C, Hori M, Williams A, et al. dGEMRIC as a function of BMI. Osteoarthritis Cartilage. 2006;14(11):1091-1097.
105. Neuman P, Tjornstrand J, Svensson J, et al. Longitudinal assessment of femoral knee cartilage quality using contrast enhanced MRI (dGEMRIC) in patients with anterior cruciate ligament injury--comparison with asymptomatic volunteers. Osteoarthritis Cartilage. 2011;19(8):977-983.
106. Price RR. The AAPM/RSNA physics tutorial for residents. MR imaging safety considerations. Radiological Society of North America. Radiographics. 1999;19(6):1641-1651.
107. Niendorf HP, Haustein J, Cornelius I, Alhassan A, Clauss W. Safety of gadolinium-DTPA: extended clinical experience. Magn Reson Med. 1991;22(2):222-228; discussion 229-232.
108. Hosseini A, Van de Velde SK, Kozanek M, et al. In-vivo time-dependent articular cartilage contact behavior of the tibiofemoral joint. Osteoarthritis Cartilage. 2010;18(7):909-916.
109. Liu F, Kozanek M, Hosseini A, et al. In vivo tibiofemoral cartilage deformation during the stance phase of gait. J Biomech. 2010;43(4):658-665.
110. Eckstein F, Lemberger B, Gratzke C, et al. In vivo cartilage deformation after different types of activity and its dependence on physical training status. Ann Rheum Dis. 2005;64(2):291-295.
111. Koo S, Rylander JH, Andriacchi TP. Knee joint kinematics during walking influences the spatial cartilage thickness distribution in the knee. J Biomech. 2011;44(7):1405-1409.
112. Mayerhoefer ME, Welsch GH, Mamisch TC, et al. The in vivo effects of unloading and compression on T1-Gd (dGEMRIC) relaxation times in healthy articular knee cartilage at 3.0 Tesla. Eur Radiol. 2010;20(2):443-449.
113. Nag D, Liney GP, Gillespie P, Sherman KP. Quantification of T(2) relaxation changes in articular cartilage with in situ mechanical loading of the knee. J Magn Reson Imaging. 2004;19(3):317-322.
114. Bingham JT, Papannagari R, Van de Velde SK, et al. In vivo cartilage contact deformation in the healthy human tibiofemoral joint. Rheumatology (Oxford). 2008;47(11):1622-1627.
115. Eckstein F, Lemberger B, Stammberger T, Englmeier KH, Reiser M. Patellar cartilage deformation in vivo after static versus dynamic loading. J Biomech. 2000;33(7):819-825.
116. Eckstein F, Tieschky M, Faber S, Englmeier KH, Reiser M. Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. Anat Embryol (Berl). 1999;200(4):419-424.
117. Eckstein F, Tieschky M, Faber SC, et al. Effect of physical exercise on cartilage volume and thickness in vivo: MR imaging study. Radiology. 1998;207(1):243-248.
118. Liess C, Lusse S, Karger N, Heller M, Gluer CC. Detection of changes in cartilage water content using MRI T2-mapping in vivo. Osteoarthritis Cartilage. 2002;10(12):907-913.
119. Hudelmaier M, Glaser C, Hohe J, et al. Age-related changes in the morphology and deformational behavior of knee joint cartilage. Arthritis Rheum. 2001;44(11):2556-2561.
120. Niehoff A, Muller M, Bruggemann L, et al. Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. Osteoarthritis Cartilage. 2011;19(8):1003-1010.
121. Boocock M, McNair P, Cicuttini F, Stuart A, Sinclair T. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. Osteoarthritis Cartilage. 2009;17(7):883-890.
122. Kersting UG, Stubendorff JJ, Schmidt MC, Bruggemann GP. Changes in knee cartilage volume and serum COMP concentration after running exercise. Osteoarthritis Cartilage. 2005;13(10):925-934.
123. Mosher TJ, Liu Y, Torok CM. Functional cartilage MRI T2 mapping: evaluating the effect of age and training on knee cartilage response to running. Osteoarthritis Cartilage. 2010;18(3):358-364.
124. Mosher TJ, Smith HE, Collins C, et al. Change in knee cartilage T2 at MR imaging after running: a feasibility study. Radiology. 2005;234(1):245-249.
125. Subburaj K, Kumar D, Souza RB, et al. The acute effect of running on knee articular cartilage and meniscus magnetic resonance relaxation times in young healthy adults. Am J Sports Med. 2012;40(9):2134-2141.
126. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Volume changes in the menisci and articular cartilage of runners: an in vivo investigation based on 3-D magnetic resonance imaging. Am J Sports Med. 2006;34(5):832-836.
127. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. Am J Sports Med. 2008;36(5):966-970.
128. Luke AC, Stehling C, Stahl R, et al. High-field magnetic resonance imaging assessment of articular cartilage before and after marathon running: does long-distance running lead to cartilage damage? Am J Sports Med. 2010;38(11):2273-2280.
129. Schueller-Weidekamm C, Schueller G, Uffmann M, Bader TR. Does marathon running cause acute lesions of the knee? Evaluation with magnetic resonance imaging. Eur Radiol. 2006;16(10):2179-2185.
130. Shellock FG, Deutsch AL, Mink JH, Kerr R. Do asymptomatic marathon runners have an increased prevalence of meniscal abnormalities? An MR study of the knee in 23 volunteers. Am J Roentgenol. 1991;157(6):1239-1241.
131. Shellock FG, Mink JH. Knees of trained long-distance runners: MR imaging before and after competition. Radiology. 1991;179(3):635-637.
132. Stahl R, Luke A, Ma CB, et al. Prevalence of pathologic findings in asymptomatic knees of marathon runners before and after a competition in comparison with physically active subjects-a 3.0 T magnetic resonance imaging study. Skeletal Radiol. 2008;37(7):627-638.
133. Stehling C, Luke A, Stahl R, et al. Meniscal T1rho and T2 measured with 3.0T MRI increases directly after running a marathon. Skeletal Radiol. 2011;40(6):725-735.
134. Son M, Goodman SB, Chen W, Hargreaves BA, Gold GE, Levenston ME. Regional variation in T1rho and T2 times in osteoarthritic human menisci: correlation with mechanical properties and matrix composition. Osteoarthritis Cartilage. 2013;21(6):796-805.
135. Krampla W, Mayrhofer R, Malcher J, Kristen KH, Urban M, Hruby W. MR imaging of the knee in marathon runners before and after competition. Skeletal Radiol. 2001;30(2):72-76.
136. Krampla WW, Newrkla SP, Kroener AH, Hruby WF. Changes on magnetic resonance tomography in the knee joints of marathon runners: a 10-year longitudinal study. Skeletal Radiol. 2008;37(7):619-626.
137. Froimson MI, Ratcliffe A, Gardner TR, Mow VC. Differences in patellofemoral joint cartilage material properties and their significance to the etiology of cartilage surface fibrillation. Osteoarthritis Cartilage. 1997;5(6):377-386.
138. Cotofana S, Eckstein F, Wirth W, et al. In vivo measures of cartilage deformation: patterns in healthy and osteoarthritic female knees using 3T MR imaging. Eur Radiol. 2011;21(6):1127-1135.

139. Shin CS, Souza RB, Kumar D, Link TM, Wyman BT, Majumdar S. In vivo tibiofemoral cartilage-to-cartilage contact area of females with medial osteoarthritis under acute loading using MRI. J Magn Reson Imaging. 2011;34(6):1405-1413.

140. Subburaj K, Souza RB, Stehling C, et al. Association of MR relaxation and cartilage deformation in knee osteoarthritis. J Orthop Res. 2012;30(6):919-926.

141. Hosseini A, Van de Velde S, Gill TJ, Li G. Tibiofemoral cartilage contact biomechanics in patients after reconstruction of a ruptured anterior cruciate ligament. J Orthop Res. 2012;30(11):1781-1788.

142. Van de Velde SK, Bingham JT, Hosseini A, et al. Increased tibiofemoral cartilage contact deformation in patients with anterior cruciate ligament deficiency. Arthritis Rheum. 2009;60(12):3693-3702.

143. Leiter JR, MacDonald L, McRae S, Davidson M, MacDonald PB. Intrinsic stresses on bone and cartilage in the normal and anterior cruciate ligament-reconstructed knee before and after a half marathon: a magnetic resonance imaging analysis. Clin J Sport Med. 2012;22(5):439-442.

144. Hinterwimmer S, Krammer M, Krotz M, et al. Cartilage atrophy in the knees of patients after seven weeks of partial load bearing. Arthritis Rheum. 2004;50(8):2516-2520.

145. Hudelmaier M, Glaser C, Hausschild A, Burgkart R, Eckstein F. Effects of joint unloading and reloading on human cartilage morphology and function, muscle cross-sectional areas, and bone density - a quantitative case report. J Musculoskelet Neuronal Interact. 2006;6(3):284-290.

146. Souza RB, Baum T, Wu S, et al. Effects of unloading on knee articular cartilage T1rho and T2 magnetic resonance imaging relaxation times: a case series. J Orthop Sports Phys Ther. 2012;42(6):511-520.

147. Liphardt AM, Mundermann A, Koo S, et al. Vibration training intervention to maintain cartilage thickness and serum concentrations of cartilage oligometric matrix protein (COMP) during immobilization. Osteoarthritis Cartilage. 2009;17(12):1598-1603.

148. Vanwanseele B, Eckstein F, Knecht H, Spaepen A, Stussi E. Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord injury. Arthritis Rheum. 2003;48(12):3377-3381.

149. Vanwanseele B, Eckstein F, Knecht H, Stussi E, Spaepen A. Knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. Arthritis Rheum. 2002;46(8):2073-2078.

150. Eckstein F, Faber S, Muhlbauer R, et al. Functional adaptation of human joints to mechanical stimuli. Osteoarthritis Cartilage. 2002;10(1):44-50.

151. Schiphof D, de Klerk BM, Kerkhof HJ, et al. Impact of different descriptions of the Kellgren and Lawrence classification criteria on the diagnosis of knee osteoarthritis. Ann Rheum Dis. 2011;70(8):1422-1427.

152. Jones G, Ding C, Glisson M, Hynes K, Ma D, Cicuttini F. Knee articular cartilage development in children: a longitudinal study of the effect of sex, growth, body composition, and physical activity. Pediatr Res. 2003;54(2):230-236.

153. Muhlbauer R, Lukasz TS, Faber TS, Stammberger T, Eckstein F. Comparison of knee joint cartilage thickness in triathletes and physically inactive volunteers based on magnetic resonance imaging and three-dimensional analysis. Am J Sports Med. 2000;28(4):541-546.

154. Gratzke C, Hudelmaier M, Hitzl W, Glaser C, Eckstein F. Knee cartilage morphologic characteristics and muscle status of professional weight lifters and sprinters: a magnetic resonance imaging study. Am J Sports Med. 2007;35(8):1346-1353.

155. Tiderius CJ, Svensson J, Leander P, Ola T, Dahlberg L. dGEMRIC (delayed gadolinium-enhanced MRI of cartilage) indicates adaptive capacity of human knee cartilage. Magn Reson Med. 2004;51(2):286-290.

156. Teichtahl AJ, Wluka AE, Wang Y, et al. Occupational activity is associated with knee cartilage morphology in females. Maturitas. 2010;66(1):72-76.

157. Foley S, Ding CH, Cicuttini F, Jones G. Physical activity and knee structural change: A longitudinal study using MRI. Med Sci Sport Exerc. 2007;39(3):426-434.

158. Hovis KK, Stehling C, Souza RB, et al. Physical activity is associated with magnetic resonance imaging-based knee cartilage T2 measurements in asymptomatic subjects with and those without osteoarthritis risk factors. Arthritis Rheum. 2011;63(8):2248-2256.

159. Stahl R, Luke A, Li X, et al. T1rho, T2 and focal knee cartilage abnormalities in physically active and sedentary healthy subjects versus early OA patients--a 3.0-Tesla MRI study. Eur Radiol. 2009;19(1):132-143.

160. Stehling C, Liebl H, Krug R, et al. Patellar cartilage: T2 values and morphologic abnormalities at 3.0-T MR imaging in relation to physical activity in asymptomatic subjects from the osteoarthritis initiative. Radiology. 2010;254(2):509-520.

161. Cotofana S, Ring-Dimitriou S, Hudelmaier M, et al. Effects of exercise intervention on knee morphology in middle-aged women: a longitudinal analysis using magnetic resonance imaging. Cells Tissues Organs. 2010;192(1):64-72.

162. Hanna F, Teichtahl AJ, Bell R, et al. The cross-sectional relationship between fortnightly exercise and knee cartilage properties in healthy adult women in midlife. Menopause. 2007;14(5):830-834.

163. Wijayaratne SP, Teichtahl AJ, Wluka AE, et al. The determinants of change in patella cartilage volume--a cohort study of healthy middle-aged women. Rheumatology (Oxford). 2008;47(9):1426-1429.

164. Racunica TL, Teichtahl AJ, Wang Y, et al. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum. 2007;57(7):1261-1268.

165. Teichtahl AJ, Davies-Tuck ML, Wluka AE, Jones G, Cicuttini FM. Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis. Osteoarthritis Cartilage. 2009;17(1):8-11.

166. Teichtahl AJ, Wluka AE, Wang Y, et al. Effect of long-term vigorous physical activity on healthy adult knee cartilage. Med Sci Sports Exerc. 2012;44(6):985-992.

167. Buck RJ, Wyman BT, Le Graverand MP, Hudelmaier M, Wirth W, Eckstein F. Osteoarthritis may not be a one-way-road of cartilage loss--comparison of spatial patterns of cartilage change between osteoarthritic and healthy knees. Osteoarthritis Cartilage. 2010;18(3):329-335.

168. Adam C, Eckstein F, Milz S, Putz R. The distribution of cartilage thickness within the joints of the lower limb of elderly individuals. J Anat. 1998;193 (Pt 2):203-214.

169. Kuettner KE, Cole AA. Cartilage degeneration in different human joints. Osteoarthritis Cartilage. 2005;13(2):93-103.

170. Daniels T, Thomas R. Etiology and biomechanics of ankle arthritis. Foot Ankle Clin. 2008;13(3):341-352, vii.

171. Koepp H, Eger W, Muehleman C, et al. Prevalence of articular cartilage degeneration in the ankle and knee joints of human organ donors. J Orthop Sci. 1999;4(6):407-412.

172.   Aurich M, Mwale F, Reiner A, et al. Collagen and proteoglycan turnover in focally damaged human ankle cartilage: evidence for a generalized response and active matrix remodeling across the entire joint surface. Arthritis Rheum. 2006;54(1):244-252.
173.   Aurich M, Squires GR, Reiner A, et al. Differential matrix degradation and turnover in early cartilage lesions of human knee and ankle joints. Arthritis Rheum. 2005;52(1):112-119.
174.   Eger W, Schumacher BL, Mollenhauer J, Kuettner KE, Cole AA. Human knee and ankle cartilage explants: catabolic differences. J Orthop Res. 2002;20(3):526-534.
175.   Schumacher BL, Su JL, Lindley KM, Kuettner KE, Cole AA. Horizontally oriented clusters of multiple chondrons in the superficial zone of ankle, but not knee articular cartilage. Anat Rec (Hoboken). 2002;266(4):241-248.
176.   Treppo S, Koepp H, Quan EC, Cole AA, Kuettner KE, Grodzinsky AJ. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. J Orthop Res. 2000;18(5):739-748.
177.   Frobell RB, Le Graverand MP, Buck R, et al. The acutely ACL injured knee assessed by MRI: changes in joint fluid, bone marrow lesions, and cartilage during the first year. Osteoarthritis Cartilage. 2009;17(2):161-167.
178.   Frobell RB. Change in cartilage thickness, posttraumatic bone marrow lesions, and joint fluid volumes after acute ACL disruption: a two-year prospective MRI study of sixty-one subjects. J Bone Joint Surg Am. 2011;93(12):1096-1103.
179.   Potter HG, Jain SK, Ma Y, Black BR, Fung S, Lyman S. Cartilage injury after acute, isolated anterior cruciate ligament tear: immediate and longitudinal effect with clinical/MRI follow-up. Am J Sports Med. 2012;40(2):276-285.
180.   Bigoni M, Sacerdote P, Turati M, et al. Acute and late changes in intraarticular cytokine levels following anterior cruciate ligament injury. J Orthop Res. 2013;31(2):315-321.
181.   Myklebust G, Bahr R. Return to play guidelines after anterior cruciate ligament surgery. Br J Sports Med. 2005;39(3):127-131.
182.   Claes S, Hermie L, Verdonk R, Bellemans J, Verdonk P. Is osteoarthritis an inevitable consequence of anterior cruciate ligament reconstruction? A meta-analysis. Knee Surg Sports Traumatol Arthrosc. 2012.
183.   Oiestad BE, Engebretsen L, Storheim K, Risberg MA. Knee Osteoarthritis After Anterior Cruciate Ligament Injury A Systematic Review. Am J Sports Med. 2009;37(7):1434-1443.
184.   Spindler KP, Parker RD, Andrish JT, et al. Prognosis and predictors of ACL reconstructions using the MOON cohort: a model for comparative effectiveness studies. J Orthop Res. 2013;31(1):2-9.
185.   Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. Am J Sports Med. 2007;35(10):1756-1769.
186.   Thomee R, Kaplan Y, Kvist J, et al. Muscle strength and hop performance criteria prior to return to sports after ACL reconstruction. Knee Surg Sports Traumatol Arthrosc. 2011;19(11):1798-1805.
187.   Kvist J. Rehabilitation following anterior cruciate ligament injury: current recommendations for sports participation. Sports Med. 2004;34(4):269-280.
188.   Hall M, Hinman RS, Wrigley TV, et al. The effects of neuromuscular exercise on medial knee joint load post-arthroscopic partial medial meniscectomy: 'SCOPEX' a randomised control trial protocol. BMC Musculoskelet Disord. 2012;13:233.
189.   Song Y, Greve JM, Carter DR, Giori NJ. Meniscectomy alters the dynamic deformational behavior and cumulative strain of tibial articular cartilage in knee joints subjected to cyclic loads. Osteoarthritis Cartilage. 2008;16(12):1545-1554.
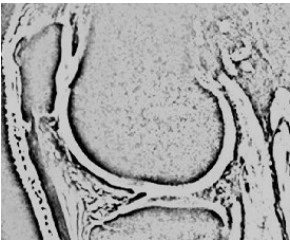
**PART ONE - EXERCISE AND
CHONDROPROTECTION
A FUNDAMENTAL APPROACH**

HUMAN ANKLE CARTILAGE DEFORMATION

AFTER DIFFERENT *IN VIVO* IMPACT CONDITIONS

ABSTRACT

Recently, the general finding of increased ankle cartilage stiffness to loading has been challenged, suggesting the need for the investigation of different *in vivo* loading conditions. Therefore, the objectives of the present study were to determine ankle (talar) cartilage deformation after *in vivo* loading using 3D volume change calculation and to establish any difference in volume change between four weight-bearing exercises. The four exercises represented increasing impact (bilateral knee bends < unilateral knee bends < drop jumps) as well as two types of loading: dynamic and static loading (i.e. unilateral knee bends and unilateral static stance). Based on MRI, 3D reconstructions of talar cartilage were generated to determine 3D volumes before and after four exercises in 13 healthy subjects (bilateral and unilateral knee bends, static unilateral stance, drop jumps). Mean talar deformation (volume decrease) was 8.3% after bilateral knee bends ($P = 0.001$), 7.7% after unilateral knee bends ($P = 0.020$), 14.6% after unilateral static stance ($P<0.001$), 12.5% after drop jumps ($P = 0.001$). Statistical analysis also revealed deformation to be significantly higher after unilateral static stance than after unilateral knee bends ($P = 0.017$). These results suggest that talar cartilage endures substantial deformation during *in vivo* loading characterized by more deformation (i.e. higher volume change) after static than after dynamic loading.

Keywords In vivo Exercise Ankle Cartilage

INTRODUCTION

The primary function of articular cartilage consists of stress dissipation, providing a frictionless surface during joint motion and improving joint surface congruence.[7] To fulfill these tasks, articular cartilage presents as a highly organized and complex tissue. The interplay between biochemical composition, ultrastructural organization and interaction between matrix constituents is generally known to characterize the tissue's biomechanical characteristics such as deformational behaviour. Being an avascular, aneural and alymfatic tissue, it is the cartilage matrix and its compounds that are of utmost importance for load transmission. This interstitial matrix consists for 70% of fluid and for 30% of structural compounds of which collagen fibrils and proteoglycan molecules are the main components. Although matrix composition varies throughout the depth of the tissue and collagens are prone to structural variation, the collagen fibrils (mainly type II) constitute a three-dimensional network that provides the tissue with tensile strength. Through linking proteins (e.g. cartilage oligomeric protein, decorin), the collagen network is attached to the proteoglycan macromolecules. The latter, in particular aggrecan, contain highly negatively charged glycosaminoglycan side chains (mainly keratin sulphate and chondroïtin sulphate) that attract water molecules and cations. Consequently, osmotic swelling pressures are created enabling cartilage to encounter compression stress.[7]

In general, cartilage *in vitro* deformational behaviour is illustrated using the linear biphasic theory. This well known theory postulates that loading the tissue leads to an instantaneous hydraulic pressurization allowing only little deformation during dynamic loading conditions. Similarly, during rapid high-impact loading, the solid collagen-proteoglycan matrix compounds would be protected as well by instantaneous rise in hydraulic pressure. In the case of static loading conditions (and over longer periods of time), fluids would gradually exude from the tissue decreasing hydraulic pressures resulting into more deformation.[7,20]

Ankle (i.e. talar) cartilage has been shown to contain a higher proportion of proteoglycans to water when compared to femoral cartilage.[3,10,16] Combined with a lower hydraulic permeability due to a smaller effective pore size, these structural features have been suggested to result into higher dynamic stiffness to loading when compared to the knee. Hence, less deformation during *in vitro* loading has been proposed to explain in part the remarkably low prevalence of primary ankle osteoarthritis.[8,10,11,15,16,26] Surprisingly, investigations using a dual fluoroscopic and magnetic resonance imaging technique to study ankle cartilage deformation in the living subject suggested an inconsistency between *in vitro* and *in vivo* conditions.[17, 28] Therefore, the study of different *in vivo* loading conditions should be pursuit.[17, 28]

In this study, talar cartilage *in vivo* deformation was investigated after four different loading conditions. The objectives were twofold. The first objective was to determine the amount of deformation of talar cartilage after *in vivo* loading. The second objective was to establish a

difference in deformation outcome between the four different exercises. The exercises under study comprised an increase in impact (i.e. bilateral loading, unilateral loading, unilateral drop jump) as well as two major types of loading (i.e. static versus dynamic loading). *In vivo* deformation was investigated by determining the difference in cartilage morphology (i.e. 3D volume) before and after each exercise.

It was hypothesized that talar cartilage deforms substantially and shows increased deformation with increasing impact. Additionally, higher volume changes were expected after static loading when compared to dynamic loading.

MATERIALS AND METHODS

Thirteen healthy able-bodied subjects (8 women, 5 men) participated voluntarily. Inclusion criteria were an age of 20–40 years old, a Body Mass Index of 20–30 kg/m^2, being injury free and performing sports on a regular basis (maximum three times/week). Exclusion criteria were a history of surgical or arthroscopic procedures, traumatic ligament injuries or chronic ankle instability, cartilage injuries or degenerative pathology to the ankle joint, a history of fractures at the lower leg or foot as well as contra-indications to MRI. On recruitment, inclusion and exclusion criteria were at first verified as reported by the subject. However, to rule out unknown cartilage lesions, MRI scans of the first visit were used to detect lesions if any. This was not the case in any of the subjects recruited.

All subjects were instructed not to practice sports the day before testing or on a testing day and to avoid running, taking stairs and lifting heavy weights 4 h preceding the actual experimental procedure.[1] The right lower limb was dominant over the left in all participants. Lower limb dominance was defined as the limb the subject would choose to kick a ball. The local Ethics Committee of the University Hospital approved the study, and informed consent was obtained from all. Subject demographics are tabulated in Table 1.

Table 1. Means (standard deviation, S.D.) and medians (percentile 25, P25 and percentile 75, P75) for subject demographics

Subject demographic (N=13; 8 female – 5 male)	Mean (S.D.)	Median (P25-P75)
Age (years)	26.7(4.5)	26.0(25.0-27.0)
Weight (kg)	72.9(11.1)	74.0(63.5-81.5)
Height (m)	1.8(0.1)	1.8(1.7-1.9)
Body Mass Index (kg/m^2)	22.4(1.5)	22.5(21.7-23.4)

Experimental procedures

Four weight-bearing exercises were examined on four separate testing days (one exercise/testing day). Whereas inter-testing intervals took at least 1 week, every testing procedure for a subject occurred on the same weekday at the same time to control for diurnal variation in cartilage thickness[23] and volume[29]. The experimental procedures took place within a 3-month period.

Weight-bearing exercises

The exercises, adapted from Eckstein et al.[5], comprised 30 bilateral knee bends until maximal ankle dorsal flexion in 1 min (Exercise 1), 30 unilateral knee bends until maximal ankle dorsal flexion in 1 min (Exercise 2), a 2-min static unilateral stance in maximal ankle dorsal flexion (Exercise 3) and 10 drop jumps from a 40 cm height landing on one foot (Exercise 4). Maximal ankle dorsal flexion was restrained to anterior rocking of the lower leg over the foot without lifting the heel from the floor while horizontally lowering the upper leg over 20° of knee flexion for static stance and to 120° of knee flexion in dynamic knee bending exercises. (Figure 1) All exercises were carried out barefoot next to the scanner under a researcher's supervision to control for a correct and standardized performance. Unilateral exercises were performed on the dominant right lower limb.

Figure 1. The four weight-bearing exercises. From *left* to *right*: 30 bilateral knee bends until maximal ankle dorsal flexion within one minute (Exercise 1), 30 unilateral knee bends until maximal ankle dorsal flexion within one minute (Exercise 2), two-minute static unilateral stance in maximal ankle dorsal flexion (Exercise 3), 10 drop jumps from a 40 cm height landing on one foot (Exercise 4). The reference knee angles used to describe the lower limb motion are displayed for the relevant exercises.

Magnetic resonance imaging of cartilage morphology

Before (pre-scan) and after each exercise (post-scan), images of the right talocrural joint were obtained using a 3 Tesla magnet (Trio Tim, Siemens, Erlangen, Germany).Hence, a sagittal 3D double echo steady state sequence with water excitation (sag 3D DESS WE) was applied with an acquisition time of 4'41".

After 1 h of physical rest,[13,17,23,28] pre-scans were performed followed by one of the four weight-bearing exercises. Within 90s after the exercise,[6,12] post-scans were started. This procedure was repeated for all four exercises separately.

Data analysis

Post-hoc image analysis

MR image stacks were subsequently segmented to generate a 3D reconstruction of talar cartilage. Using a solid modeling software package (Mimics® version 12.11, Materialise, Leuven, Belgium), a semi-automatical segmentation procedure was implemented based on grey value-oriented thresholds (lower and upper thresholds set at 105–629, respectively) and a slice-by-slice manual correction to digitize talar cartilage by masking. Manual correction was preceded by a region growing algorithm to dispose of abundant voxels. Image reconstruction in the three planes enabled segmentation in the sagittal (for anterior and posterior aspects of the trochlear surface) and coronal (for medial and lateral talar surfaces) section orientation. Figure2 displays the segmentation of the talar cartilage layer by means of masking.
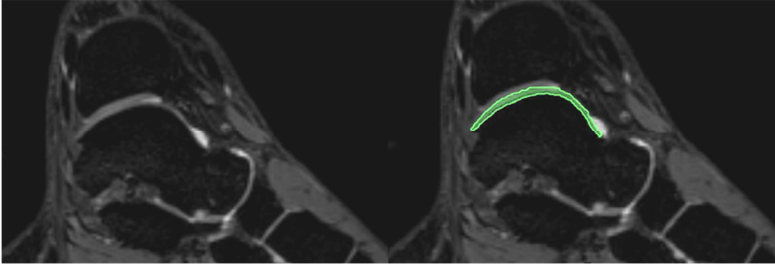
Figure 2. Segmentation of the talar cartilage layer by means of masks. On the *left*: image without the mask; on the *right*: image with the mask and contour line. Images are screenshots taken from the Mimics software interface. (Mimics[®], Materialise, Leuven, Belgium)

Subsequently, based on a marching cubes algorithm and contour interpolation taking possible partial volume effects into account, 3D cartilage plates were reconstructed and 3D volumes were calculated for pre- and post-scans. 3D volumes were calculated summing the pertinent voxels within the obtained binary volumes. All functions were implemented in the software package.

In a pilot study, intra-tester reliability and inter-scan short-term precision error in calculation talar volumetric measures attained an ICC of 0.99 and Root Mean Square of the Coefficients of Variation (RMS CV)[4] of 2.7%,respectively. Consequently, all images were read by a single observer in a paired order blinded to time of scanning.[4]

Statistical analysis

3D volume change percentages for the four exercises each were calculated using the following equation: $[((3D \text{ volume pre-scan} - 3D \text{ volume post-scan})/3D \text{ volume pre-scan}) \times 100]$.[2,5] Descriptive statistics were calculated. As the prerequisites for Mauchly's test of sphericity were met ($P > 0.05$), a two-factor analysis of variance with repeated measures (General Linear Model, GLM) was applied to compare percentage changes in volume after and between exercises. Within subject factors were 'time of scanning' (pre and post) and 'exercise' (exercise one to four). 'Gender' was allocated as between subject factor. To adjust for multiple comparisons of main effects, a Bonferroni post-hoc test was implemented. Level of significance for all tests was set at $\alpha < 0.05$. SPSS statistical package (version 17.0, Chicago, IL, USA) was used for all analyses.

RESULTS

Shown in Figure 3 are the relative measures of cartilage volume changes after the four exercises each. These changes (mean ± SD) display talar cartilage volume decreases of 8.3% ± 6.7 after Exercise one, 7.7% ± 8.9 after Exercise two, 14.6% ± 9.2 after Exercise three and 12.5% ± 8.2 after Exercise four.
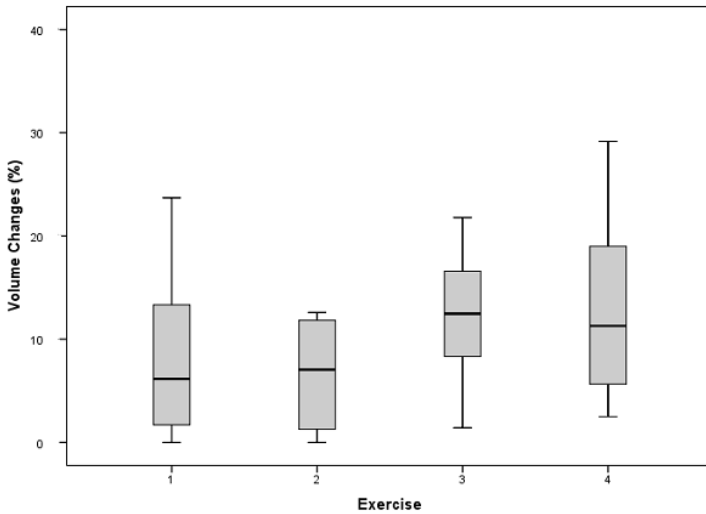
Figure 3. Whisker Box plots representing the distribution in 3D volume change after each exercise. Each box plot displays median, minimum, maximum, first and third quartile of the volume change percentages.

For all four exercises, repeated measures analysis revealed these mean changes to be statistically significant (Figure 3; Exercise 1: P = 0.001, Exercise 2: P = 0.020,Exercise 3: P<0.001, Exercise 4: P = 0.001). However, as can be seen in Table 2, pair-wise comparison between exercises showed only cartilage volume decrease to be significantly different between Exercise three and Exercise two (P = 0.017). Additionally, in Figure 4, an example is displayed of the 3D volume change after Exercise 2 and 3 within one subject. Given the number of subjects available, no other statistically significant differences could be established between exercises. In addition, 'gender' did not demonstrate to significantly interact with volume changes after (gender x time of scanning; non-significant (NS)) and between exercises (gender x exercise; NS). In Table 3, means (SD) and medians (percentile 25 (P25)– percentile 75 (P75)) of volume change percentages for each exercise are presented for the entire sample as well as stratified according to gender.
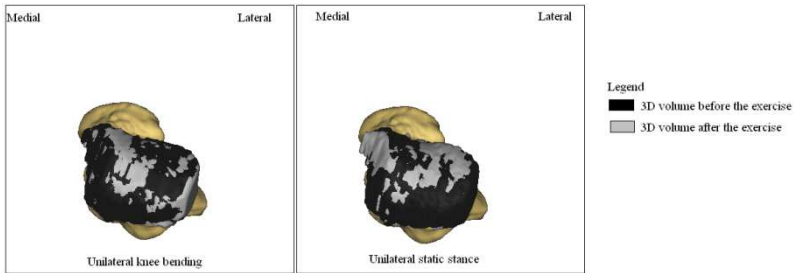
Figure 4. Example of the 3D volume changes after exercise 2 and exercise 3 within one subject. The 3D layers in each figure are superimposed on the talar bone matching the cartilage – bone interface. Visible layers represent the highest volumes in that area after the exercise in this example. Consequently, wherever *black shades* are present, volumes have decreased after the exercise. *Note* the more uniform pressure distribution after the static exercise when compared to the dynamic exercise.

Table 2. Mean differences, standard errors of the mean (S.E.M.) and *P*-values for the pair-wise comparison between the four exercises.

Pair-wise comparison		Mean Difference	S.E.M.	*P*-value
Exercise 1	Exercise 2	-12.8	46.5	NS
	Exercise 3	90.3	35.0	NS
	Exercise 4	57.2	25.4	NS
Exercise 2	Exercise 1	12.8	46.5	NS
	Exercise 3	103.1	27.7	0.017*
	Exercise 4	70.1	36.1	NS
Exercise 3	Exercise 1	-90.3	35.0	NS
	Exercise 2	-103.1	27.7	0.017*
	Exercise 4	-33.1	26.3	NS
Exercise 4	Exercise 1	-57.2	25.4	NS
	Exercise 2	-70.1	36.1	NS
	Exercise 3	33.1	26.3	NS

P-values are a result of the two-factor GLM with repeated measures. *: significance at 0.05% error level. NS: nonsignificant.

Table 3. Means (standard deviations, S.D.), medians (percentile 25, P25 and percentile75, P75) of volume change percentages of the total sample and volume change percentages stratified by gender for all four exercises.

	Exercise 1		Exercise 2		Exercise 3		Exercise 4	
	Mean (S.D.)	Median (P25-P75)	Mean (S.D.)	Median (P25-P75)	Mean (S.D.)	Median (P25-P75)	Mean (S.D.)	Median (P25-P75)
Total	8.3(6.7)	7.2(3.0-13.3)	7.7(8.9)	7.3(1.3-11.6)	14.6(9.2)	12.8(8.8-19.5)	12.5(8.2)	10.1(5.5-19.0)
Female	7.5(8.2)	6.2(1.5-12.3)	7.7(10.9)	6.5(1.8-10.1)	13.7(10.8)	13.4(3.9-20.7)	13.8(9.3)	11.3(5.3-22.2)
Male	9.6(5.0)	7.6(5.5-14.8)	7.6 (5.5)	7.5 (2.9-12.3)	16.0(7.0)	12.8(11.6-22.0)	10.4(6.3)	10.1(4.3-16.6)

DISCUSSION

The most important finding of this study was that talar cartilage deforms substantially after different *in vivo* impact conditions showing more deformation after static than after dynamic loading. Recently, a discrepancy between *in vitro* and *in vivo* outcomes for human talocrural cartilage deformation was suggested,[17? 29] recommending *in vivo* investigation of various loading conditions.

Therefore, the first objective of this study was to determine the extent of talar cartilage deformation after four weight-bearing exercises in healthy subjects. (Figure 1) The results obtained demonstrate significant mean volume decreases ranging from 7.7 to 14.6% after all four exercises.(Figure 3) Because this is the first study implementing 3D volume changes of distinct talar cartilage plates to investigate *in vivo* deformation, comparison with existing literature remains impeded. In contrast, a series of studies have already documented on knee cartilage (i.e. tibiofemoral) *in vivo* deformation after various activities, including knee bending,[5] static squatting,[5] running[2,13] and jumping[5]. Since these reports cover volume decreases from 1.2 to 7.2%, the results of the current study suggest that talar cartilage deforms substantially after *in vivo* loading conditions. The present finding supports the work of Wan et al.[29] and Li et al.[17] who suggested that, based on real-time peak contact compressive strains in the overlapping tibio-talar layer, ankle cartilage is prone to considerable deformation during daily activities. Under body weight, these authors captured peak deformational strains of 34.5[29] and 38%[17] compared to a similar technique exploited on the tibiofemoral contact areas reporting 20 and 30% depending upon knee flexion angle[1]. Interestingly, these findings do not appear to concur with *in vitro* and cadaver specimen studies. Based on *in vitro* biomechanical testing, the extensive work of Kuettner and Cole[10,16] and Treppo et al.[26] provided evidence for increased ankle cartilage stiffness with reduced permeability.

To the best of our knowledge, there is no sound explanation available yet for the apparent difference in deformation between *in vitro* and *in vivo* test conditions. However, *in vivo* conditions display some characteristics that cannot be entirely met during *in vitro* tests and, hence, might influence deformation outcome. In this respect, interesting factors are the unknown physiological loads exerted onto the joint (i.e. loading type and magnitude), complexity and variability of load distribution during dynamic activities including variability of loading areas involved, joint lubrication, incongruence of the joint surface, local cartilage thickness variability over the joint surface, cartilage mechanical properties, mechanics of cartilage-cartilage contact areas, et cetera. Additionally, one might hypothesize that it is the continuous interplay between these factors, especially local cartilage thickness distribution, surface area involved, type and magnitude of loading and the manner to express deformation rate (e.g. volume, local thickness) that defines the observed outcomes irrespective of cartilage thickness at baseline. Schumacher et al.[21] showed a higher prevalence of planar multiple chondrons in the talar superficial layer

when compared to the uniformly distributed isolated cells or doublets in the superficial tibiofemoral cartilage layer in human joint specimen. This observation underlines the importance of joint specific load and force distribution at the joint surface.[14,21] Although there have been no reports published yet on *in vivo* local thickness changes of talar cartilage, one might suggest that substantial volume changes result from higher intra-articular stresses to be attenuated.[22,24] These higher intra-articular stresses might be present due to the relatively small articular surfaces and the congruous configuration of the talocrural joint, especially under high loads.[14,22,25,27] Taken together with the relatively small range of movement feasible approximately the entire surface is stressed during locomotion,[14,22] presumably leading to the overall considerable mean volume changes observed in this study. Nonetheless, more investigation is needed to corroborate the suggested substantial talar cartilage *in vivo* deformation in view of the lower vulnerability of the talocrural joint to osteoarthritis affliction. In this respect, future research should also implement recovery rate to study the tissue's resiliency after loading.

The second objective of this study was to establish whether talar cartilage deformational behaviour differed between impact conditions. The current results convey higher talar cartilage volume decreases after static than after dynamic loading ($P = 0.017$, Figure 4). This finding is supported by the biphasic theory in that static loading, in contrast to dynamic loading, allows deformational responses of the extra-cellular matrix to more easily adapt to the imposed load. As a result, interstitial fluids eventually exude through the porous matrix decreasing hydraulic pressures which leads to larger deformations of the tissue without a large pressure surge within its matrix.[6,9,24]

In this study, no other significant differences between exercises (i.e. with increasing impact) could be established. Two plausible explanations might be the relatively small sample size and/or the loading regimens not being distinctive enough to show differences in volume changes with regard to ankle cartilage deformation.

Limitations of this study are the moderate control of age and gender distribution on the one hand, and the relatively small sample size on the other hand. The standard deviations of the measured volume changes suggest considerable inter-individual variability in deformational behaviour. Although cartilage deformation has already been shown to exhibit high inter-individual variability,[9,18,22] moderate control of gender distribution and age[4,6] might have added to the variance encountered in the present results.

This study population indeed presents with an unequal gender distribution (eight women versus five men). As already suggested by previous research,[2] the present analyses, however, showed that gender did not systematically affect 3D volume changes after (NS) and between exercises (NS). Although statistical adjustment might be limited by sample size, the stratified volume changes in Table 3 do not show a systematic drift in outcomes according to gender.

Furthermore, subjects were recruited displaying a considerable range in years of age (minimum 20-maximum 39 years). Next to altered movement strategies and progressive cartilage thinning, ageing might present with increased cross-linking between collagen fibres. Due to the deposition of pentosidine, a product of non-enzymatic glycolization, cartilage becomes more brittle and might exhibit increased stiffness to loading.[12] Nonetheless, this study's subjects were deliberately chosen to be relatively young (<40 years).

In addition, several other factors might be responsible for the scatter in data as well. Next to differences in cartilage mechanical properties,[26] possible causes might include differences in movement strategy and control,[6] talocrural joint configuration and contact area,[6,14,19] cartilage surface geometry,[6] strength[6] and flexibility of (calf) muscle–tendon units, subject lifestyle.[22] Nonetheless, it should be highlighted that since these authors opted for a repeated measures statistical model to perform the comparative analyses, statistical significance is not influenced by inter-individual variability.

Finally, the present study comprised of a relatively small sample size. Given the mean and median volume changes observed (Table 3), the presence of possible outliers, however, did not critically effect a drift in outcome. Nevertheless, these authors acknowledge that implementation of larger samples is needed endorsing the present findings.

Clinically, these insights might prove useful in rehabilitation strategies following focal cartilage injuries and repair strategies that should consider the considerable overall volume changes (representing a considerable surface area involved) talar cartilage might be subjected to in daily life and sports.

CONCLUSION

This study investigated *in vivo* deformation of ankle (talar) cartilage after various impact conditions. The 3D talar cartilage volume changes established suggested that considerable deformation might occur. Additionally, these results lend credence to higher deformation after static than after dynamic loading. Considering this study's limitations, these results might prove useful in comprehending the loads imposed upon ankle cartilage in subjects engaged in exercise and rehabilitation.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

1. Bingham JT, Papannagari R, Van de Velde SK, Gross C, Gill TJ, Felson DT, Rubash HE, Li G (2008) In vivo cartilage contact deformation in the healthy human tibiofemoral joint. Rheumatology (Oxford) 47:1622–1627
2. Boocock M, McNair P, Cicuttini F, Stuart A, Sinclair T (2009)The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. Osteoarthritis Cartilage 17:883–890
3. Daniels T, Thomas R (2008) Etiology and biomechanics of ankle arthritis. Foot Ankle Clin 13:341–352
4. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C(2006) Magnetic resonance imaging (MRI) of articular cartilagein knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage 14(Suppl A):A46–A75
5. Eckstein F, Lemberger B, Gratzke C, Hudelmaier M, Glaser C,Englmeier KH, Reiser M (2005) In vivo cartilage deformation after different types of activity and its dependence on physical training status. Ann Rheum Dis 64:291–295
6. Eckstein F, Lemberger B, Stammberger T, Englmeier KH, Reiser M (2000) Patellar cartilage deformation in vivo after static versus dynamic loading. J Biomech 33:819–825
7. Eckstein F, Reiser M, Englmeier KH, Putz R (2001) In vivo morphometry and functional analysis of human articular cartilage with quantitative magnetic resonance imaging–from image to data. from data to theory Anat Embryol (Berl) 203:147–173
8. Fetter NL, Leddy HA, Guilak F, Nunley JA (2006) Composition and transport properties of human ankle and knee cartilage.J Orthop Res 24:211–219
9. Herberhold C, Faber S, Stammberger T, Steinlechner M, Putz R,Englmeier KH, Reiser M, Eckstein F (1999) In situ measurement of articular cartilage deformation in intact femoropatellar joints under static loading. J Biomech 32:1287–1295
10. Hendren L, Beeson P (2009) A review of the differences between normal and osteoarthritis articular cartilage in human knee and ankle joints. Foot (Edinb) 19:171–176
11. Huch K, Kuettner KE, Dieppe P (1997) Osteoarthritis in ankle and knee joints. Semin Arthritis Rheum 26:667–674
12. Hudelmaier M, Glaser C, Hohe J, Englmeier KH, Reiser M, Putz R, Eckstein F (2001) Age-related changes in the morphology and deformational behavior of knee joint cartilage. Arthritis Rheum 44:2556–2561
13. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB (2006) Volume changes in the menisci and articular cartilage of runners: an in vivo investigation based on 3-D magnetic resonance imaging. Am J Sports Med 34:832–836
14. Kleipool RP, Blankevoort L (2010) The relation between geometry and function of the ankle joint complex: a biomechanical review. Knee Surg Sports Traumatol Arthrosc 18(5):618–627
15. Koepp H, Eger W, Muehleman C, Valdellon A, Buckwalter JA, Kuettner KE, Cole AA (1999) Prevalence of articular cartilage degeneration in the ankle and knee joints of human organ donors. J Orthop Sci 4:407–412
16. Kuettner KE, Cole AA (2005) Cartilage degeneration in different human joints. Osteoarthritis Cartilage 13:93–103
17. Li G, Wan L, Kozanek M (2008) Determination of real-time in vivo cartilage contact deformation in the ankle joint. J Biomech 41:128–136
18. Lyyra T, Kiviranta I, Vaatainen U, Helminen HJ, Jurvelin JS (1999) In vivo characterization of indentation stiffness of articular cartilage in the normal human knee. J Biomed Mater Res 48:482–487
19. Matricali GA, Bartels W, Labey L, Dereymaeker GP, Luyten FP, Vander Sloten J (2009) High inter-specimen variability of baseline data for the tibio-talar contact area. Clin Biomech (Bristol,Avon) 24:117–120
20. Mow VC, Holmes MH, Lai WM (1984) Fluid transport and mechanical properties of articular cartilage: a review. J Biomech 17:377–394
21. Schumacher BL, Su JL, Lindley KM, Kuetttner KE, Cole AA (2002) Horizontally oriented clusters of multiple chondrons in the superficial zone of ankle, but not knee articular cartilage. Anat Rec 266:241–248
22. Seedhom BB (2006) Conditioning of cartilage during normal activities is an important factor in the development of osteoarthritis.Rheumatology (Oxford) 45:146–149
23. Sitoci K, Hudelmaier M, Glaser C et al (2003) Noctural changes of cartilage morphology in healthy subjects. Osteoarthritis Cartilage 11:S95

24. Suh JK, Li Z, Woo SL (1995) Dynamic behavior of a biphasic cartilage model under cyclic compressive loading. J Biomech 28:357–364
25. Suckel A, Muller O, Wachter N, Kluba T (2010) In vitro measurement of intraarticular pressure in the ankle joint. Knee Surg Sports Traumatol Arthrosc 18(5):664–668
26. Treppo S, Koepp H, Quan EC, Cole AA, Kuettner KE, Grodzinsky AJ (2000) Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. J Orthop Res 18:739–748
27. van Dijk CN, Reilingh ML, Zengerink M, van Bergen CJA (2010) Osteochondral defects in the ankle: why painful? Knee Surg Sports Traumatol Arthrosc 18(5):570–580
28. Wan L, de Asla RJ, Rubash HE, Li G (2008) In vivo cartilage contact deformation of human ankle joints under full body weight. J Orthop Res 26:1081–1089
29. Waterton JC, Solloway S, Foster JE, Keen MC, Gandy S, Middleton BJ, Maciewicz RA, Watt I, Dieppe PA, Taylor CJ (2000) Diurnal variation in the femoral articular cartilage of the knee in young adult humans. Magn Reson Med 43:126–132

EFFECTS OF *IN VIVO* EXERCISE ON ANKLE CARTILAGE DEFORMATION AND RECOVERY IN HEALTHY VOLUNTEERS: AN EXPERIMENTAL STUDY

ABSTRACT

Objective: To monitor ankle cartilage 3D volume changes after *in vivo* exercise and during recovery.

Method: Based on 3D MRI, 3D volumes of talar and tibial cartilage were calculated before and after 30 bilateral knee bends in 12 healthy volunteers. 3D volumes were calculated at five time points (one pre- and four post-scans) determining deformation and recovery for both cartilage plates of interest. Post-scans ran immediately after the exercise and were repeated according to a 15 min interval. 3D volumes were subjected to repeated measures GLM. Additionally, relative surface area use during deformation was compared between plates using a Wilcoxon Signed Ranks test and its correlation with deformation was investigated using Spearman's rho.

Results: Mean 3D volume change percentages for talar cartilage after the exercise were: -10.41%, -8.18%, -5.61% and -3.90%. For tibial cartilage mean changes were: -5.97%, -5.75%, +0.89% and +1.51%. For talar cartilage changes were significant, except following 30 min post-exercise. For tibial cartilage no changes were significant. At all time points, no significant differences in relative volume changes between both cartilage plates existed. Although no significant differences in relative surface area use between plates were revealed, a moderate to strong correlation with deformation existed.

Conclusion: Ankle cartilage endures substantial deformation after *in vivo* loading that was restored within 30 min for the talus. Overall cartilage contact area involvement might be associated with cartilage quality maintenance in the upper ankle. Talar cartilage is suggested to play a critical role in intra-articular shock attenuation when compared to tibial cartilage.

Key words Ankle Hyaline Cartilage Exercise Deformational Behavior

INTRODUCTION

In Western society, osteoarthritis (OA) is one of the most frequent causes of pain, malfunction, and disability in adults. Due to the aging population, the prevalence of OA is expected to increase up to 40% the next 10 years labelling OA the fourth leading cause of disability.[1,2] Therefore, knowledge on predictors for disease onset and progression are paramount in furthering OA management and prevention.

Mechanical factors have long been implicated in this disease's aetiology.[3-6] On the one hand, lack of cartilage conditioning to frequent loading has been hypothesized to predispose for degeneration when encountered with impulsive loads.[4] On the other hand, joint tissue vulnerability to biomechanical insults has been suggested to depend in part upon the tissue's resilience.[5] Given the intriguingly low ankle idiopathic OA prevalence as compared to the knee,[7-11] the study of ankle cartilage resilience to loading might enhance insights into its contribution in cartilage quality maintenance. Insight into joint degeneration aetiology requires knowledge of cartilage deformation in this joint.[12] In this regard, the extensive work of Kuettner & Cole and co-workers[9] using human cadaver specimen showed ankle (i.e., talar) cartilage to contain a higher proportion of proteoglycans to water when compared to knee (i.e., femoral) cartilage of the same cadaver. Combined with a lower hydraulic permeability due to a smaller effective pore size,[10] these structural features have been suggested to result into higher dynamic stiffness to loading when compared to the knee. Hence, less deformation during *in vitro* loading has been proposed to explain in part the remarkably low prevalence of idiopathic ankle OA.[9] Surprisingly, the first *in vivo* studies reporting on ankle cartilage deformation under body weight suggested that ankle cartilage may be undergoing large deformations during daily activities.[12,13] Using a dual-orthogonal fluoroscopic and magnetic resonance imaging (MRI) technique, considerable cartilage deformational strains were registered in the overlapping tibio-talar layers. Additionally, these authors' previous study reported considerable talar cartilage volume changes after four different exercises when compared to the available reports on knee joints.[14] Hence, this apparent contradiction between *in vitro* and *in vivo* loading responses urges further investigation. The study of cartilage deformational behaviour after *in vivo* loading conditions provides a means to encompass *in vivo* resiliency. Cartilage deformational behaviour entails that changes in 3D morphology (e.g., volume) are monitored before and after a weightbearing exercise.[15] Registration of recovery after loading additionally allows for a comprehensive evaluation of cartilage resilience capacity. *In vivo* studies addressing recovery,[16,17] however, are few in number, especially regarding the ankle joint. This is - to our knowledge - the first study monitoring cartilage recovery processes in the upper ankle following *in vivo* loading bouts.

The objectives of this study were twofold. The first objective was to investigate the changes in 3D talar and tibial cartilage volumes after an *in vivo* weight-bearing exercise and during recovery and, hence, determine the time required to restore initial volumes (i.e., recovery time).

The second objective was to determine whether the two cartilage plates of interest displayed similar tendencies in deformation and recovery times by means of volume change comparisons at the different time points under study. It was hypothesized that ankle cartilage endures considerable deformation after *in vivo* loading.[12,14] Since talar cartilage is known to show decreased stiffness and increased permeability in creep indentation experiments when compared to distal tibial cartilage,[18] higher talar than tibial cartilage volume changes were expected to be observed. Considering ankle cartilage decreased hydraulic permeability,[9,10,19] recovery time was hypothesized to proceed relatively slow for both cartilage plates displaying dominant talar cartilage involvement in intra-articular stress attenuation.[18] Finally, in view of ankle cartilage stiffness, it was hypothesized that surface areas involved would contribute to ankle cartilage considerable volume changes.[14]

MATERIALS AND METHODS

Subjects

Twelve healthy able-bodied subjects (six men, six women) participated voluntarily. All subjects were recruited from the local community or university campus. Inclusion criteria were: age 20-40 years, Body Mass Index (BMI) 20-30 kg/m^2, injury free at the time of study, sports participation maximum three times/week, no changes in regular life style the week prior to the actual study appointment. Exclusion criteria were: history of surgical or arthroscopic procedures, traumatic ligament injuries or chronic ankle instability, cartilage injury or degenerative pathology to the ankle joint, a history of fractures at the lower leg or foot as well as contraindications to MRI. On recruitment, eligibility was verified using a standard questionnaire. All subjects were instructed not to practice sports the day before testing or on the testing day and to avoid running, lifting heavy weights and taking stairs 4 h preceding the actual experimental procedures.[14,20] The right lower limb was the dominant limb in all participants and was defined as the limb the subject would choose to kick a ball.[14,21,22] Informed consents ratified by the local ethics committee were obtained from all subjects. Subject demographics are depicted in Table 1.

Table 1. Means (S.D.) for subject demographics

Parameter	Means (S.D.)
Age	29.08 (5.02)
BMI	22.08 (1.75)
Physical Activity Score*	8.49 (0.98)

* Physical Activity Scores were determined using the reliable and valid Baecke questionnaire quantifying physical activity level during work, sports and leisure time activities. [13,22,23]

Experimental procedures

One weight-bearing exercise was examined. For every subject the testing procedures occurred at the same time of day.

In vivo exercise

The exercise consisted of 30 knee bends until maximal ankle dorsal flexion in 1 min. Maximal dorsal flexion was restrained to anterior rocking of the lower leg over the foot without heel lifts while lowering the upper leg horizontally. To control for a correct and standardized performance, the exercise was carried out under a researcher's supervision and performed barefoot next to the scanner magnet.[14,24]

MRI of cartilage morphology

Before (one pre-scan; tpre) and after the exercise (four post-scans;tpostt0-15-30-45), high-resolution images of the right talocrural joint were obtained with a dedicated phased array high-resolution 8-channel Foot-Ankle coil (Invivo, Gainesville, FL, USA) on a 3 T Trio Tim magnet (Siemens medical solutions, Erlangen, Germany). Hence, a sagittal 3D double echo steady state sequence was applied with fat suppression by means of water excitation (sag3D DESS WE). The following parameters were implemented: partition thickness 0.4mm,104 partitions, echo time 5.5 ms, repetition time 15.6 ms, flip angle 28°, field of view 105mm and matrix 384 pixels (in-plane resolution 0.3x0.3, interpolated to 0.125x 0.125)[25,26], acquisition time 07'19". After 1 h of standardized physical rest,[14,17,24,27] pre-scans were performed followed by the exercise under study. Within 90 s after exercise cessation,[14,24] the first post-scan is started (i.e., tpostt0) and repeated with 15min-intervals up to 45 min after the exercise (i.e., tpostt15-30-45). The sequence of events is displayed in Figure 1.
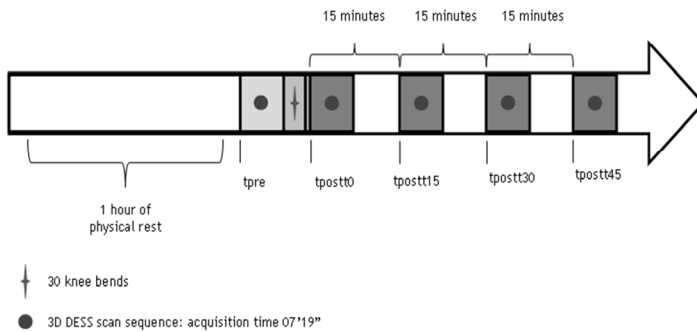
Figure 1. Sequence of events during the experimental procedures

Data analysis

Three-dimensional reconstruction, volume calculation, model registration and surface area calculation were performed using a commercial solid modelling software package (Mimics version 13.1, Materialise, Leuven, Belgium). No custom codes were used.

Talar and tibial cartilage 3D reconstruction and volume calculation MR image stacks were subsequently segmented to generate a 3D reconstruction of talar and tibial cartilage. A semi-automatical segmentation procedure was implemented based on grey value-oriented threshold (lower and upper threshold set at 105-533 respectively) and a slice-by-slice manual correction to digitize talar and tibial cartilage by masking (Figure 2). Manual correction was preceded by a region growing algorithm to dispose of abundant voxels. Subsequently, applying contour interpolation, 3D cartilage plates were reconstructed and 3D volumes were calculated for pre- and post-scans. 3D volumes were calculated summing the pertinent voxels within the obtained binary volumes.[14]
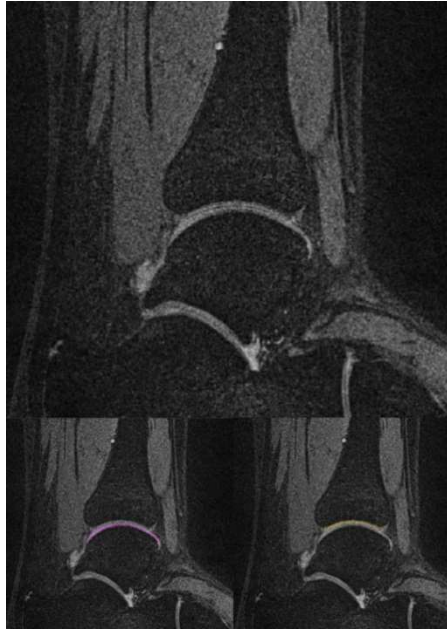
Figure 2. sag3D DESS WE image of tibial and talar cartilage. *Top*: without masks; *bottom left*: with talar mask; *bottom right*: with tibial mask

Determination of relative surface area use for talar and tibial cartilage

For every pre-3D volume, total coverage area was determined by means of surface triangulation. To calculate the area of predominantly loaded surfaces during the exercise, the first post-3D volume was imported as an STereoLithography (STL)-format and superimposed on the pre-3D volume. Registration of models was guided by means of navigating their respecting contours matching the cartilage-bone interface using the index scan images as a reference. Predominantly loaded surfaces were identified as those regions where post-3D volumes remained covered by the pre-3D volumes.[14] Using orthogonal cut, regions of interest were distracted from the pre-3D model and their distinct surface areas were determined as calculated by the software package. (Figure 3)

Based on four test-retest measurements conducted in all participants prior to the actual experiment,[15,26,28] 3D volumetric measurements' intra-tester reliability and inter-scan short-term precision error for talar cartilage attained an Intra-Class Correlation Coefficient (ICC)=0.99 and Root Mean Square Coefficient of Variation (RMS CV)=3.3% respectively and for tibial cartilage an ICC=0.98 and RMS CV=4.8 % respectively. All segmentations were performed by a single

researcher with 2 years of experience in cartilage segmentation at the time of analysis and who was blinded to the time sequence of scanning.

Statistical analysis

Absolute 3D volumes for talar and tibial cartilage were calculated at all time points. Relative 3D volume change percentages were calculated for each post-scan relative to the pre-scan using the following equation: [((3D volume post-scan - 3D volume pre-scan)/3D volume pre-scan) x100].[14,24,27] To compare changes in volume after the exercise and between cartilage plates, a General Linear Model (GLM) with repeated measures was applied. Within-subject factors were 'time of scanning' (t_{pre} and t_{post}0-15-30-45) and 'cartilage plate' (talar and tibial). 'Gender' was allocated as between-subject factor. To adjust for multiple comparisons of main effects, a Bonferroni post-hoc test was implemented. Relative surface area use was calculated using the following equation: [(predominantly loaded surface/total cartilage coverage area)x100]. To investigate the correlation between relative surface area use and 3D volume change, a Spearman's rho correlation coefficient was calculated. To test the hypothesis that significant changes exist in relative surface area use between plates, a Wilcoxon Signed Ranks test was applied. Level of significance for all tests was set at $a < 0.05$. PASW statistical package (version 18.0, Chicago, IL, USA) was used for all analyses.
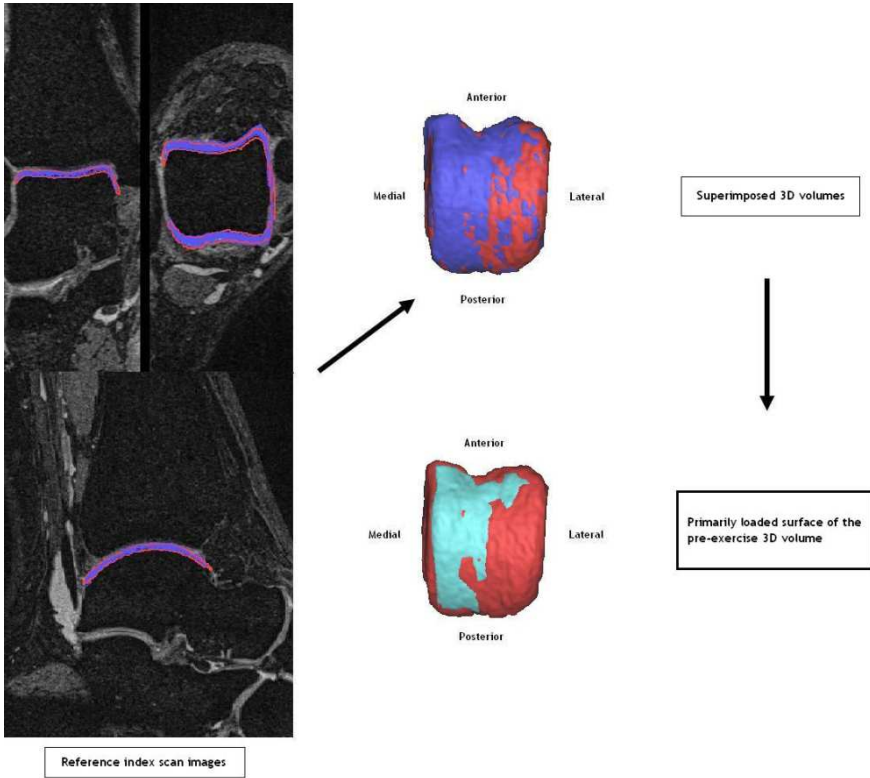
Figure 3. Illustration of the determination of relative surface area use for talar cartilage plates. Images are screenshots taken from the Mimics user interface. Red volumes are the post-exercise 3D volumes, dark blue volume is the pre-exerise volume, light blue volume is the primarily loaded surface of the pre-exercise volume. Wherever light blue shades are present, volumes have decreased after the exercise.[14] Similar procedures were applied on tibial cartilage.

RESULTS

Absolute 3D volumes of talar and tibial cartilage at the five time points (i.e., tpre, tpostt0-15-30-45)

Absolute mean (S.D.) talar cartilage 3D volumes at the five consecutive time points were: 2,207.20 (460.86) mm^3, 1,966.79 (382.95) mm^3, 2,017.73 (394.88) mm^3, 2,083.98 (464.19) mm^3, 2,119.11 (446.27) mm^3. Accordingly, absolute mean (S.D.) tibial cartilage 3D volumes were: 1,427.49 (305.31) mm^3, 1,342.27 (228.45) mm^3, 1,335.21 (268.46) mm^3, 1,434.63 (335.41) mm^3, 1,454.62 (349.84) mm^3. In Figure 4, the absolute mean volumes course is displayed for talar and tibial cartilage.

Relative 3D volume changes: deformation and recovery time of talar and tibial cartilage (i.e., tpostt0-15-30-45)

Relative mean (S.D.) talar 3D volume change percentages at the four post-exercise time points respectively were: -10.41% (6.89;P=0.006), -8.18% (6.07; P=0.009), -5.61% (6.39; P=0.216), and -3.90% (5.52; P=0.300). All volume changes compared to baseline were significant except for the volume changes calculated at the two last time points (i.e., tpostt30-45). Accordingly, relative mean (S.D.) tibial 3D volume change percentages were: -5.97% (9.61; P=0.072), -5.75% (7.94;P=0.364), +0.89% (8.71; P= 0.611), and +1.51% (7.43; P=0.428). No volume changes were significant when compared to baseline.

Given the number of subjects included, no significant between-subject effects for 'gender' were observed for both talar and tibial volume changes (P=0.776 and P=0.965 respectively). Mean (S.D.), median (95% Confidence Interval (CI)) as well as each subject's individual volume change percentages for talar and tibial cartilage and stratified according to gender are displayed in Tables 2 and 3.
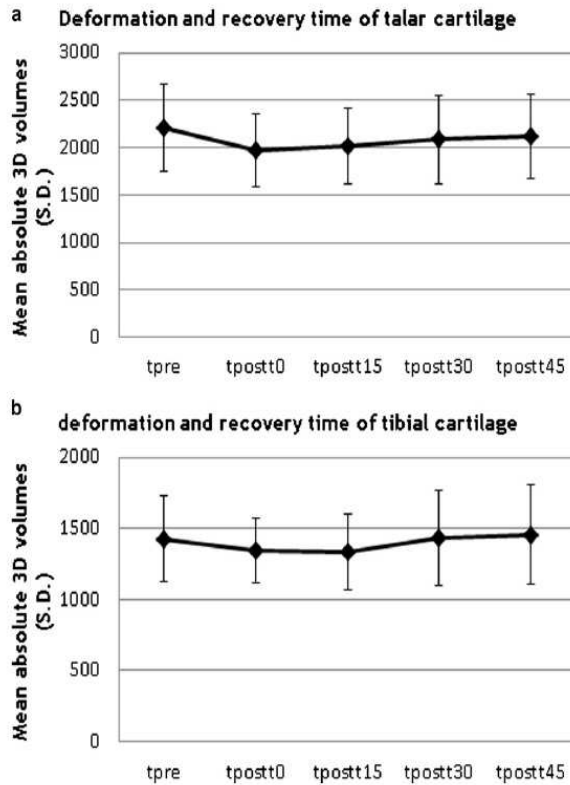
Figure 4. The course of deformation and recovery time for talar (a) and tibial (b) cartilage displayed using means of absolute 3D volumes (mm^3) (S.D.) at the five time points under study. tpre: 3D volume before the exercise; tpostt0-15-30-45: 3D volume immediately, 15 minutes, 30 minutes, 45 minutes after the exercise respectively.

Comparison of deformation and recovery time between talar and tibial cartilage (i.e., tpostt0-15-30-45)

No significant differences between plates were revealed at all time points (P=0.103, P=0.346, P=0.163, P=0.194 for tpostt0-15-30-45 respectively).

Relative surface areas used and the relationship with deformation in both talar and tibial cartilage (i.e., tpostt0)

For talar surfaces a mean (S.D.) 35.47 (10.25)% of the available surface was loaded, for tibial cartilage a mean (S.D.) 46.25 (22.02)%. The Wilcoxon Signed Ranks test revealed no

significant difference in relative use of surfaces between plates (P=0.176). Spearman's rho correlation analysis revealed a moderate to strong correlation between relative surface area use and 3D volume decrease observed at tpostt0 (i.e., talar cartilage: r_s=0.60, P=0.044; tibial cartilage: r_s=0.71, P=0.036).

Table 2. Mean (S.D.) and median (95 % CI) talar volume change percentages compared to baseline for the entire study sample as well as stratified according to gender, and for individual subjects for the four post-exercise time points.

	tpostt0	tpostt15	tpostt30	tpostt45
Total (N=12)				
Mean (S.D.)	-10.41 (6.89)	-8.18 (6.07)	-5.61 (6.39)	-3.90 (5.52)
Median (95%CI)	-9.09 (-18.72,-5.04)	-6.81 (-11.45,-3.13)	-4.26 (-6.90,-1.36)	-2.30 (-5.56,-0.58)
Female (N=6)				
Mean (S.D.)	-8.66 (7.96)	-6.90 (7.70)	-6.30 (7.44)	-4.33 (7.81)
Median (95% CI)	-5.69 (-23.67,-2.82)	-3.25 (-21.84,-1.74)	-4.16 (-21.16,-1.36)	-1.54 (-20.10,0.00)
Male (N=6)				
Mean (S.D.)	-12.17 (5.81)	-9.47 (4.21)	-4.91 (5.78)	-3.47 (2.35)
Median (95%CI)	-11.18 (-19.32,-5.03)	-10.63 (-15.12,-3.97)	-4.90 (-14.27,2.65)	-3.07(-6.72,-0.79)
Subject				
1	-5.04	-3.97	-1.16	-.79
2	-10.62	-10.45	2.65	-5.56
3	-18.71	-10.82	-6.36	-4.06
4	-7.56	-5.03	-3.43	-2.08
5	-19.32	-15.12	-14.27	-6.72
6	-11.74	-11.45	-6.90	-1.62
7	-11.20	-8.58	-5.12	-.58
8	-23.67	-21.84	-21.15	-20.10
9	-6.05	-3.37	-3.23	-2.51
10	-2.82	-2.71	-1.89	-.16
11	-5.32	-3.13	-1.36	.00
12	-2.90	-1.74	-5.08	-2.63

Note that mean outcomes are prone to drift in data to a limited extent. Medians, however, remain to display considerable volume changes according to similar courses.

Table 3. Mean (S.D.) and median (95 % CI) tibial volume change percentages compared to baseline for the entire study sample as well as stratified according to gender, and for individual subjects for the four post-exercise time points.

	tpostt0	tpostt15	tpostt30	tpostt45
Total (N=12)				
Mean (S.D.)	-5.97 (9.61)	-5.75 (7.94)	+0.89 (8.71)	+1.51(7.43)
Median (95%CI)	-6.65 (-14.55,2.56)	-4.46 (-14.24,-0.38)	+1.81 (-8.37,9.25)	+1.50 (-6.15,7.90)
Female (N=6)				
Mean (S.D.)	-4.23 (10.17)	-4.25 (7.33)	-4.37 (12.53)	-0.94 (6.99)
Median (95% CI)	-4.86 (-18.10,6.48)	-4.98 (-14.24,7.047)	+3.72 (-9.25,26.39)	-0.93 (-9.54,10.80)
Male (N=6)				
Mean (S.D.)	-6.99 (10.95)	-8.26 (9.94)	+0.66 (10.20)	+3.96 (7.62)
Median (95%CI)	-7.30 (-22.52,7.96)	-4.44 (-22.53, 1.02)	+0.45 (-13.97,11.62)	+5.45 (-9.70, 12.64)
Subject				
1	-6.57	-6.34	+1.96	+7.90
2	-22.52	-21.53	-7.37	+3.91
3	+7.96	-1.53	+14.97	+12.64
4	+1.78	+1.02	+11.88	+7.00
5	-14.55	-14.37	-10.62	-9.70
6	-8.02	-1.79	+1.85	+2.03
7	-10.93	-7.98	-4.67	-1.86
8	-18.10	-14.24	-9.76	-9.54
9	-2.99	-2.59	-6.18	-6.15
10	+2.56	-7.38	+10.25	+10.80
11	+10.80	+7.05	+6.57	.00
12	-6.73	-.38	+1.77	+1.08

Note that mean outcomes are prone to drift in data to a limited extent. Medians, however, remain to display considerable volume changes according to similar courses.

DISCUSSION

The main findings of this study suggest considerable deformation for talar as well as tibial cartilage after 30 knee bends. Talar cartilage recovered within 30 min after exercise cessation. For tibial cartilage, no volume changes were significant.

The first objective was to monitor deformation and recovery time after 30 knee bends for talar and tibial cartilage respectively. Present talar and tibial cartilage deformation fits with a previous *in vivo* study reporting mean talar volume decreases of 8.3% after a similar exercise.[14] Compared to significant volume decreases of 1.2-7.2% captured in tibiofemoral compartments after a variety of exercises (e.g., Refs.[17,24,27]), considerable deformation is observed in both cartilage plates investigated in this study. Absence of significance within the tibial cartilage plate, however, might be due to the combination of smaller volume changes to be measured with relatively larger precision errors, thus, requiring a relatively larger sample to reach 95% confidence. Additionally, as described below, scan duration and/or intervals might have hampered capturing tibial cartilage involvement in intra-articular stress attenuation. Although applying a different technique to monitor *in vivo* deformational responses, Wan et al.[12] and Li et al.[13] similarly concluded with ankle cartilage being subjected to substantial deformation in daily life. Using a dual fluoroscopic and MRI technique, these authors determined real-time peak compressive strains (i.e., defined as the cartilage penetration divided by the thickness of the two overlapping cartilage layers at each vertex) of 34.5% and 38% in the overlapping tibio-talar cartilage layers under bodyweight. As the same technique documented on peak deformational strains of 22-30% and 10.5-12.6%in the tibiofemoral compartment,[20,29] the notion of ankle cartilage deforming substantially might be supported.

Surprisingly, *in vivo* observation does not appear to agree with previous *in vitro* biomechanical studies. *In vitro* studies (e.g., unconfined compression measurements, indentation probe testing) showed ankle (talar) cartilage to present with increased stiffness to loading (i.e., higher dynamic stiffness and lower permeability) when compared to knee (femoral) cartilage.[9,19,30,31] However, *in vitro* and *in vivo* measurements do not necessarily conflict. Outcome of *in vivo* deformation is suggested to depend on several factors other than local material properties alone.[6,14] In this respect, *in vivo* conditions display some characteristics that cannot be met during *in vitro* tests and, hence, might influence deformation outcome. Factors that come into play are the unknown physiological loads exerted onto the joint, variability and complexity of load distribution during dynamic activities including surface areas involved, joint lubrication, (in-)congruence, variability in cartilage thickness distribution, mechanics of cartilage-cartilage contact, etc.[14] Additionally, actual *in vivo* outcome depends upon the manner in which deformation is expressed (i.e., overall volume change, local thickness change, deformational strains in overlapping layers). Cartilage material properties such as dynamic stiffness and permeability are thus suggested to contribute to *in vivo* deformational outcomes in this study, however, these properties are not the

sole determining factors. Actual changes in overall volume depend upon [local thickness changes x surface areas loaded]. In view of ankle cartilage increased stiffness, these authors previously proposed that the considerable overall volume changes are more likely to be caused by relatively extensive use of surface areas during joint articulation, rather than by considerable local thickness changes.[14] In this regard, the ankle joint is known to adapt a state of increased congruence when loaded in order to assume a position of inherent stability.[32,33] As congruence is, next to bony constitution (i.e., tight ankle mortise) attended to by the overlapping cartilage layers,[34] small articular surfaces and limited range of motion might give way to substantial relative cartilage surface area involved.[4,35] The fact that these authors revealed a moderate to strong correlation between 'degree of deformation' and 'relative surface area used' for both talar and tibial cartilage in the present study supports this hypothesis. Hosseini et al.[29] similarly suggested that cartilage-cartilage contact brings about the difference in *in vivo* deformational responses determined between ankle and knee joints. Alternatively, notwithstanding its stiffness, ankle cartilage might just experience larger deformation because of the higher intra-articular stresses to be attenuated by the articular surfaces when compared to other joints. Nonetheless, for a complete comprehension of the present findings, functional intra-individual comparisons between joints remain appropriate as well as the incorporation of *in vivo* local thickness change computations.

Next to deformation, this study also includes recovery time. As pressure is relieved, fluid influx effects cartilage recovery according to a biphasic exponential function.[36] In this study, talar cartilage volume changes were significant until 30 min after 30 knee bends. Patellar cartilage volumes have been shown to require more than 90 min for volume restoration after 100 knee bends.[16] Sixty minutes after a 20 km run no significant volume changes could be detected anymore in tibial knee cartilages of experienced runners.[17] Considering the difference in loading regimen, current talar cartilage recovery might be suggested comparable or even slightly slower than knee cartilage recovery. Nonetheless, recovery proceeded gradually, approximately linear in time.[16] Relatively slow recovery times reflect decreased hydraulic permeability characterizing ankle cartilage superficial layers.[16] As low ankle cartilage permeability has been associated with increased *in vitro* stiffness to loading,[9,10,16,19] this observation might underline the role of surface area involvement in the degree of deformation discovered.

Although no changes within plates were significant, tibial cartilage remarkably displayed positive volume changes following 15 min after exercise cessation. Remaining recovery from loading in the resting state prior to the exercise seems reasonable to be argued upon. However, as a 1 h rest period is commonly applied in the literature (e.g., Refs.[14,17,24,27]), this study's participants were instructed to refrain from excessive loading before the experimental procedures. Additionally, during scanning procedures, axial loading was prevented by instructing the participants to keep their feet in a relaxed position within the Foot-Ankle coil. Furthermore, if

tibial cartilage demands extended recovery before exercise, volume changes would not resolve this fast within post-exercise recovery time spans that ~equal pre-exercise recovery. For these reasons, these authors propose that mechanisms other than unresolved pre-exercise recovery, were likely to have contributed to this volume increment. In this respect, negative pressurization resulting from different cartilage plates' recovery times within the congruent talocrural joint, is proposed to induce an additional 'swelling' of tibial cartilage.[37] Alternatively, volume increases might be effected by fluid displacement from talar to tibial cartilage.[37] Because of differences in Poisson's ratio between fibular, tibial and talar surfaces, propensity for more fluid transport through the solid matrix of the tibial and talar cartilage as opposed to fibular cartilage is indicated.[18] Additional increases in volume are physiologically feasible as cartilage has already been described to be subjected to an equilibrium compression state during daily life.[38] Finally, considerable variation in cases of little deformation and/or positive volume changes measured with precision errors up to 4.0% has been previously reported in the literature (e.g., Ref.[6]).

The second objective was to compare the course of deformation and recovery between both cartilage plates of interest. In this study, no significant differences in volume change percentages between plates were resolved after the exercise and at all the following time points under study. Differences in significant time-effects *within* plates, however, suggest potential differences *between* plates that possibly could not be statistically proven due to the relatively limited sample size. In this respect, a tendency is noted towards smaller tibial cartilage deformation when compared to talar cartilage. As these authors could not distinguish in relative surface area use between plates, smaller tibial cartilage deformation is most likely due to its decreased permeability and increased stiffness when compared to talar cartilage.[18] Hence, larger deformation of talar cartilage resulting into an apparently slower recovery process might endorse the notion of talar cartilage more critically involved in intra-articular stress attenuation during joint articulation than tibial cartilage.[18] As decreased tibial cartilage deformation possibly accounts for decreased involvement in cushioning intra-articular stresses, higher shear - and compression stresses for the talar surfaces might be encountered.[18] Athanasiou et al.[18] proposed that disparities in the mechanical properties between two articulation surfaces produce dissimilar strain fields. The fact that several pathologic processes are mostly manifested in the talus (i.e., transchondral fractures of the talus, OCD lesions) should support this hypothesis. Furthermore, it is these authors' contention that the relatively limited involvement of tibial cartilage in cushioning impact loads by means of less deformation, adds up to the difficulties in detecting significant volume changes during deformation and recovery in this study as mentioned before.

Unlike dual-orthogonal fluoroscopic and MRI techniques providing with real-time monitoring,[12,13,20,29,43] deformation in this study was evaluated through post-exercise effects to avoid motion artifacts MR imaging is susceptible to. Consequently, scan duration might

underestimate true deformation and/or miss early recovery. As the majority of volume gain should be registered during early recovery,[36] talar cartilage volume decreases were significant until 30 min after exercise cessation. As in between the first and second post-scan (~twice the scan duration), no significant difference in volume could be achieved (mean difference: $50.95mm^3$; P=0.135), these authors suggest that recovery occurred slow enough to be captured by the scan sequence. On the other hand, tibial cartilage was suggested to show limited contribution to shock attenuation[18] by means of deformational responses. Although the course in absolute volumes suggests restoration of the pre-exercise state before 30 min after exercise cessation (Table 3), scan sequences and/or intervals were possibly too long since no significant difference in volume could be established between all distinct time points.

Additionally, as high-resolution imaging is paramount to produce accurate measurements, thin cartilage layers are known to produce relatively larger precision errors.[26,39,40] In this regard, the combination of the precision error and the registered (or expected) volume change is crucial to allow for statistical significance to be established. As a guideline, the minimal interval of change that can be detected with 95% confidence in a single individual is 2.8 times the precision error[41]. Hence, it is reasonable that talar cartilage changes reached significance where tibial cartilage did not. Although this study was the first including single tibial cartilage plates, attaining significant effects for "time" in case of larger errors requires larger samples to increase the likelihood for significance. Notwithstanding the ability to detect talar cartilage deformation (i.e., tpostt0), relevant precision errors were too extensive to resolve late recovery (from tpostt30) changes with 95% confidence. Nonetheless, early recovery of talar cartilage is not missed which encompasses the most important and critical changes after pressure release.[36] Although the importance of precision should be acknowledged, one needs to mention that the present precision errors concur with[26] or were smaller[37,39,42] than ranges reported previously when applying high-resolution ankle cartilage morphology imaging.

In view of overall volume changes, this study did not include local thickness change calculations next to loaded surface areas. Assessing local thickness changes would enable (1) confirming the hypothesis that extent of deformation is primarily driven by surface area use or not, (2) exploring whether load transfer is characterized as being (in-)homogonous. Nonetheless, the latter has been addressed before[43] and was considered beyond the scope of this study. Additionally, given the circumstances of thin cartilage within a congruent joint, these authors suggest that overall 3D volume changes might be preferred over local thickness in successfully detecting morphometric differences within single plates. In this respect, measurement of local thickness changes would rely even more on high precision data processing which is recognized as an inherent technical challenge when dealing with thin cartilage layers.

CONCLUSION

The present results reveal considerable *in vivo* deformation for both talar and tibial cartilage that might be primarily driven by relatively extensive surface areas involved in joint articulation. The suggested substantial volume changes recovered relatively slow, more specifically within 30 min for talar cartilage. Tendencies towards smaller deformation for tibial when compared to talar cartilage and slower recovery occurring in the latter were disclosed, suggesting critical involvement of talar cartilage in shock attenuation in the upper ankle. Limitations in sample size, precision error, scan duration and/or intervals possibly hampered adequate monitoring of tibial cartilage deformational behaviour. In a key next step, the study of cartilage deformational behaviour in the OA patient should be addressed. Additionally, other recreational or sports-specific activities such as running or jumping, warrant attention.

AUTHOR CONTRIBUTIONS

Van Ginckel Ans: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patients, collection and assembly of data.

Roosen Philip: critical revision of the article for important intellectual content, final approval of the article.

Almqvist Karl Fredrik: conception and design, critical revision of the article for important intellectual content, final approval of the article.

Verstraete Koenraad: conception and design, critical revision of the article for important intellectual content, technical support, final approval of the article.

Witvrouw Erik: conception and design, critical revision of the article for important intellectual content, final approval of the article.

ROLE OF THE FUNDING SOURCE

CONFLICTS OF INTEREST

No conflicts of interest were declared.

ACKNOWLEDGMENTS

REFERENCES

1. Belo JN, Berger MY, Reijman M, Koes BW, Bierma-Zeinstra.Prognostic factors of progression of osteoarthritis of the knee:a systematic review of observational studies. Arthritis Rheum 2007;57(1):13e26.
2. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions.Bull World Health Organ 2003;81(9):646e56.
3. Brandt KD, Dieppe P, Radin E. Etiopathogenesis of osteoarthritis.Med Clin North Am 2009;93(1):1e24. xv.
4. Seedhom BB. Conditioning of cartilage during normal activities is an important factor in the development of osteoarthritis. Rheumatology (Oxford) 2006;45(2):146e9.
5. Arden N, Nevitt MC. Osteoarthritis: epidemiology. Best Pract Res Clin Rheumatol 2006;20(1):3e25.
6. Cotofana S, Eckstein F, Wirth W, Souza RB, Li X, Wyman B, et al. In vivo measures of cartilage deformation: patterns in healthy and osteoarthritic female knees using 3T MR imaging. Eur Radiol 2011; doi: 10.1007/s00330-011-2057-y.
7. Huch K, Kuettner KE, Dieppe P. Osteoarthritis in ankle and knee joints. Semin Arthritis Rheum 1997;26:667e74.
8. Koepp H, Eger W, Muehleman C, Valdellon A, Buckwalter JE,Kuettner KE, et al. Prevalence of articular cartilage degeneration in the ankle and knee joints of human organ donors. J Orthop Sci 1999;4(6):407e12.
9. Kuettner KE, Cole AA. Cartilage degeneration in different human joints. Osteoarthritis Cartilage 2005;13(2):93e103.
10. Fetter NL, Leddy HA, Guilak F, Nunley JA. Composition and transport properties of human ankle and knee cartilage. J Orthop Res 2006;24(2):211e9.
11. Hendren L, Beeson P. A review of the differences between normal and osteoarthritis articular cartilage in human knee and ankle joints. Foot (Edinb) 2009;19:171e6.
12. Wan L, de Asla RJ, Rubash HE, Li G. In vivo cartilage contact deformation of human ankle joints under full body weight. J Orthop Res 2008;26(8):1081e9.
13. Li G, Wan L, Kozanek M. Determination of real-time in-vivo cartilage contact deformation in the ankle joint. J Biomech 2008;41(1):128e36.
14. Van Ginckel A, Almqvist F, Verstraete K, Roosen P, Witvrouw E. Human ankle cartilage deformation after different in vivo impact conditions. Knee Surg Sports Traumatol Arthrosc 2011;19(1):137e43.
15. Eckstein F, Hudelmaier M, Putz R. The effects of exercise on human articular cartilage. J Anat 2006;208(4):491e512.
16. Eckstein F, Tieschky M, Faber S, Englmeier KH, Reiser M. Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. Anat Embryol (Berl) 1999;200(4):419e24.
17. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. Am J Sports Med 2008;36(5):966e70.
18. Athanasiou KA, Niederauer GG, Schenck Jr RC. Biomechanical topography of human ankle cartilage. Ann Biomed Eng 1995;23(5):697e704.
19. Treppo S, Koepp H, Quan EC, Cole AA, Kuetner KE, Grodzinsky AJ. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. J Orthop Res 2000;18(5):739e48.
20. Bingham JT, Papannagari R, Van de Velde SK, Gross C, Gill TJ, Felson DT, et al. In vivo cartilage contact deformation in the healthy human tibiofemoral joint. Rheumatology (Oxford) 2008;47(11):1622e7.
21. Van Ginckel A, Thijs Y, Ghanizadeh Hesar N, Mahieu N, De Clercq D, Roosen P, et al. Intrinsic gait-related risk factors for Achilles tendinopathy in novice runners: a prospective study. Gait Posture 2009;29:387e91.
22. Van Ginckel A, Baelde N, Almqvist KF, Roosen P, McNair P,Witvrouw E. Functional adaptation of knee cartilage in asymptomatic female novice runners compared to sedentary controls. A longitudinal analysis using delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC). Osteoarthritis Cartilage 2010;18(12):1564e9.
23. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36(5):936e42.

24. Eckstein F, Lemberger B, Gratzke C, Hudelmaier M, Glaser C, Englmeier KH, et al. In vivo cartilage deformation after different types of activity and its dependence on physical training status. Ann Rheum Dis 2005;64(2):291e5.

25. Graichen H, Springer V, Flaman T, Stammberger T, Glaser C, Englmeier KH, et al. Validation of high-resolution water-excitation magnetic resonance imaging for quantitative assessment of thin cartilage layers. Osteoarthritis Cartilage 2000;8(2):106e14.

26. Al-Ali D, Graichen H, Faber S, Englmeier KH, Reiser M, Eckstein F. Quantitative cartilage imaging of the human hind foot: precision and inter-subject variability. J Orthop Res 2002;20(2):249e56.

27. Boocock M, McNair P, Cicuttini F, Stuart A, Sinclair T. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. Osteoarthritis Cartilage 2009;17(7):883e90.

28. Glüer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. Osteoporos Int 1995;5(4):262e70.

29. Hosseini A, Van de Velde SK, Kozanek M, Gill TJ, Grodzinsky AJ, Rubash HE, et al. In-vivo time-dependent articular cartilage contact behavior of the tibiofemoral joint. Osteoarthritis Cartilage 2010;18:909e16.

30. Shepherd DE, Seedhom BB. Thickness of human articular cartilage in joints of the lower limb. Ann Rheum Dis 1999;58(1):27e34.

31. Swann AC, Seedhom BB. The stiffness of normal articular cartilage and the predominant acting stress levels: implications for the aetiology of osteoarthrosis. Br J Rheumatol 1993;32(1):16e25.

32. Daniels T, Thomas R. Etiology and biomechanics of ankle arthritis. Foot Ankle Clin 2008;13:341e52. vii.

33. Wynarsky GT, Greenwald AS. Mathematical model of the human ankle joint. J Biomech 1983;16(4):241e51.

34. Froimson MI, Ratcliffe A, Gardner TR, Mow VC. Differences in patellofemoral joint cartilage material properties and their significance to the etiology of cartilage surface fibrillation. Osteoarthritis Cartilage 1997;5(6):377e86.

35. Millington S, Grabner M, Wozelka R, Hurwitz SR, Crandall JR. A stereophotographic study of ankle joint contact area. J Orthop Res 2007;25:1465e73.

36. Rubenstein JD, Kim JK, Henkelman RM. Effects of compression and recovery on bovine articular cartilage: appearance on MR images. Radiology 1996;201:843e50.

37. Waterton JC, Solloway S, Foster JE, Keen MC, Gandy S,Middleton BJ, et al. Diurnal variation in the femoral articular cartilage of the knee in young adult humans. Magn Reson Med 2000;43:126e32.

38. Sitoci K, Hudelmaier M, Glaser C, Englmeier KH, Reiser M,Eckstein F. Nocturnal changes of cartilage morphology in healthy subjects. Osteoarthritis Cartilage 2003;11:S95.

39. Welsch GH, Mamisch TC, Weber M, Horger W, Bohndorf K,Trattnig S. High-resolution morphological and biochemical imaging of articular cartilage of the ankle joint at 3.0 T using a new dedicated phased array coil: in vivo reproducibility study. Skeletal Radiol 2008;37(6):519e26.

40. Peterfy CG, van Dijke CF, Nguyen A, Conninck TJ, Kneeland BJ,Tirman PFJ, et al. Quantification of the volume of articular cartilage in the metacarpophalangeal joints of the hand: accuracy and precision of three-dimensional MR imaging. AJR 1995;165:371e5.

41. Cummings SR, Black D. Should perimenopausal women be screened for osteoporosis? Ann Intern Med 1986;104:817e23.

42. Millington SA, Li B, Tang J, Trattnig S, Crandall JR, Hurwitz SR, et al. Quantitative and topographical evaluation of ankle articular cartilage using high resolution MRI. J Orthop Res 2007;25(2):143e51.

43. Wan L, de Asla RJ, Rubash HE, Li G. Determination of in-vivo articular cartilage contact areas of human talocrural joint under weightbearing conditions. Osteoarthritis Cartilage 2006;14(12):1294e301.

FUNCTIONAL ADAPTATION OF KNEE CARTILAGE IN ASYMPTOMATIC FEMALE NOVICE RUNNERS COMPARED TO SEDENTARY CONTROLS. A LONGITUDINAL ANALYSIS USING DELAYED GADOLINIUM ENHANCED MAGNETIC RESONANCE IMAGING OF CARTILAGE (dGEMRIC)

ABSTRACT

Objective: To longitudinally estimate the change in glycosaminoglycan content of knee cartilage in asymptomatic untrained female novice runners participating in a Start To Run program (STR) compared to sedentary controls.

Method: Nine females enrolling in a 10-week STR and 10 sedentary controls participated voluntarily. Prior to and after the 10-week period, both groups were subjected to dGEMRIC imaging. dGEMRIC indices of knee cartilage were determined at baseline and for the change after the 10-week period in both groups. Based on a self-reported weekly log, physical activity change during the study was depicted as decreased, unchanged or increased. The Mann-Whitney *U* and Kruskal-Wallis tests were applied to test the hypotheses that dGEMRIC changes occurred between groups and according to physical activity changes respectively.

Results: No significant differences were established between groups for dGEMRIC indices at baseline (*P*=0.541). A significant positive change of the median dGEMRIC index in the runners group was demonstrated when compared to the controls [+11.66 ms (95% CI: -25.29, 44.43) vs -9.56 ms (95% CI:-29.55, 5.83), P=0.006]. The change in dGEMRIC index differed significantly according to physical activity change (*P*=0.014), showing an increase in dGEMRIC index with increasing physical activity.

Conclusion: Since cartilage appears to positively respond to moderate running when compared to a sedentary lifestyle, this running scheme might be considered a valuable tool in osteoarthritis prevention strategies. Caution is warranted when applying these results to a wider population and to longer training periods.

Key words Knee Cartilage Running dGEMRIC Osteoarthritis Prevention Female

INTRODUCTION

Worldwide, running is gaining popularity because of its salutatory benefits on cardiorespiratory fitness, weight control, and psychosocial health.[1] Additionally, an athletic lifestyle has been associated with a reduced risk of type II diabetes mellitus and of cancer to the reproductive system, breast and colon.[1] Even though endurance running has been reported to come along with overuse injury,[1,2] the effects of running on joints remain equivocal. Next to possible increases in bone density,[1,3,4] highly repetitive loading, in time, was generally thought to deplete the joint of lubricating glycoproteins, disrupt the collagen network and to slowly break down the cartilage causing microfractures in the underlying bones.[5] However, several studies have already investigated the association in prolonged running and osteoarthritis (OA) of the knee and hip showing conflicting results.[3-13] While some studies showed no association between running and an increased prevalence of OA,[3-11] others contrarily indicated an increased risk for knee and hip OA.[12,13] Furthermore, an extensive cohort of community-dwelling older adults could not associate recreational physical activity (e.g., walking, jogging) with increased nor decreased risk of OA.[14] The disparity in outcomes can be suggested being attributed to mixed subject characteristics or analysis methods insensitive to cartilage tissue itself (e.g., X-ray).[15] Nevertheless, since OA is becoming the leading cause of disability in adults in the industrialized world,[16] strategies to preserve joint health have been requested over the years of which exercise (and running) has been one of the proposed means.[17-19]

Developments in Magnetic Resonance Imaging (MRI) allow monitoring cartilage macroscopic (morphology: e.g., volume and thickness) and ultra-structural changes (biochemical composition: e.g., glycosamino-glycan (GAG) content) accurately and precisely over time.[20] Recently, however, a longitudinal study could not show cartilage morphology changes in middle aged women after a 3-month endurance or strength program compared to autogenic training.[21] Since this observation concurs with the hypothesis that human adult cartilage is not likely to increase in thickness in response to an exercise regime,[22] one might suggest that the possible benefits of (running) exercise occur at an ultra-structural, qualitative level; the GAG content.

In this respect, no study has yet been published investigating functional adaptation of human knee cartilage due to running by means of changes in GAG content in a longitudinal design. Hence, these results might contribute in understanding the value of moderate running in view of OA prevention strategies. A commonly used technology to estimate GAG content is the delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC) technique using the anionic contrast agent gadolinium diethylene triamine penta-acetic acid (Gd-DTPA^{2-}).[23,24] When injected intravenously, and given sufficient time, the anionic contrast agent distributes inversely to the fixed negative charge associated with the GAG content. Gd-DTPA^{2-} therefore distributes in relatively higher concentrations in regions of low GAG, and vice versa. Since Gd-DTPA^{2-} has a concentration dependent effect on the MRI parameter T1, T1 imaging in the

presence of Gd-DTPA^{2-} (T1Gd or dGEMRIC index) reflects the cartilage Gd-DTPA^{2-} concentration and, hence, GAG concentration.[25]

Applying the dGEMRIC technique, the objective of this study was to investigate the change in dGEMRIC index over time in a cohort of untrained asymptomatic female novice runners participating in a Start To Run program (STR) compared to sedentary controls. It was hypothesized that the group of runners experienced chondroprotective effects of running exercise on knee cartilage when compared to the sedentary controls. This beneficial effect was expected to be shown by a positive dGEMRIC index change in the novice runners when compared to the controls.

MATERIALS AND METHODS

Prior to and after a 10-week STR, asymptomatic female novice runners were subjected to a dGEMRIC analysis of knee cartilage. Accordingly, sedentary controls were tested prior to and after a 10-week period. Consequently, for each subject the change in dGEMRIC index of knee cartilage was calculated and compared between groups.

Subjects

Two groups were recruited on a voluntary basis: (1) nine novice runners and (2) 10 sedentary controls. This study was approved by the relevant local Ethics Committees and all subjects granted their consent to participate. Ethics procedures followed were in accordance with the Helsinki Declaration. For both groups, the inclusion criteria at baseline were a sedentary lifestyle (i.e., not being regularly involved in sports activities for the last 3-5 years), a sedentary occupation (e.g., desk work), age 20-40 years, Body Mass Index (BMI) 20-30 kg/m^2 and female gender. Exclusion criteria were a history of knee complaints, knee internal derangements, surgical and arthroscopic procedures on the knee joint, known presence of degenerative knee pathology, contra-indications for MRI and the dGEMRIC technique in particular. All subjects used contraceptives.

For the runners, this STR program was their first attempt to engage in recreational running activities.[2] All runners recruited were enrolled to participate in the same STR organized in April 2009 in the same Track and Field club. Sedentary controls were recruited from the local community or university campus by oral and written advertisement according to similar Physical Activity Scores. On recruitment, eligibility of the subjects was verified using a standard questionnaire. Physical activity score in particular, was determined using the reliable and valid

Baecke Questionnaire.[2,26] This questionnaire measures physical activity level by quantifying 'work', 'sports' and 'leisure' activities using a five-point scale(1=never and 5=always). By counting up the scores of the three distinct dimensions each subject's total physical activity score was calculated. The sedentary controls were not individually matched to the novice runners. Subject demographics are listed in Table 1.

Table 1. Medians (95% Confidence Intervals (CI)) and *P*-values of the baseline characteristics of the novice runners compared to the control group.

Parameter	Control group (N = 10)	Novice runners group (N = 9)	*P*-value [a]
	Medians (95% CI)	Medians (95% CI)	
Body Mass Index (BMI) (kg/m^2)	22.85 (20.00,26.40)	22.20 (20.00,29.30)	0.964
Age (years)	25.00 (22.00,34.00)	26.00 (22.00,34.00)	0.515
Physical Activity Score	6.96 (5.00,8.73)	7.00 (5.75,8.25)	0.965

[a] *P*-values are the result of the nonparametric Mann-Whitney *U* test.

MRI

Prior to and after the STR subjects were invited to an MRI session. Four hours prior to the MRI appointment, subjects were instructed to restrain from taking stairs, running and lifting heavy weights.[27,28]

A 1.5 T magnet (Siemens Medical Solutions, Erlangen, Germany) and a dedicated 8-channel knee coil were used for cartilage imaging. At the start of each session, the subjects were subjected to 30-45 min physical rest. For the dGEMRIC technique, a double dose (0.2 mmol/kg) of Gd-DTPA^{2-} (Magnevist, Bayern Schering, Germany) was administered slowly into the right antecubital vein followed by a saline flush with the subject lying supine.[29] After injection, the subjects walked for 15 min to facilitate contrast distribution in the cartilage.[29,30] Ninety minutes after injection,[29,30] two-dimensional sagittal single slice dGEMRIC images were obtained for the medial knee compartment. These dGEMRIC images consisted of sets of inversion recovery (IR) images with different inversion times (TR=1800 ms, TE=14 ms, TI=50-100-200-400-800-1600 ms, matrix 256x256, FOV 130x130, slice thickness 3 mm). Sagittal slices were centered on the medial femoral condyle using a standard series of localizer images in the three planes. Along with the IR sequence, sagittal proton density images with a similar voxel size were acquired for the purpose of visual guidance during image processing (i.e., T1 calculation for the dGEMRIC

index).[29] Scanning and slice positioning were performed by a qualified and experienced musculoskeletal radiologist. Patient positioning was standardized using the position of the knee joint according to the reference points on the knee coil. Knee joints were scanned in extension with rigid foam placed around the lower leg and pads around the knee joint to prevent additional movement. In all subjects, the right dominant knee was scanned. Dominance of the lower leg was defined as the leg the subject would choose to kick a ball.[28]

Intervention period

During the 10-week period, all novice runners participated in a standardized STR. The STR coaches novice runners to achieve the goal of jogging 5 km (±30 min) within a training period of 10 weeks. This initiative is supervised by the Flemish Track and Field Association and is organized in qualified Track and Field clubs. Participants are trained in a group by a qualified STR coach three times a week. In this study, the coach was a qualified physiotherapist. The STR comprises a gradual build-up of interspersed running and walking units during which the participants are encouraged to jog at their own comfortable speed.[2] The training scheme is online available in the Supplementary material.

To standardize cushioning properties of footwear, all runners wore the same type of neutral running shoe during training (Landreth Gel, Asics Benelux). Additionally, all runners filled out a weekly training log registering training compliance (i.e., participated training units per week/total amount of training units),running surface [grass, athletics track, (hard) woodland, asphalt, other (specify)], absence from training and reason, other concomitant sports/leisure activities (type of activity and duration),possible (knee) complaints. After 10 weeks, runners were subjected to a test during which they had to run laps continuously without resting for a distance of 5 km.

Accordingly, during a 10-week period, sedentary controls were instructed to carry on with their usual lifestyle. Concomitant leisure or sports activities were registered in a weekly log. If usual activity level was restricted controls were instructed to report this as well. Based on the weekly logs change in physical activity during the 10-week period for each subject was depicted as unchanged, increased or decreased.[18]

Post-hoc data analysis

T1 calculation for the dGEMRIC index dGEMRIC indices prior to and after the 10-week period were based on the T1Gd.[25] The change in dGEMRIC index was calculated using the formula: (dGEMRIC index post-dGEMRIC index pre).

Using MRIMapper (©2006, MIT, Boston) run on Matlab (version 7.9, The Mathworks, Natick, MA), T1 maps were generated based on a pixel-wise, exponential three-parameter fit of the T1-(IR) images.[29,30] Subsequently, mean T1 values for the Region Of Interest (ROI) were determined in that ROIs were drawn on the T1 map on the medial femoral cartilage overlying the posterior horn of the meniscus as described previously(Figure 1).[18,31] This ROI covered cartilage full thickness and has been shown to present with low intra- and inter-observer variabilities. Additionally, since this region is known for encountering most of the weight-bearing and is one of the primary locations for knee OA onset,[31] this ROI was of particular interest. In the present study, intra-rater reliability and variability in drawing this ROI attained an intra-class correlation coefficient (ICC) of 0.98 and RMS CV of 0.02 respectively. T1 maps were manually processed in pairs by one researcher with 2 years of practice in cartilage segmentation at the time of analysis, and who was blinded to the time of scanning. Because of the range in BMI (min. 20- max. 30), for all mean T1 values, the T1-corrected was determined as put forward by Tiderius et al.[32]

Statistical analysis and power calculation

A mean difference in dGEMRIC index of 42 ms[33] between inactive and moderately active subjects can be expected clinically. Consequently, to attain such a difference and to reject the null hypothesis (i.e., no difference between groups exists) with a standard power of 80% and a<0.05, one needs to include at least six subjects in both groups.

Prior to the statistical analysis, all outcome variables (i.e., dGEMRIC index at baseline, dGEMRIC index change, subject demographics) were subjected to the Shapiro-Wilk test for normality testing revealing a nonparametric distribution ($P<0.05$). Consequently, the nonparametric Mann-Whitney U test was applied to test the hypothesis that significant differences in dGEMRIC index change occurred between the novice runners and controls.[18] The Kruskal-Wallis test was used to test the hypothesis that differences in dGEMRIC change occurred between the three categories in physical activity change (unchanged, decreased, and increased). In this regard, Spearman's rho correlation coefficients were calculated as well. Level of significance was set at a< 0.05. PASW (version 18.0, Chicago, Illinois) was used for the statistical analyses.
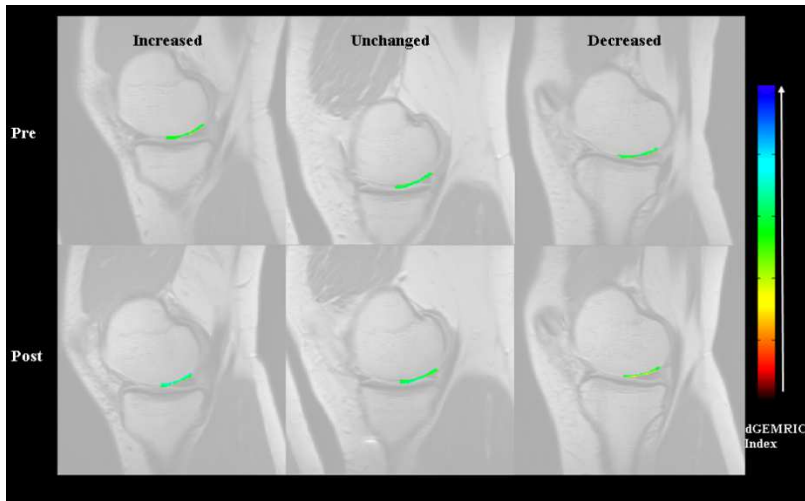
Figure 1. Color-coded maps displaying an example of the dGEMRIC change for the three categories of self-reported physical activity change: increased (novice runner), unchanged (control group), decreased (control group). Additionally, the ROI under study is illustrated.

RESULTS

At baseline, no statistical significant differences were established between both groups for age (P=0.515), BMI (P=0.964), and physical activity score (P=0.965) (Table 1). Similarly, no significant differences between groups were shown for the dGEMRIC indices at baseline (P=0.541) (Table 2).

Table 2. Medians (95% Confidence Intervals (CI)) and P-values of the dGEMRIC indices at baseline and of the change in dGEMRIC indices after the ten week period for the novice runners group and control group.

Parameter	Control group (N = 10)	Novice runners group (N = 8)	P-value [a]
	Medians (95% CI)	Medians (95% CI)	
dGEMRIC index at baseline (ms)	584.38 (276.82,616.560)	598.48 (255.10,651.80)	0.541
dGEMRIC change after the ten week period (ms)	-9.56 (-29.55,5.83)	+11.66 (-25.29,44.43)	0.006

[a] P-values are the result of the non-parametric Mann-Whitney U test.

At the end of the 10-week period, eight runners succeeded the final running test and were scanned a second time. One runner dropped out of the study because of sustained shin splints reported during the third week of the program whereas the other runners did not report any complaint. Compliance to the running scheme was 89%. During the 10 weeks, running surface consisted for 54% of participated training units of grass, for 23% of asphalt, for 19% of (hard) woodland, and for 4% of athletics track. In all eight runners, physical activity increased due to participation in the STR program. Based on the log, runners reported, next to the STR, no unusual change in their leisure time activities. All controls met the second MRI appointment. Based on their weekly log, all of them reported no change in physical activity except for four subjects. These four controls reported decreased activity because of upcoming exams or sickness. A significant difference between the runners and controls was found for the change in dGEMRIC index after the 10-week period [+11.66 ms (95% confidence interval (CI): -25.29, 44.43) vs -9.56 ms (95% CI: -29.55, 5.83), P=0.006, Table 2]. The Kruskal-Wallis test revealed significant differences in dGEMRIC change according to physical activity change category (P=0.014). Median dGEMRIC index changes were -26.24 ms (95% CI: -29.55, -12.19), 4.34 ms (95% CI: -6.94, 5.83), 11.66 ms (95% CI: -25.29, 44.43) for the decreased, unchanged and increased category respectively.

Spearman's rho analysis revealed the relationship between self-reported physical activity change and dGEMRIC index change to display a positive significant correlation (r_s=0.741, P<0.001). In Figures 1 and 2, the dGEMRIC index changes are stratified according to self-reported physical activity change by either using scatter plots or color-coded maps.
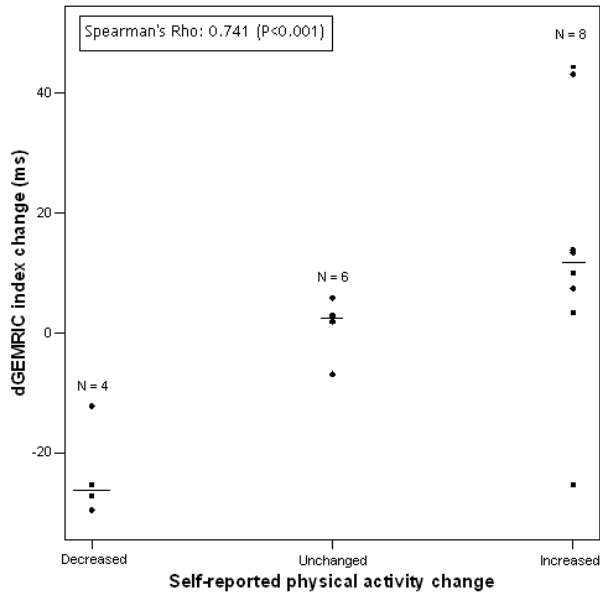
Figure 2. Scatter plots showing individual data points and medians (bars) of the medial femoral cartilage dGEMRIC changes for both the runners (i.e., "Increased") and controls (i.e., "Decreased" and "Unchanged") stratified according to change in physical activity level. Additionally, Spearman's rho outcome for the non-parametric statistical correlation between dGEMRIC change and the three categories of physical activity change is presented. This correlation coefficient reveals a good to strong positive significant correlation between dGEMRIC change and physical activity change.

DISCUSSION

The most important finding of the present study was that the change in dGEMRIC index after the 10-week period revealed a positive change in the novice runners when compared to the sedentary controls. Since the change in dGEMRIC indices registered was significantly different according to self-reported change in physical activity, these authors suggest that increasing physical activity was associated with positive dGEMRIC index changes, and *vice versa*.

This study to our knowledge is the first longitudinal design to address the ultra-structural response of cartilage to running in humans. The present results can be supported by the cross-sectional comparison of the dGEMRIC index between sedentary subjects, recreational runners and elite runners performed by Tiderius et al.[33] Reporting mean indices (S.D.) of 382 (33) ms, 424 (22) ms and 476 (36) ms respectively, Tiderius et al. substantiate functional adaptation capacity of cartilage with increasing running level. Although not running, nor in asymptomatic subjects, Roos et al.[18] similarly presented positive effects on the mean dGEMRIC index change (S.D.) of medial femoral cartilage in post-meniscectomized patients undergoing a 4-month

weight bearing exercise program (+15 (45) ms in the exercise group vs -15 (32) ms in the control group) endorsing the notion that moderate exercise can positively affect the dGEMRIC index.

GAGs are known for being important structural matrix compounds in regulating the cartilage tissue's endo-osmotic swelling pressure and thus, the tissue's compressive strength.[34] Therefore, GAG content could be put forward as a surrogate marker for cartilage quality. The positive change of the dGEMRIC index in the novice runners when compared to the sedentary controls allows conjecture about concordant ultra-structural adaptations of cartilage occurring in subjects withstanding higher mechanical demands during the 10-week period. However, one might argue that the difference in dGEMRIC indices observed in the runners group in this study does not appear to meet the expected changes in dGEMRIC index of 42 ms. The median difference in dGEMRIC index after the 10-week period between groups attained 47.69 ms (95% CI: 17.16, 102.96), hence, confirming the expected estimate. Since this difference, however, is driven by an imbalance in physical activity change between groups, the established significance cannot be considered solely in view of the runners group but always in relation to the sedentary lifestyle characterized by inactivity or even decreased activity. Consequently, combined with the positive significant correlation established between physical activity change and dGEMRIC index change, these results remain to endorse the possible chondroprotective effect of the running scheme.

The effect of physical exercise on knee joints is known to display inter-individual differences.[15] Despite the efforts to select a specific subset of individuals in the present study, the main outcome remains to display substantial variation (Table 2). In this regard, Figure 2 underlines the importance of physical activity change in - but does not entirely explain - the variance in index changes observed. Next to physical activity/sedentary lifestyle, (female) gender, BMI<30, age <40 (pre-menopausal), no known history of knee injury and cartilage degeneration, there are other factors defining a subject's responsiveness to exercise.

The main limitations of this study comprise the reproducibility and validity of dGEMRIC technique in the long-term and the limited sample size. With reproducibility of T1 measurements within the range of 5-8%[35] and 10-15%[29], sources of long-term analysis inaccuracy are mainly patient and slice positioning faults and/or segmentation error due to the smaller areas (i.e., fewer pixels) of the ROI under study. Our segmentation precision (RMS CV=0.02) falls within ranges of those previously reported by Tiderius et al..[31] In addition, segmentation and the scanning procedures for both groups were performed by the same tester and the same trained technicians respectively. An advantage of a statistical comparison between groups encompasses that the established differences in dGEMRIC change are prone to the same measurement errors and these errors are therefore counterbalanced.[18] Additionally, T1 quantification is influenced by contrast agent distribution primarily regulated by extra-cellular

water in the lean and adipose tissues.[32] Consequently, long-term evaluation might be confounded by alterations in body composition over time and due to the training regimen. These authors acknowledge that, next to BMI measurements during the two test appointments, no other measures were acquired (e.g., bio-electric impedance, DEXA scan) to evaluate body composition. Nonetheless, BMI has been shown to be associated with $Gd-PTA^{2-}$ plasma concentrations without changes in $Gd-PTA^{2-}$ kinetics.[32] Although circumspection remains warranted, no significant changes in BMI after the 10-week period were observed in this study (median BMI change=0.20; 95% CI: 0.00,0.49; P=0.910). As the same dosage was administered to the subject twice, delivery at the cartilage plate was likely to be similar during the two test appointments. Finally, the study sample size was relatively limited. Larger sample sizes would have reduced variability or might have allowed taking confounding factors into account. Although confirmation in larger samples is needed, these results suggest similar (i.e., chondroprotective) effects of moderate physical activity as proposed by previous cross-sectional and longitudinal studies applying direct or indirect measures for cartilage status in larger study populations.[17,36]

CONCLUSION

These results suggest that a gradually built up running scheme causes a chondroprotective effect on the knee when compared to a sedentary lifestyle in a specific subset of asymptomatic subjects. This effect is shown by a positive change in dGEMRIC index (i.e.,estimation of GAG content) in the novice runners when compared to the sedentary controls. Consequently, such a moderate running scheme might be proposed valuable in OA prevention strategies. Nonetheless, caution is advised when interpolating these results to a wider variety of individuals and to longer training periods.

AUTHOR CONTRIBUTIONS

Van Ginckel, Ans: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patients, collection and assembly of data.

Baelde, Nick: final approval of the article, provision of study materials or patients, collection and assembly of data, administrative, technical or logistic support.

Almqvist, Fredrik: conception and design, critical revision of the article for important intellectual content, final approval of the article.

Roosen, Philip: conception and design, critical revision of the article for important intellectual content, final approval of the article.

McNair, Peter: conception and design, final approval of the article.

Witvrouw, Erik: conception and design, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in online version at doi: 10.1016/j.joca. 2010. 10.007.

REFERENCES

1. Marti B. Health effects of recreational running in women. Some epidemiological and preventive aspects. Sports Med 1991;11:20e51.
2. Van Ginckel A, Thijs Y, Ghanizadeh Hesar N, Mahieu N,De Clercq D, Roosen Ph, et al. Intrinsic gait-related risk factors for Achilles tendinopathy in novice runners: a prospective study. Gait Posture 2009;29:387e91.
3. Lane NE, Bloch DA, Hubert HB, Jones HH, Marshall Jr WH, Wood PD, et al. Long-distance running, bone density, and osteoarthritis. JAMA 1986;255:1147e51.
4. Lane NE, Oehlert JW, Bloch DA, Fries JF. The relationship of running to osteoarthritis of the knee and hip and bone mineral density of the lumbar spine: a 9-year longitudinal study. J Rheumatol 1998;25:334e41.
5. Fries JF, Singh G, Morfeld D, Hubert HB, Lane NE, Brown BW. Running and the development of disability with age. Ann Intern Med 1994;121:502e9.
6. Lane NE, Michel B, Bjorkengren A, Oehlert J, Shi H, Bloch DA,et al. The risk of osteoarthritis with running and aging: a 5-year longitudinal study. J Rheumatol 1993;20:461e8.
7. Lahr DD. Does running exercise cause osteoarthritis? Md Med J 1996;45:641e4.
8. Panush RS, Hanson CS, Caldwell JR, Longley S, Stork J,Thoburn R. Is running associated with osteoarthritis? An eightyear follow-up study. J Clin Rheum 1995;1:35e9.
9. Konradsen L, Hansen EM, Søndergaard L. Long distance running and osteoarthrosis. Am J Sports Med 1990;18:379e81.
10. Chakravarty EF, Hubert HB, Lingala VB, Zatarain E, Fries JF.Long distance running and knee osteoarthritis. A prospective study. Am J Prev Med 2008;35:133e8.
11. Krampla WW, Newrkla SP, Kroener AH, Hruby WF. Changes on magnetic resonance tomography in the knee joints of marathon runners: a 10-year longitudinal study. Skeletal Radiol 2008;37:619e26.
12. Marti B, Knobloch M, Tschopp A, Jucker A, Howald H. Is excessive running predictive of degenerative hip disease? Controlled study of former elite athletes. Br Med J 1989;299:91e3.
13. Spector TD, Harris PA, Hart DJ, Cicuttini FM, Nandra D, Etherington J, et al. Risk of osteoarthritis associated with longterm weight-bearing sports. A radiologic survey of the hips and knees in female ex-athletes and population controls. Arthritis Rheum 1996;39:988e95.
14. Felson DT, Niu J, Clancy M, Sack B, Aliabadi P, Zhang Y. Effect of recreational physical activities on the development of knee osteoarthritis in older adults of different weights: the Framingham Study. Arthritis Rheum 2007;57:6e12.
15. Urquhart DM, Soufan C, Teichtahl AJ, Wluka AE, Hanna F, Cicuttini FM. Factors that may mediate the relationship between physical activity and the risk for developing knee osteoarthritis. Arthritis Res Ther 2008;10:1e10.
16. Mollenhauer JA, Erdmann S. Introduction: molecular and biomechanical basis of osteoarthritis. Cell Mol Life Sci 2002;59:3e4.
17. Rogers LQ, Macera CA, Hootman JA, Ainsworth BE, Blair SN. The association between joint stress from physical activity and self-reported osteoarthritis: an analysis of the Cooper Clinic data. Osteoarthritis Cartilage 2002;10:617e22.
18. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage. A four-month,randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum 2005;52:3507e14.
19. Helminen HJ. Sports, loading of cartilage, osteoarthritis and its prevention. Scand J Med Sci Sports 2009;19:143e5.
20. Burstein D, Gray M. New MRI techniques for imaging cartilage. J Bone Joint Surg Am 2003;85:70e7.
21. Cotofana S, Ring-Dimitriou S, Hudelmaier M, Himmer M,Wirth W, Sänger AM, et al. Effects of exercise intervention on knee morphology in middle-aged women: a longitudinal analysis using magnetic resonance imaging. Cells Tissues Organs 2010;192(1):67e72.
22. Eckstein F, Faber S, Mühlbauer R, Hohe J, Englmeier KH,Reiser M, et al. Functional adaptation of human joints to mechanical stimuli. Osteoarthritis Cartilage 2002;10:44e50.
23. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 1999;41:857e65.
24. Gray ML, Burstein D, Kim YJ, Maroudas A. Magnetic resonance imaging of cartilage glycosaminoglycan: basic principles,imaging technique, and clinical applications. J Orthop Res 2008;23:281e91.
25. Williams A, Mikulis B, Krishnan N, Gray M, McKenzie C, Burstein D. Suitability of T1GD as the "dGEMRIC index" at 1.5 T and 3.0 T. Magn Reson Med 2007;58:830e4.
26. Phillipaerts RM, Lefevre J. Reliability and validity of three physical activity questionnaires in Flemish males. Am J Epidemiol 1998;147:982e90.
27. Bingham JT, Papannagari R, Van de Velde SK, Gross C, Gill TJ, Felson DT, et al. In vivo cartilage contact deformation in the healthy human tibiofemoral joint. Rheumatology (Oxford) 2008;47:1622e7.
28. Van Ginckel A, Almqvist F, Verstraete K, Roosen Ph, Witvrouw E. Human ankle cartilage deformation after different in vivo impact conditions. Knee Surg Sports Traumatol Arthrosc 2010. doi:10.007/s00167-010-1159-4.29.
29. Burstein D, Velyvis J, Scott KT, Stock KW, Kim YJ, Jaramillo D, et al. Protocol issues for delayed Gd(DTPA)2_ enhanced MRI (dGEMRIC) for clinical evaluations of articular cartilage. Magn Reson Med 2001;45:36e41.
30. Williams A, Gillis A, McKenzie C, Po B, Sharma L, Micheli L, et al. Glycosaminoglycan distribution in cartilage as determined by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): potential clinical applications. AJR 2004;182:167e72.
31. Tiderius CJ, Tjörnstrand J, Akeson P, Södersten K, Dahlberg L, Leander P. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): intra- and interobserver variability in standardized drawing regions of interest. Acta Radiol 2004;45:628e34.
32. Tiderius CJ, Hori M, Williams A, Sharma L, Prasad PV, Finnell M, et al. dGEMRIC as a function of BMI. Osteoarthritis Cartilage 2006;14:1091e7.
33. Tiderius CJ, Svensson J, Leander P, Thorsson O, Dahlberg L. dGEMRIC (delayed gadolinium-enhanced MRI of cartilage)indicates adaptive capacity of human knee cartilage. Magn Reson Med 2004;51:286e90.
34. Mow VC, Holmes MH, Lai WM. Fluid transport and mechanical properties of articular cartilage: a review. J Biomech 1984;17:377e94.

35.   Multanen J, Rauvala E, Lammentausta E, Ojala R, Häkkinen A, Nieminen MT, et al. Reproducibility of imaging human knee cartilage by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) at 1.5 Tesla. Osteoarthritis Cartilage 2009;17: 559e64.

36.   Urquhart DM, Tobing JFL, Hanna FS, Berry P, Wluka AE, Ding C,et al. What is the effect of physical activity on the knee joint? A systematic review. Med Sci Sports Exerc 2010;10.1249/ MSS.0b013e3181ef5bf8

SUPPLEMENTARY MATERIAL

Start to Run program for novice runners

5 kilometres (3.11 miles) within 10 weeks

+ = 1 minute (min) of running
0 = 1 minute (min) of walking

Week	Day	Training	Total
Week 1	1	+0+0++00++00+++000	18'
	2	rest	
	3	+0+0++00+++000+++000	20'
	4	rest	
	5	+0++00++00+++000+++000	22 '
	6+7	rest	
Week 2	1	+0++00++00+++000+++000	22 '
	2	rest	
	3	++00+++000+++000+++000	22'
	4	rest	
	5	+0++00+++000+++000+++000	24 '
	6+7	rest	
Week 3+4	1	+0++00+++000+++000+++00	24 '
	2	rest	
	3	++00++0++0++0++0++0++0++0	25 '
	4	rest	
	5	+0++00++++000++++000+++++0	27 '
	6+7	rest	
Week 5+6	1	++00+++00+++++000+++++000++++00	32 '
	2	rest	
	3	++0+++00++++++00++++++00+++++++00	33 '
	4	rest	
	5	++00++++00+++++00++++++00+++++++00	34 '
	6+7	rest	
Week 7	1	+++++0++++++00+++++++00+++++++++0	32 '
	2	rest	
	3	++++++++0++++++++00++++++++0++++++++0	37 '
	4	rest	
	5	++++++++++00++++++++++00++++++++++++0	37 '
	6+7	rest	
Week 8+9	1	++++++++++++++++00+++++++++++++++00	34 '
	2	rest	
	3	++++++++++0+++++++++++++0+++++++++++++0	37 '
	4	rest	
	5	++++++++++0++++++++++++++++++++++0	32 '
	6+7	rest	
Week 10	1	30 min of jogging/ 1 or 2 min of walking by choice	
	2	rest	
	3	32 min of jogging/ 1 or 2 min of walking by choice	
	4	rest	
	5	30 min of jogging/ no walking	
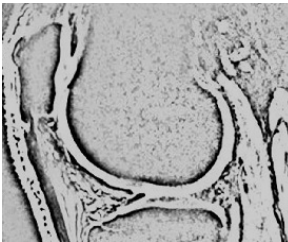
PART TWO - EXERCISE AND CHONDROPROTECTION CLINICAL IMPLEMENTATION IN INDIVIDUALS AT RISK FOR - OR DIAGNOSED WITH EARLY RADIOGRAPHIC OA

CARTILAGE ADAPTATION AFTER ANTERIOR CRUCIATE LIGAMENT INJURY AND RECONSTRUCTION: IMPLICATIONS FOR CLINICAL MANAGEMENT AND RESEARCH?

A SYSTEMATIC REVIEW OF LONGITUDINAL MRI STUDIES

ABSTRACT

Objective: To summarize the current evidence of magnetic resonance imaging (MRI)-measured cartilage adaptations following anterior cruciate ligament (ACL) reconstruction and of the potential factors that might influence these changes, including the effect of treatment on the course of cartilage change (i.e., surgical vs nonsurgical treatment).

Methods: A literature search was conducted in seven electronic databases extracting 12 full-text articles. These articles reported on *in vivo* MRI-related cartilage longitudinal follow-up after ACL injury and reconstruction in "young" adults. Eligibility and methodological quality was rated by two independent reviewers. A best-evidence synthesis was performed for reported factors influencing cartilage changes.

Results: Methodological quality was heterogenous amongst articles (i.e., score range:31.6-78.9%). Macroscopic changes were detectable as from 2 years follow-up next to or preceded by ultra-structural and functional (i.e., contact-deformation) changes, both in the lateral and medial compartment. Moderate-to-strong evidence was presented for meniscal lesion or meniscectomy, presence of bone marrow lesions (BMLs), time from injury, and persisting altered biomechanics, possibly affecting cartilage change after ACL reconstruction. First-year morphological change was more aggravated in ACL reconstruction compared to nonsurgical treatment.

Conclusion: In view of osteoarthritis (OA) prevention after ACL reconstruction, careful attention should be paid to the rehabilitation process and to the decision on when to allow return to sports. These decisions should also consider cartilage fragility and functional adaptations after surgery. In this respect, the first years following surgery are of paramount importance for prevention or treatment strategies that aim at impediment of further matrix deterioration. Considering the low number of studies and the methodological caveats, more research is needed.

Key words Anterior Cruciate Ligament Reconstruction Cartilage Magnetic Resonance Imaging Osteoarthritis Knee Prevention

INTRODUCTION

Although debated,[1-3] anterior cruciate ligament (ACL) reconstruction is offered to those patients actively engaged in cutting, jumping or pivoting sports and/or other functionally demanding activities. The purpose is to improve stability in a mechanically unstable knee and to reduce the risk of subsequent meniscal or chondral damage.[2,4] Long-term radiographic studies, however, suggest that ACL reconstruction may not protect against the development of post-traumatic osteoarthritis (OA).[5]

In view of OA prevention, careful attention should be paid to the rehabilitation process and to the decision on when to allow return to sports.[2,6] In view of cartilage deterioration due to (injurious or surgical) trauma and/or biomechanical disturbances (e.g., excessive anterior/lateral tibial translation and rotation, decreased knee extension),[7-17] one of the key components to guide these decisions - next to graft fixation and functional improvement - should also be the course of cartilage adaptation after surgery. However, reliable and valid methods are needed to measure cartilage adaptation *in vivo*.

This systematic review pursued two main research questions. First, how does cartilage status change over time in patients who underwent ACL reconstruction? Second, if reported, which factors might affect rate of change? To understand the effect of surgery on cartilage remodeling, the effect of treatment (i.e., surgical vs nonsurgical) was additionally investigated. Hence, longitudinal follow-up studies were systematically collected reporting on any magnetic resonance imaging (MRI)-measured cartilage parameter evaluated in ACL injury and reconstruction.

METHODS

This systematic review was performed according to the Prisma Statement and was confined to a quality analysis.[18] Because of study heterogeneity, statistical pooling was refrained from and, as an alternative, a best-evidence synthesis was implemented.[19,20]

Information sources and literature search

Boolean searches were conducted in seven electronic databases (PubMed, SportDiscus, CINAHL, Biomedical reference collection: comprehensive, Biomed Central, Science Direct via Scirus, Web of Science) using search strategies in accordance with the semantics of each

database (Appendix 1). Key - if applicable MeSH - search terms and synonyms were entered separately in two main filters which were ultimately combined. The two filters focused on:

1. *Assessed outcome*: OA, knee OR knee OA OR knee osteoarthritides OR chronic disease(s) OR disease progression(s) OR cartilage OR cartilage, articular OR joint disease(s) OR cartilage disease(s)

2. *Patients/intervention*: ACL reconstruction OR ACL/surgery OR ACL/injuries

Study selection process and eligibility criteria

Figure 1 displays the flow diagram of the study selection process. An initial search (on March 22nd, 2012) identified 5.338 records. After removal of duplicates and irrelevant titles, the remaining abstracts (n=506) were rated for eligibility according to seven inclusion criteria:

1. Published in an Institute of Science Index (ISI)-indexed journal

2. Original research report with retrievable abstract and full-text

3. Human *In vivo* study

4. Cartilage-related follow-up after ACL injury and/or reconstruction

5. Should include "young adults", excluding studies specifically focusing on skeletally immature or middle-aged patients

6. Should include at least two consecutive MRI readings within ACL injured and/or reconstructed knees

7. Published in English, French, German

Two independent readers (AVG, EW) screened abstracts both blinded for author names. To be included, all eligibility criteria should be met. In case of disagreement or doubt, records were discussed and consensus was reached. Additionally, newly on-line published and potentially eligible articles were considered up until September 1st, 2012 (n=2). As such, 16 full-text articles were assessed, excluding another four at this stage because of incompliance with criterion 4 and 6. Subsequently, targeted hand-searches in the reference lists of included articles were also performed. Finally, 12 studies were included in the qualitative analysis.

Quality appraisal

A customized three-composite "Total Quality Score (TQS)" was used (Table I, Appendix 2). The TQS assessed reporting adequacy, external/internal validity and power[21] and is based on general methodological requirements as put forward by the Downs and Black Quality Index.[22] Whereas the Quality Index proved reliable and valid, MRI-specific and clinical criteria were added to adjust this index to this field of study. The TQS for all included studies was determined by two readers (AVG, EW) that reached final consensus in case of disagreement or doubt. Based on two repeats performed by both readers on the included studies (n=12), intra- and inter-rater reliability was evaluated for each question separately (n=29). Consequently, considering the 29 separate items, intra- and inter-rater reliability was good-to-excellent (Intra-class Correlation Coefficient (ICC) from 0.71 to 1.00) and moderate-to-excellent (ICC from 0.45 to 1.00) respectively. When compared to the Quality Index, Bland-Altman plots revealed highly correlative (r=0.96, *P*<0.001) but consistently lower TQS scores. The TQS was based on the following three components:

1. *General study quality*: 17 criteria from the Quality Index[22]

2. *Field-specific methodological features – MRI acquisition and post-processing*: eigth criteria on the minimal methodological requirements of quantitative MRI studies[23]

3. *Field-specific methodological features – clinical considerations*: four criteria derived from the Coleman Methodology Score[24]

Criteria were scored ranging from 2 to 0 with (1) "yes: 1", "no: 0", or "unable to determine: 0", or (2) "yes: 2", "partially: 1", "no: 0", or "unable to determine: 0"[22], resulting into a maximum score of 38 points. If a criterion was not a requirement, the study was granted "not applicable" and the specific item was not considered in the final score. Consequently, score percentages were calculated and classified in view of the percentile-50 (P50) distribution of all scores defining "low quality" and "high quality" as "<P50" or ">P50" respectively.[19]

Data extraction

Data extraction was performed by one reader (AVG) including (1) patient characteristics, (2) surgical characteristics including outcome (Appendix 4), (3) cartilage change, (4) reference group, (5) MRI acquisition (data not shown) and post-processing, (6) baseline factors influencing the rate of cartilage change. In case of pooled cohorts, distribution of factors over individuals that underwent either operative or nonoperative treatment or adjustment for treatment should be clear. Only those factors were listed that were reported to significantly influence cartilage outcomes.

The data-extraction process was performed independently of the quality appraisal. While this systematic review did not proceed to a formal meta-analysis including statistical analyses on the extracted data, consistency of the data-extraction process was not separately verified.

Best-evidence synthesis

Evidence was rated as adapted from Van Tulder et al.[20]: (1) strong: generally consistent findings among multiple high-quality studies, (2) moderate: generally consistent findings among multiple low-quality studies and/or one high-quality study, (3) limited: one low-quality study, (4) conflicting: inconsistent findings among multiple studies.

Figure 1. Flow diagram of the study selection process adapted from Moher et al.[18]

Identification

Records identified through 7 electronic database searching
(n = 5.338)

Additional records identified through other sources: manual reference check eligible full-text articles included in the qualitative synthesis
(n = 0)

Screening

Records after duplicates removed
(n = 963)

Records screened after removal of irrelevant titles
(n = 504)

Records excluded
(n = 488)

Eligibility

Full-text articles assessed for eligibility
(n = 16)

Full-text articles excluded, with reasons
(n = 4)

- n = 65: no A1 journal
- n = 5: original report, abstract could not be retrieved
- n = 14: no original report, no abstract available
- n = 50: no original report: reviews and case reports
- n = 7: cadaveric/animal studies (not mentioned in the title)
- n = 242: no follow-up related to cartilage after ACL reconstruction
- n = 105: no MRI included for cartilage evaluation

- n = 3: no consecutive MRI readings
- n = 1: MRI follow-up not specific after ACL patients or reconstruction

Included

Studies included in qualitative synthesis
(n = 12)

Table 1. TQS shortlist: Overview of the three composites with answer options

Criteria	Answer
General: Reporting outcomes, external validity, internal validity[¶]	**Max. 19 pts**
1. Hypothesis/aim/objective clearly described?	Y/N
2. Main outcomes clearly described?	Y/N
3. Main characteristics of the patients clearly described?	Y/P/N
4. Distributions of principal confounders clearly described?	Y/P/N
5. Main findings clearly described?	Y/N
6. Provision numerical estimates of random variability for the main outcomes?	Y/N
7. Characteristics of the patients lost to follow-up been described?	Y/N
8. Report of actual probability values except where probability is less than 0.001?	Y/N
9. Subjects asked to participate in the study representative?	Y/N/U
10. Analysis adjusted for different lengths of follow-up of patients?	Y/N/U
11. Statistical tests appropriate?	Y/N/U
12. Measures accurate (valid and reliable)?	Y/N/U
13. Subjects recruited from the same population?	Y/N/U
14. Subjects recruited over the same period of time?	Y/N/U
15. Adequate adjustment for confounding?	Y/N/U
16. Losses of patients to follow-up taken into account?	Y/N/U
17. Power analysis performed?	Y/N/U
Field-specific methodological features - MRI acquisition and image-analysis: Reporting and internal validity[$]	**Max. 13 pts**
18. For quantitative imaging, loading conditions of the knee during or prior to imaging described?	Y/N
19. Magnetic field strength, scanner and coil type described/appropriate?	Y/P/N/U
20. Imaging sequence and parameters/technique described/appropriate?	Y/P/N/U
21. Anatomic regions/sub-regions clearly described?	Y/N
22. Detailed methodological description for calculation quantitative and semi-qualitative parameters described/appropriate?	Y/P/N/U
23. Longitudinal data read in pairs and blinded for sequence acquisition in view of follow-up? OR Longitudinal data read ad random and blinded to subject ID	Y/P/N/U
24. Measures of precision/reproducibility for acquisition and/or post-processing mentioned?	Y/N
25. Number of readers, level of experience and measure of reliability of reader intervention described?	Y/P/N
Field-specific methodological features – Clinical considerations: Reporting[¥]	**Max 6 pts**
26. Rehabilitation clearly described?	Y/P/N
27. Graft use and surgical technique clearly described?	Y/P/N
28. Number of surgeons involved clearly described?	Y/N
29. Management of concomitant injuries described?	Y/N

[¶] Criteria and qualifications adapted from Black and Downs [22] [$] Criteria based on Eckstein et al. [23] (2006). [¥] Criteria 26,27, 29 derived/adapted from the Coleman methodology [24]. Questions 1-2,5-8,18,21,24, 28-29: "Y/Yes"= score 1, "N/No"=score 0. Questions 3-4,25-27: "Y/Yes"=score 2, "P/Partially"=score 1, "N/No"=score 0. Questions 9-17: "Y/Yes"= score 1, "N/No"=score 0, "U/Unable to determine"=score 0. Questions 19-20, 22-23: "Y/Yes"=score 2, "P/Partially"=score 1, "N/No"=score 0, "U/Unable to determine"=score 0.

RESULTS

Description of studies

All 12 studies were considered observational longitudinal studies and were published from 1999 onwards with the majority being published recently (2008-2013). Four studies included both patients that underwent surgical or nonsurgical treatment. [12,25-27]

One study used a 1.0T magnet,[28] five used 1.5T,[12,25-27,29] and three studies applied 3T imaging.[30-32] Three studies reported mixed use of either 1.5T and 0.5T,[33] 1.5T and 3T[34] or 1.0T and 1.5T magnets.[35] One study did not apply consistent sequence types between consecutive baseline and follow-up.[33]

Sample sizes ranged from 8 to 54 ACL reconstructed patients with an estimated average age of 28.7 years. Apart from two studies,[30-31] body mass index (BMI) was not reported for ACL reconstructed patients. Patients were predominantly male.

Hamstrings and bone-patellar tendon-bone (BPTB) autografts were each used as the only graft choice in two studies.[32-35] The other studies reported mixed graft choices entailing hamstrings and BPTB autografts,[25-26] hamstrings, BPTB, and quadriceps tendon autografts,[28] hamstrings autografts, tibialis posterior and Achilles tendon allografts,[31] or hamstrings and BPTB autografts and Achilles tendon allografts.[12]

Baseline patient and surgical characteristics are presented in Table 2 and Appendix 4 respectively.

Quality appraisal

TQS ranged from 31.6% to 78.9% . Six studies were depicted as "low quality", [28-30,33-35] and six studies as "high" quality. [12,25-27,31-32] Lowest scores were attained for general external and internal validity, power, and MRI-related reporting and internal validity. (Appendix 3, Table 3)

Table 2. Characteristics of ACL-reconstructed patients in included studies (n=12)

Authors	N subjects	Gender M/F	Age baseline Average (Range,S.D.)	BMI Average (Range,S.D.)
Faber (1999)	23	18M/5F	30(20-49)	NR
Costa-Paz (2001)	21	15M/6F	31(20-58)	NR
Weninger (2008)	54	31M/14F	27.6(17-48)	NR
Frobell (2009)	34	NR*	NR*	NR
Arnoldi (2011)	9	7M/2F	35(12)	NR
Frobell (2011)	45	NR*	NR*	NR*
Li (2011)	12	7M/5F	34(27-45)	24.1(2.5)
Neuman (2011)	14	NR*	NR*	NR*
Potter (2012)	26	NR	35.1(8.2)	NR
	(28 knees)	14M/14F		
Theologis (2011)	9	5M/4F	35.4(6.0, 27-45)	23.1(2.1)
Hosseini (2012)	8	5M/3F	(19-38)	NR
Lee (2013)	36	30M/6F	34.5(19-60)	NR

"NR": Not Reported, "NR*": data not separately reported for ACL-reconstructed patients in the cohort.

Cartilage changes in view of follow-up time

In Tables 4 to 7, cartilage changes are listed in view of follow-up time and baseline joint status. Follow-up ranged from 2 weeks[30] to 11 years[12].

Semi-quantitative morphology

Two studies used the MRI-modified Outerbridge score,[12,28] and three studies reported on Whole-Organ MRI Score (WORMS) scores.[29,31,34] Three out of five were low-quality studies.[28-29,34] At 1 year follow-up, Li et al.[31] reported no change. After an average follow-up of 2.2 years from surgery, Lee et al.[34] detected progressive cartilage degeneration in 26.7% of all investigated sites, or improvement in 5% of sites. After an average of 2.8 years from surgery, Weninger et al. documented[28] cartilage degeneration in 68.9% of patients. After an average of 3.7 years from surgery, Arnoldi et al.[29] could not detect significant changes in prevalence of cartilage defects. Potter et al.[12] displayed progressive cartilage loss in femoral, tibial, patellar and trochlear cartilage registered up to 11 years post-injury. (Table 4)

Table 3. Extracted data on MRI-related reporting and internal validity: post-processing algorithms in quantitative imaging methods

Authors	Method	Segmentation	Processing algorithms	Registration images?	2D/3D	Laminar or zonal?	Reproducibility Precision error
Faber (1999)	Subjective thickness	No	No	NA	2D/3D	No	NR
Costa-Paz (2001)	Subjective thickness	No	No	NA	2D	No	NR
Frobell (2009-2011)	Thickness, volume, surface area	3D region-growing and knowledge-based 3D deformable model, automatic feature-based atlas for ROI, piece-wise mesh based tracking, trimming.[∞]	3D surface mesh models Volume, surface area: integration polygonal surface and triangulation, Thickness: normal distance to opposite surface[∞]	Yes	3D	Yes	NR
Arnoldi (2011)	Thickness, volume	B-spline snakes	NR	NA	3D	No	CV: 3.3-3.5%
Li (2011)	T1rho	Bezier splines and edge detection[∞]	Mono-exponential two-parameter nonlinear least square fit	Yes	3D	Yes	NR
Neuman (2011)	T2 dGEMRIC	Manual	Bi-exponential three-parameter fit	NR	2D	No	RMS CV: 5-8%[∞]
Potter (2012)	T2	Functool 3.1 GE software	Mono-exponential, two-parameter nonlinear least squares fit	NR	2D	Yes	NR
Theologis (2011)	T1rho	Bezier splines and edge detection	Mono-exponential two-parameter nonlinear least squares fit	Yes	3D	Yes	NR
Hosseini (2012)	Thickness	Rhinoceros software package	3D surface mesh models. Thickness: Euclidean distance (surface to cartilage-bone interface)	Yes	3D	No	NR

[∞]Reported by reference. (RMS) CV: (Root Mean Square) Coefficient of Variation. NR: "Not Reported". NA: "Not Applicable".ROI: Region Of Interest.

Quantitative morphology

Two studies reported on subjective thickness changes,[33,35] whereas three studies applied 3D computation of cartilage volume, thickness, or area.[25-26,29] Similarly, three out of five were low-quality studies.[29,33,35] At 1 year follow-up, Frobell et al.[25] noted a nonsignificant reduction in cartilage area of the trochlear femur and an increase in cartilage volume and thickness of the central medial femur. After 2 years, cartilage thickening of the central medial femur and thinning of the trochlear femur significantly progressed accompanied by significant thinning in the posterior medial and lateral femur.[26] After an average of 2.8 years from surgery, Costa-Paz et al.[35] noted cartilage thinning in 23.8% of patients. After an average of 3.7 years follow-up, Arnoldi et al.[29] described no significant changes. After an average of 6 years from surgery, Faber et al.[33] described significant cartilage thinning of the lateral femur in 56.5% of patients. (Table 5)

Estimates of collagen and water

Two high-quality studies applied T2 mapping.[12,31] After 1 year, Li et al.[31] did not detect significant T2 increases. From 1 up to 11 years post-injury, Potter et al.[12] registered significant progression of T2 values in lateral femoral cartilage and superficial and deep patellar cartilage. (Table 6)

Estimates of proteoglycan (PG)/glycosaminoglycan (GAG) content

Two studies reported on changes in T1rho values[30,31] and one study used the delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) index.[27] Two out of three were high-quality studies.[27,31] Up to 1 year, Theologis et al.[30] revealed significant T1rho elevations in bone marrow lesion (BML)-overlying cartilage when compared to adjacent cartilage in the lateral tibial full-thickness and superficial layer. In contrast, significant T1rho decreases were established in full-thickness as well as superficial and deep BML- overlying cartilage of the lateral femur. At 1 year follow-up, Li et al.[31] monitored significantly elevated T1rho values in both full-thickness as well as superficial cartilage layers of the medial weight-bearing femur and tibia. After an average of 2 years from injury, when compared to healthy controls, Neuman et al.[27] reported an overall decrease in dGEMRIC indices in lateral and medial femoral cartilage in the patient group both at baseline and follow-up, despite the patients' attempts to recover. (Table 6)

Functional properties: deformational behavior

At 6 months post-surgery, a high-quality study by Hosseini et al.[32] showed, at lower knee flexion angles, a 42% and a 29% increase in contact-deformation in respectively the medial and lateral compartment in the reconstructed knee when compared to the healthy contra-lateral knee at baseline. Despite this difference, an attempt to recover was noted when comparing the reconstructed knee to the post-injury condition (i.e., cartilage contact deformation in the medial compartment of 29±9% and 27±3%, and in the lateral compartment of 33±6% and 31±3% in the ACL-deficient and reconstructed knee respectively) (Table 7)

Potential factors affecting rate of cartilage change (best-evidence synthesis)

Bone Marrow Lesions (BML) (moderate evidence)

Four of the included studies associated initial BML (location, type, size/volume) with location and occurrence of cartilage thinning/increased cartilage loss, depression or increased T1rho values at 2 weeks up to 11 years follow-up.[12,30,33,35] In this regard, Potter et al.[12] established that the intial BML size was significantly associated with increased cartilage loss the first 3 years in the lateral tibia and the first 2 years in the lateral femur. In the lateral tibia, Theologis et al.[30] found a significant positive correlation between BML volume and percentage increase in T1rho values of the cartilage overlying the BML relative to the surrounding cartilage up to 1 year from injury (r=0.74).

Meniscal injury/meniscectomy (strong evidence)

Medial meniscal lesions at baseline showed increased T1rho and T2 values in the ipsilateral femur at 1 year follow-up.[31] In support, lateral/medial meniscal tears corresponded with lower femoral cartilage dGEMRIC indices at on average 2 years follow-up from injury.[27] Partial meniscectomy also led to lower femoral cartilage dGEMRIC indices.[27]

Time from injury (moderate evidence)

Regardless of surgical intervention, Potter et al.[12] established that, when compared to baseline (i.e., post-injury), the risk of cartilage loss doubled from year 1 for the lateral femur, lateral tibia,

and medial femur, and tripled for the patella. By years 7 to 11 after injury, the risk of cartilage loss for lateral femur was 50 times that of baseline, 30 times that for the patella, and 19 times for the medial femur.

Biomechanical factors (moderate evidence)

One study linked lack of biomechanics restoration after reconstruction to shifts in contact points toward regions of thinner cartilage displaying increased contact-deformation, especially at lower flexion angles.[32]

Surgical vs nonsurgical treatment

At 1 year after injury, ACL reconstruction was directly and significantly related to a reduction in cartilage area of the trochlear femur and to an increase in cartilage volume and thickness of the central medial femur.[25] After 2 years, treatment was no longer related to any of the changes in cartilage morphology.[26] Similarly, Neuman al.[27] reported a similar course in dGEMRIC index changes in both patients that underwent surgical or nonsurgical treatment after an average of 2 years from injury. Based on 11 years follow-up, Potter et al.[12] established higher Odd's ratios for cartilage loss in the medial tibia in nonsurgical compared to surgical treatment.

Table 4. Cartilage changes relative to baseline as an assessed MRI outcome in view of average follow-up time and baseline joint status (cartilage and concomitant injuries or procedures): semi-quantitative morphology

Authors	FU (years)	Parameter	Reference	ROI	Change	Baseline Joint Status			
						Cartilage	Meniscus	BML	Other
Li (2011)	1	WORMS	Baseline	General	=	Yes, WORMS:	Yes	Yes	K/L 1
				FL	NR	1,3			Osteophytes
				FM	NR	1			
				TL	NR	1,2,3			
				TM	NR	1			
				Ftr	NR	NR			
				P	NR	NR			
						NR			
Lee (2012)	2.2	WORMS	Baseline	General	-	-	Yes	NR	NR
				P(M-L)	=,↑	NR			
				FL(ant-post-centr)	=,↑,↓				
				FM (ant-post-centr)	=,↑,↓				
				TL(ant-post-centr)	=,↑,↓				
				TM (ant-post-centr)	=,↑,↓				
Weninger (2008)	2.8	Outerbridge	Baseline	General	↓	Yes, Outerbridge	Yes	Yes	NR
				FL	NR	-			
				FM	NR	2			
				TL	NR	3			
				TM	NR	-			
Arnoldi. (2011)	3.7	WORMS	Baseline	General	=	Yes, WORMS:	Yes	Yes	Ligament
				FL	NR	NR			Sub-articular cyst
				FM	NR	NR			
				TL	NR	NR			
				TM	NR	NR			
				P	NR	NR			
Potter (2012)	11	Outerbridge	Baseline	General	-	Yes, Outerbridge:	No	Yes	Ligament
				FL	↑	1.8			Popliteus tendon
				FM	↑	0.0-0.5			Lateral meniscal fascicle
				TL	↑	3.0			Meniscocapsular separation
				TM	↑	0.0			
				P	↑	0.5-1.0			
				Ftr	↑	0.0-0.5			

FU: Follow-Up time in average years. ROI: Region Of Interest. WORMS: Whole-Organ Magnetic Resonance Imaging Score. K/L. Kellgren/Lawrence grade. BML: Bone Marrow Lesion. NR="Not Reported". P (M-L):Patella (Medial-Lateral). FL/FM: Femur Lateral/Femur Medial. "=": no (significant) difference compared to reference. "↑/↓": (significant) increase (i.e., worsening)/decrease (i.e., improvement) compared to reference. Baseline joint status includes the presence of cartilage abnormalities (i.e., "Yes" indicated by parameter and/or degree; "No", indicated by parameter and/or degree; "NR"), meniscal involvement (i.e., Yes/No/NR), Presence of BML (i.e., Yes/No/NR), or Other (i.e., specified concomitant injuries).

104

Table 5. Cartilage changes relative to baseline as an assessed MRI outcome in view of average follow-up time and baseline joint status (cartilage and concomitant injuries or procedures): quantitative morphology

Authors	FU (years)	Parameter	Reference	ROI	Change	Baseline joint status			
						Cartilage	Meniscus	BML	Other
Frobell (2009)	1	Volume/thickness/ surface area	Baseline in patients treated with surgery or no surgery	General FL(total,centr) FM (total, centr, periph) TL TM Ftr P	- = = = = = =	NR	Yes	Yes	Cortical depression fractures
Frobell (2011)	2	Thickness	Baseline in patients treated with surgery or no surgery	General FL(centr) FL(post) FM (cent) FM (post) TL TM Ftr P	- = → ← → = = → =	No full-thickness lesions	Yes	Yes	Cortical depression fractures Meniscocapusular separation
Costa-Paz (2001)	2.8	Subjective Thickness	Baseline	General FL FM TL TM	→ NR NR NR NR	No arthroscopic lesions	NR	Yes	NR
Arnoldi (2011)	3.7	Volume/Thickness	Baseline	General FL FM TL TM P	- = = = = =	Yes, WORMS NR NR NR NR NR	Yes	Yes	Ligament Sub-articular cyst
Faber (1999)	6	Subjective Thickness	Baseline	General FL TL	- → =	No arthroscopic lesions	Yes	Yes	NR

FU: Follow-Up time in average years. ROI: Region Of Interest. WORMS: Whole-Organ Magnetic Resonance Imaging Score. BML: Bone Marrow Lesion. NR="Not Reported". P:Patella. FL/FM/Ftr: Femur Lateral/Femur Medial/Femoral Trochlea. Centr: central; periph: peripheral; post: posterior. "=": no (significant) difference compared to reference. "↑/↓": (significant) increase/decrease compared to reference. Both increases and decreases in quantitative morphology (i.e., thickness and volume) are associated with worsening of cartilage status. Baseline joint status includes the presence of cartilage abnormalities (i.e., "Yes", indicated by parameter and/or degree; "No", indicated by parameter and/or degree"); "NR meniscal involvement (i.e., Yes/No/NR), Presence of BML (i.e., Yes/No/NR), or Other (i.e., specified concomitant injuries).

105

Table 6. Cartilage changes relative to baseline as an assessed MRI outcome in view of average follow-up time and baseline joint status (cartilage and concomitant injuries or procedures): estimates of collagen/water, and PG/GAG content.

Authors	FU (years)	Parameter	Reference	ROI	Change	Baseline Joint Status			
						Cartilage	Meniscus	BML	Other
Li (2011)	1	T2/T1rho	Baseline in healthy controls	General	-	Yes, T2/T1rho	Yes	Yes	K/L 1 Osteophytes
				FL (total, sup, deep)	=/=	=			
				FM (total, sup)	=/↑	=			
				FM(deep)	=/=	=			
				TL (total, sup)	=/=	=/↑			
				TL (deep)	=/↑	↑/=			
				TM (total, sup)	=/↑	=			
				TM(deep)	=				
Theologis (2011)	Up to 1	T1rho in cartilage overlying BML	Surrounding cartilage	General	-	Yes, T1rho	No	Yes	No
				FL	→	→			
				FLsup	→	→			
				FLdeep	→	→			
				FM (total, sup, deep)	=	=			
				TL	←	←			
				TLsup	←	←			
				TLdeep	=	=			
				TM (total, sup, deep)	=	=			
Neuman (2011)	2	dGEMRIC	Baseline in patients treated with surgery or no surgery	General	-	No lesions in ROI	Yes	Yes	NR
				FL	←				
				FM	=				
Potter (2012)	1-11	T2	Baseline in patients treated with surgery or no surgery	General	-	Yes, Outerbridge	No	Yes	Ligament Popliteus tendon Lateral meniscal fascicle Meniscocapsular separation
				FL	←	1.8			
				TL	=	3.0			
				P (sup, deep)	←	0.5-1.0			

FU: Follow-Up time in average years. ROI: Region Of Interest. BML: Bone Marrow Lesion. K/L: Kellgren/Lawrence grade. NR="Not Reported". P:Patella. FL/FM: Femur Lateral/Femur Medial. Sup: superficial. "=": no (significant) difference compared to reference. "↑/↓": (significant) increase (i.e., worsening in case of T2or T1rho and improvement in case of dGEMRIC index)/decrease (i.e., improvement in case of T2 or T1rho and worsening in case of dGEMRIC index) compared to reference. Baseline joint status includes the presence of cartilage abnormalities (i.e., "Yes", indicated by parameter and/or degree; "No", indicated by parameter and/or degree; "NR"), meniscal involvement (i.e., Yes/No/NR), presence of BML (i.e., Yes/No/NR), or other (i.e., specified concomitant injuries).

Table 7. Cartilage changes relative to baseline as an assessed MRI outcome in view of average follow-up time and baseline joint status (cartilage and concomitant injuries or procedures): functional properties – deformational behavior

Authors	FU (years)	Parameter	Reference	ROI	Change	Baseline Joint Status			
						Cartilage	Meniscus	BML	Other
Hosseini (2012)	0.5	Contact deformation	Baseline contra-lateral intact knee	General Lateral Compartment Medial Compartment	- ↑ ↑	No visible lesions	No	No	No

FU: Follow-Up time in average years. ROI: Region Of Interest. BML: Bone Marrow Lesion. "=": no (significant) difference compared to reference. "↑/↓": (significant) increase (i.e., worsening /decrease (i.e., improvement) compared to reference. Baseline joint status includes the presence of cartilage abnormalities (i.e., "Yes" indicated by parameter and/or degree; "No", indicated by parameter and/or degree; "NR"), meniscal involvement (i.e., Yes/No/NR), Presence of BML (i.e., Yes/No/NR), or Other (i.e., specified concomitant injuries).

DISCUSSION

Next to baseline influencing factors, the main goal of this systematic review was to summarize the MRI-detected evidence of cartilage adaptation after ACL reconstruction.To understand the effect of surgery on the course of cartilage adaptation, this systematic review additionally investigated the effect of treatment (i.e., operative vs nonoperative). The main conclusions regarding clinical management and research directions are tabulated in Table 8.

Table 8. Take Home Messages for clinical management and future research directions

Clinical management	Future research directions
• Chondral defects are commonly detected in ACL injured and reconstructed knees.	• Longitudinal follow-up studies of cartilage ultra-structural changes during the first year(s) following injury or reconstruction. UTE and UTE-T2* and T1rho imaging may be more sensitive than standard T2 mapping in this respect.
• Gross MRI-detected morphological change requires approximately 2 years.	
• Prevention should focus on ultra-structural deterioration accelerating cartilage loss.	• Validation of MRI biomarkers in long-term studies in view of the prediction of future radiographic and/or symptomatic OA.
• In the lateral compartment, morphological and/or ultra-structural damage most likely progresses from blunt trauma onwards. Medially, changes presumably start during the first year, hitherto recorded the soonest at 3 weeks follow-up.	• Prospective risk factor studies to support identification of patients treated with ACL reconstruction at risk for accelerated cartilage degeneration.
• Moderate-to-strong evidence exist for baseline factors meniscal lesion/ meniscectomy, BML, time from injury and persistent altered biomechanics as influencing rate of cartilage change after ACL reconstruction.	• High quality (multi-center) Randomized Controlled Trial (RCT)s on the efficacy and safety of biological, surgical, and rehabilitation techniques in mediating cartilage morphological and ultra-structural deterioration following ACL injury and reconstruction both in the short- and long-term.
• (Late) Post-operative rehabilitation should also consider cartilage status in return to play decisions.	
• ACL reconstructed knees may benefit from longer recovery than nonsurgically treated knees. After 1 year, treatment effects disappear and, so far, no treatment option appears convincingly superior in view of structural longevity of the knee.	

While MRI evaluation is the measure of interest, several methodological issues require consideration. Next to insufficient field strength (<1.0T) in one study, three studies implemented mixed field strengths and/or sequence types throughout consecutive baseline and follow-up.[33-35]

These inconsistencies jeopardize longitudinal morphological assessment.[36-41] Quantitative morphology was rated on 2D[33,35] or 3D image stacks.[25-26,29,33] As opposed to 2D Fast Spin Echo ((F)SE) imaging, 3D Spoiled Gradient echo Recalled acquisition (SPGR)/Fast Low Angle Shot (FLASH) or Dual Echo in the Steady State (DESS) sequences allow thinner sections with near-isotropic high-resolution that avoid partial volume averaging and allow analysis independent of slice orientation or localization.[36-38,41] Hence, computerized 3D quantification is superior over 2D or subjective evaluation. Although the reported 3D techniques are appropriate, measures of reproducibility were hardly described (Table 3). A recent systematic review by Hunter et al.[42] confirmed that both semi-quantitative and quantitative morphological methods perform with moderate-to-excellent intra- and inter-reader consistency and good responsiveness to longitudinal change. However, present variability of quantitative techniques attained up to a coefficient of variation (CV) of 3.5% (Table 3), limiting detection of significant change within the first year (i.e., expected mean relative changes: -2.2% to +3.3%[25-26]). Despite the majority of low-quality studies, the course of morphological adaptation described below is supported by the few high-quality trials.[12,25-26,31]

Apart from morphology, compositional imaging techniques such as T2, T1rho mapping and dGEMRIC imaging were appraised. T2 mapping is sensitive to changes in hydration (or, nearly equivalently collagen concentration) as well as to organization of the anisotropic arrangement of the collagen fibrils in the extra-cellular matrix. Early cartilage degeneration, reflected by increased matrix permeability, appears as an increase in T2.[36-38,41,43] T1rho mapping is suggested to provide superior sensitivity to early deterioration compared to standard T2 mapping, especially when applying laminar analyses.[31] While reported nonspecific, T1rho relaxation times inversely relate to PG depletion.[36-38,41,43] dGEMRIC, T1 imaging in the presence of GdDTPA^{2-} (i.e., T1Gd or dGEMRIC index), reflects cartilage GdDTPA^{2-}concentration, and, hence indirectly, GAG concentration. Low dGEMRIC indices are commonly observed in areas of cartilage degeneration.[36-38,41,43-44]

Whereas both T2 and T1rho analyses may have benefited from a multi-exponential decay model,[43,45-46] Ultra-short TE (UTE) and UTE-T2* imaging techniques may have been more sensitive than standard T2 mapping in detecting early matrix changes toward the cartilage-bone interface).[47] An increased sensitivity for change of T2* compared to standard T2 has already been shown in ACL-reconstructed knees as soon as 6 months post-surgery.[6] Whereas T1rho quantification may have been less orientation-dependent,[44,46-48] magic angle effects may have affected T2 outcomes.[44] Despite all influencing factors, relative changes were interpreted instead of actual values to allow for comparison between studies. As dGEMRIC index quantification depends, next to GAG content, on contrast supply and distribution within the tissue, matrix permeability may have gradually changed during follow-up warranting circumspection in the interpretation of index change.[49] Apart from Neuman et al.[27], no

compositional imaging study reported measures of reproducibility (Table 3). Variability (i.e., CV) in T2, T1rho and dGEMRIC indices is documented to range from 1 to 9%,[6,50-51] 3.3-8.5%,[51-52] and 5-8%[27] respectively, appropriate in view of the expected differences during the first years (i.e., -3.4% to +17.6%[27,31]).

This review determined that MRI-detectable progressive macroscopic change after ACL reconstruction requires on average 2 years. The absence of substantial baseline cartilaginous injury did not seem protective against progressive degeneration when time reaches or elapses 2-year follow-up.[26,33,35] Noted both medially and laterally, macroscopic changes appeared more evident in the femur than in the tibia.[25-26,33-34] In support, animal models documented that ACL transection resulted in higher thickness increases in femoral than tibial cartilage.[53-54] The corresponding decrease in compressive stiffness might render femoral cartilage more susceptible to surface fibrillation[55] possibly explaining the location of most evident morphological change.[53-57]

Before or simultaneous with macroscopic change, cartilage in ACL-reconstructed knees suffers from compositional adaptations. Changes in matrix constituents may present as remnants of blunt trauma and afterward as maintained by the biochemical environment within the knee, co-existing injuries, surgical procedures and persistent biomechanical alterations. Baseline elevated T2, T1rho values and decreased dGEMRIC indices in the lateral tibia or femur are presumably resulting from blunt trauma and tissue edema.[7,27,31] In this regard, impact traumata cause ultra-structural and morphologic changes (i.e., surface fraying and delamination, tidemark disruption, accumulation of unbound water, PG loss)[7,58] and are likely accompanied by BML or cortical depression fractures on MRI.[59] These concomitant baseline injuries were frequently reported and, hence, support that blunt trauma led to the ultra-structural baseline changes captured by MRI. Interestingly, in the lateral femur, Theologis et al.[30] reported decreased T1rho values in BML-overlying cartilage suggestive of increased relative PG contents. This study mainly compared weight-bearing to non-weight-bearing regions within the same knee with the latter possibly presenting with higher T1rho values because of the natural topographical variation in GAG contents.[48,60]

During the first year(s), healing attempts in the lateral compartment are noted (i.e., increase in dGEMRIC index, decrease in T2 and contact-deformation),[7,27,31-32] however based on limited follow-up (i.e. up to an average of 2 years from injury) as deterioration appears to progress nonetheless. In this regard, signs of incomplete recovery are pronounced by progressive cartilage defects in all compartments accompanied by T2 prolongation in the lateral femur and patella from the first year onwards[12] and by maintenance or development of ultra-structural, morphological and functional changes medially recorded the soonest at 3 weeks after

injury.[7,12,25-27,31-32,34] Early medial deterioration presumably results from net GAG loss rather than trauma-induced tissue edema suggesting global biochemical disturbance in the ACL-injured joint.[7] Although the medial compartment is not likely involved in blunt trauma, it often develops OA in the long-term.[61-64]

The prevalence of radiographic patella-femoral (PF) OA is reported to range from 11 to 90% following 2-15 years after ACL surgery.[65-67] In this study, six articles[12,25-26,29,31,34] included investigation of the patella and/or femoral trochlea, four of those revealing considerable PF involvement in morphological[25-26,34] and ultra-structural changes.[12] PF cartilage damage might result from impaction of joint surfaces and/or from inflammatory responses upon injury or surgery.[66] Additionally, insufficient restoration of knee biomechanics or patellar orientation, accompanied by possible extension Range Of Motion (ROM) or quadriceps strength deficits, may affect PF joint contact areas and loading patterns increasing its vulnerability toward degeneration.[66-69]

Moderate-to-strong evidence was provided for meniscal lesions/meniscectomy, time from injury, BML and altered biomechanics as potentially influencing cartilage change following reconstruction. Association sizes (e.g., Odd's Ratio) were not consistently presented but were rather reported by *P*-values and/or averages. Nonetheless, in long-term studies of ACL reconstruction or OA, meniscal involvement,[5,62-64,71-73] BML[74] and length of follow-up[63,75] persist as risk factors for MRI-detected cartilage degeneration or radiographic OA. As reconstruction (combined with partial medial meniscectomy) only partially restores knee biomechanics,[13-17,76-77] cartilage-cartilage contact points may shift toward regions of thinner cartilage not sufficiently adapted to cope with impact or shear stresses.[32,78] Next to shifts in contact area, MRI cartilage T2 and thickness analyses in animal models additionally proposed that medial meniscectomy resulted in increased contact stress.[79-80] As revealed by finite element modeling, altered contact stresses may impair cartilage fluid pressurization, dissipation and load-transferring properties.[81] Finally, BMLs are hypothesized to reduce the stress-dissipating capacities of the cartilage-subchondral bone unit and to impede nutritional flow toward the cartilage tissue potentially contributing to quality degradation.[82] Four of the presently evaluated studies investigated cohorts that included both individuals that underwent operative and nonoperative treatment.[12,25-27] With respect to these studies, caution may be warranted when directly applying factors potentially influencing rate of cartilage change onto ACL-reconstruction alone because of the suggested treatment effects on cartilage status in the early years of follow-up. In this regard, despite protection against subsequent meniscal procedures, ACL reconstruction presented with pronounced morphological changes during the first year when compared to nonsurgical treatment.[25] When time progressed, treatment effects disappeared or even displayed protective effects against cartilage loss in cases treated with isolated reconstruction.[12,26-27] Supplementary BML and/or prolonged inflammatory cascades caused by surgery might cause slower resolution

of BML and joint fluid volumes during the first year[25] inviting speculation on the need for extended recovery in ACL reconstruction.[6,25-26] Nonetheless, cartilage in both patients that underwent surgical or nonsurgical treatment evolves toward early arthritic changes [26] and neither of both treatment options convincingly safeguards structural longevity of the knee so far.[83] Therefore, in view of these treatment effects during the early years of follow-up, this systematic review only considered those risk factors in the best-evidence synthesis for which distribution over operated and nonoperated patients could be clearly discerned or for which adjustment for treatment was made clear. Hence, risk factors are not limited to those presented here and more research is needed identifying patients at risk for accelerated cartilage disease after ACL reconstruction.

MRI-measured morphological changes, low dGEMRIC indices, and increased T2 are associated with accelerated cartilage degeneration, radiographic OA or total knee arthroplasty.[84-87] Although confirmation in future long-term studies on radiographic and/or symptomatic OA following ACL-injury remains warranted, the present early arthritic changes are considered important in view of future joint deterioration. As during the early phase cartilage might be more susceptible to treatment and prevention strategies,[88] speculation on biological, surgical and rehabilitation interventions effecting chondroprotection is tempting. One needs to stress that these interventions require well-designed short- and long-term clinical trials to confirm efficacy and safety in (ACL-injured) patients. Proposed biological treatments may include symptomatic slow acting drugs, biophysical stimulation modalities, viscosupplementation, blood derivates, mesenchymal cell based therapies, and stimulation or inhibition of respectively anabolic and catabolic pathways.[89] Whereas in view of restoring joint kinematics anatomic double-bundle reconstruction may be preferred, surgical interventions may also involve cartilage repair or meniscal preservation or restoration procedures (i.e., meniscus repair or replacement).[90-92] Altered biomechanics including gait, affects both limbs and is – of the identified influencing factors – the only potentially modifiable post-surgery.[93-95] Apart from graft positioning,[14,90] neuromuscular and/or quadriceps (eccentric) strength training may remedy altered gait while potentially positively influencing GAG content.[95-96] Additionally, specific gait-retraining focusing on cadence and stride frequency preferably directed by a metronome[97] could be useful next to the potential use of insole or shoe modification.[98] Furthermore, joint and cartilage vulnerability, especially in case of BML or meniscal involvement, should be considered in return to sports approvals. In this regard, depending upon the athlete's profile and type of sports, return to play takes place at on average 6 months from surgery. At this point in time, diminished cartilage quality and *in vivo* resiliency was revealed in ACL-reconstructed patients especially in those resuming sports before 5 months after surgery.[6] Hence, one might argue that cartilage may be at risk for further deterioration when imposed with high(er) impact loads that

typically occur during sports. Ideally, adding a feasible MRI protocol to functional tests may support return to play decisions. As a weak correlation exists between symptoms and joint health,[99] in this review, no baseline clinical factors (Appendix 4) related to cartilage status. Interestingly, although cause-effect interpretation remains unclear, Potter et al.[12] linked increased cartilage loss to decreased patient-reported activity-related scores at follow-up.

CONCLUSION

In ACL reconstruction, cartilage macroscopic changes were detectable after approximately 2 years follow-up. In view of OA prevention, braking (early) deterioration of matrix constituents is key. In the lateral compartment, ultra-structural and morphological damage most likely progresses from blunt trauma onwards. Medially, changes presumably start during the first year, hitherto recorded the soonest at 3 weeks follow-up. These results may have implications on future research directions, prevention and treatment including return to play decisions. Important factors are meniscal lesions/meniscectomy, BML, time from injury, persistent altered biomechanics. First-year morphological changes were more pronounced in knees that underwent reconstruction compared to nonsurgical treatment.

AUTHOR CONTRIBUTION

Van Ginckel, Ans: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, collection and assembly of data.

Verdonk, Peter: conception and design, critical revision of the article for important intellectual content, final approval of the article.

Witvrouw, Erik: conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article.

ROLE OF THE FUNDING SOURCE

the data; in writing of the manuscript; and in the submission to submit the manuscript for publication.

CONFLICTS OF INTEREST

Peter Verdonk receives consultancy and lecture fees, payment for development of educational presentations and travel expenses/accommodation/meeting expenses and owns stock/stock options from Smith and Nephew. The other authors did not declare any conflict of interest.

ACKNOWLEDGMENTS

REFERENCES

1. Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS. A randomized trial of treatment for acute anterior cruciate ligament tears. N Engl J Med 2010;363(4):331-42.
2. Renström PA. Eight clinical conundrums relating to anterior cruciate ligament (ACL) injury in sport: recent evidence and a personal reflection. Br J Sports Med 2013;47(6):367-72.
3. Richmond JC, Lubowitz JH, Poehling GG. Prompt operative intervention reduces long-term osteoarthritis after knee anterior cruciate ligament tear. Arthroscopy 2010;26(10):1368-9.
4. Myklebust G, Bahr R. Return to play guidelines after anterior cruciate ligament surgery. Br J Sports Med 2005; 39(3):127-31.
5. Claes S, Hermie L, Verdonk R, Bellemans J, Verdonk P. Is osteoarthritis an inevitable consequence of anterior cruciate ligament reconstruction? A meta-analysis. Knee Surg Sports Traumatol Arthrosc 2012;DOI 10.1007/s00167-012-2251-8.
6. Van Ginckel A, Verdonk P, Victor J, Witvrouw E. Cartilage status in relation to return to sports. Am J Sports Med 2013; 41(3):550-559.
7. Tiderius CJ, Olsson LE, Nyquist F, Dahlberg L. Cartilage glycosaminoglycan loss in the acute phase after an anterior cruciate ligament injury - Delayed gadolinium-enhanced magnetic resonance imaging of cartilage and synovial fluid analysis. Arthritis Rheum 2005;52:120-7.
8. Swärd P, Frobell R, Englund M, Roos H, Struglics A. Cartilage and bone markers and inflammatory cytokines are increased in synovial fluid in the acute phase of knee injury (hemarthrosis) - a cross-sectional analysis. Osteoarthritis Cartilage 2012;20(11):1302-8.
9. Nelson F, Billinghurst RC, Pidoux I, Reiner A, Langworthy M, McDermott M, et al. Early post-traumatic osteoarthritis-like changes in human articular cartilage following rupture of the anterior cruciate ligament. Osteoarthritis Cartilage 2006;14(2):114-9.
10. Fleming BC, Oksendahl HL, Mehan WA, Portnoy R, Fadale PD, Hulstyn MJ.Delayed Gadolinium-Enhanced MR Imaging of Cartilage (dGEMRIC) following ACL injury. Osteoarthritis Cartilage 2010;18(5):662-7.
11. Bolbos RI, Ma CB, Link TM, Majumdar S, Li XJ. In Vivo T1 rho Quantitative Assessment of Knee Cartilage After Anterior Cruciate Ligament Injury Using 3 Tesla Magnetic Resonance Imaging. Invest Radiol 2008;43:782-8.
12. Potter HG, Jain SK, Ma Y, Black BR, Fung S, Lyman S. Cartilage injury after acute, isolated anterior cruciate ligament tear: immediate and longitudinal effect with clinical/MRI follow-up. Am J Sports Med 2012;40:276.
13. Scanlan SF, Chaudhari AM, Dyrby CO, Andriacchi TP. Differences in tibial rotation during walking in ACL reconstructed and healthy contralateral knees. J Biomech 2010;43(9):1817-22.
14. Stergiou N, Ristanis S, Moraiti C, Georgoulis AD. Tibial rotation in anterior cruciate ligament (ACL)-deficient and ACL-reconstructed knees: a theoretical proposition for the development of osteoarthritis. Sports Med 2007;37(7):601-13.

15. Carpenter R, Majumdar S, Ma C. Magnetic resonance imaging of 3-dimensional in vivo tibiofemoral kinematics in anterior cruciate ligament-reconstructed knees. Arthroscopy 2009; 25:760-6.

16. Haughom B, Schairer W, Souza RB, Carpenter D, Ma CB, Li X. Abnormal tibiofemoral kinematics following ACL reconstruction are associated with early cartilage matrix degeneration measured by MRI T1rho. Knee 2012 Aug;19(4):482-7.

17. Schairer WW, Haughom BD, Morse LJ, Li X, Ma CB. Magnetic resonance imaging evaluation of knee kinematics after anterior cruciate ligament reconstruction with anteromedial and transtibial femoral tunnel drilling techniques. Arthroscopy 2011;27(12):1663-70.

18. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6(7):e1000097.

19. Yusuf E, Kortekaas MC, Watt I, Huizinga TW, Kloppenburg M. Do knee abnormalities visualized on MRI explain knee pain in knee osteoarthritis? A systematic review. Ann Rheum Dis 2011;70(1):60-7.

20. van Tulder M, Furlan A, Bombardier C, Bouter L; Editorial Board of the Cochrane Collaboration Back Review Group. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine 2003;28(12):1290-9.

21. Mallen C, Peat G, Croft P. Quality assessment of observational studies is not commonplace in systematic reviews. J Clin Epidemiol 2006;59:765-9.

22. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomized and non-randomised studies of health care interventions. J Epidemiol Community Health 1998;52:377-84.

23. Eckstein F, Ateshian G, Burgkart R, Burstein D, Cicuttini F, Dardzinski B, et al. Proposal for a nomenclature for magnetic resonance imaging based measures of articular cartilage in osteoarthritis. Osteoarthritis Cartilage 2006;14(10):974-83.

24. Coleman BD, Khan KM, Maffulli N, Cook JL, Wark JD. Studies of surgical outcome after patellar tendinopathy: clinical significance of methodological deficiencies and guidelines for future studies. Victorian Institute of Sport Tendon Study Group. Scand J Med Sci Sports 2000;10(1):2-11.

25. Frobell RB, Le Graverand MP, Buck R, Roos EM, Roos HP, Tamez-Pena J, et al. The acutely ACL injured knee assessed by MRI: changes in joint fluid, bone marrow lesions, and cartilage during the first year. Osteoarthritis Cartilage 2009;17:161-7.

26. Frobell RB. Change in cartilage thickness, posttraumatic bone marrow lesions, and joint fluid volumes after acute ACL disruption: a two-year prospective MRI study of sixty-one subjects. J Bone Joint Surg Am 2011;93:1096-103.

27. Neuman P, Tjornstrand J, Svensson J, Ragnarsson C, Roos H, Englund M, et al. Longitudinal assessment of femoral knee cartilage quality using contrast enhanced MRI (dGEMRIC) in patients with anterior cruciate ligament injury - comparison with asymptomatic volunteers. Osteoarthritis Cartilage 2011;19:977-83.

28. Weninger P, Zifko B, Liska M, Spitaler R, Pelinka H, Hertz H. Anterior cruciate ligament reconstruction using autografts and double biodegradable femoral cross-pin fixation: functional, radiographic and MRI outcome after 2-year minimum follow-up. Knee Surg Sports Traumatol Arthrosc 2008;16:988-95.

29. Arnoldi AP, Weckbach S, Nussbickel C, Horng A, Nobauer I, Zysk S, et al. MRI Based Volumetric Assessment of Knee Cartilage after ACL-Reconstruction, Correlated with Qualitative Morphologic Changes in the Joint and with Clinical Outcome. Is there Evidence for Early Posttraumatic Degeneration? Rofo 2011;183:1138-44.

30. Theologis AA, Kuo D, Cheng J, Bolbos RI, Carballido-Gamio J, Ma CB, et al. Evaluation of bone bruises and associated cartilage in anterior cruciate ligament-injured and -reconstructed knees using quantitative t(1rho) magnetic resonance imaging: 1-year cohort study. Arthroscopy 2011;27:65-76.

31. Li X, Kuo D, Theologis A, Carballido-Gamio J, Stehling C, Link TM, et al. Cartilage in anterior cruciate ligament-reconstructed knees: MR imaging T1rho and T2--initial experience with 1-year follow-up. Radiology 2011;258:505-14.

32. Hosseini A, Van de Velde S, Gill TJ, Li G. Tibiofemoral cartilage contact biomechanics in patients after reconstruction of a ruptured anterior cruciate ligament. J Orthop Res 2012;30(11):1781-8.

33. Faber KJ, Dill JR, Amendola A, Thain L, Spouge A, Fowler PJ. Occult osteochondral lesions after anterior cruciate ligament rupture. Six-year magnetic resonance imaging follow-up study. Am J Sports Med 1999;27:489-94.

34. Lee YS, Jeong YM, Sim JA, Kwak JH, Kim KH, Nam SW, et al. Specific compartmental analysis of cartilage status in double-bundle ACL reconstruction patients: a comparative study using pre- and postoperative MR images. Knee Surg Sports Traumatol Arthrosc 2013;21(3):702-7.

35. Costa-Paz M, Muscolo DL, Ayerza M, Makino A, Aponte-Tinao L. Magnetic resonance imaging follow-up study of bone bruises associated with anterior cruciate ligament ruptures. Arthroscopy 2001;17:445-9.

36. Link TM. MR imaging in osteoarthritis: hardware, coils, and sequences. Radiol Clin North Am 2009;47(4):617-32.

37. Crema MD, Roemer FW, Guermazi A. Magnetic resonance imaging in knee osteoarthritis research: semiquantitative and compositional assessment. Magn Reson Imaging Clin N Am;19 (2):295-321.

38. Roemer FW, Crema MD, Trattnig S, Guermazi A. Advances in imaging of osteoarthritis and cartilage. Radiology 2011;260(2):332-54.

39. Moriya S, Miki Y, Matsuno Y, Okada M. Three-dimensional double-echo steady-state (3D-DESS) magnetic resonance imaging of the knee: establishment of flip angles for evaluation of cartilage at 1.5 T and 3.0 T. Acta Radiol 2012;53(7):790-4.

40. Hudelmaier M, Glaser C, Pfau C, Eckstein F. Comparison between different implementations of the 3D FLASH sequence for knee cartilage quantification. MAGMA 2012;25(4):305-12.

41. Guermazi A, Roemer FW, Hayashi D. Imaging of osteoarthritis: update from a radiological perspective. Curr Opin Rheumatol 2011;23(5):484-91.

42. Hunter DJ, Zhang W, Conaghan PG, Hirko K, Menashe L, Reichmann WM, et al.Responsiveness and reliability of MRI in knee osteoarthritis: a meta-analysis of published evidence. Osteoarthritis Cartilage 2011;19(5):589-605.

43. Burstein D, Gray M, Mosher T, Dardzinski B. Measures of molecular composition and structure in osteoarthritis. Radiol Clin North Am 2009;47(4):675-86.

44. Williams A, Gillis A, McKenzie C, Po B, Sharma L, Micheli L, et al. Glycosaminoglycan distribution in cartilage as determined by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): potential clinical applications. AJR Am J Roentgenol 2004;182(1):167-72.

45. Raya JG, Dietrich O, Horng A, Weber J, Reiser MF, Glaser C. T2 measurement in articular cartilage: impact of the fitting method on accuracy and precision at low SNR. Magn Reson Med 2010;63(1):181-93.

46. Wang N, Xia Y. Dependencies of multi-component T2 and T1ρ relaxation on the anisotropy of collagen fibrils in bovine nasal cartilage. J Magn Reson 2011;212(1):124-32.

47.   Du J, Diaz E, Carl M, Bae W, Chung CB, Bydder GM. Ultrashort echo time imaging with bicomponent analysis. Magn Reson Med 2012 Mar;67(3):645-9.

48.   Li X, Cheng J, Lin K, Saadat E, Bolbos RI, Jobke B, et al. Quantitative MRI using T1ρ and T2 in human osteoarthritic cartilage specimens: correlation with biochemical measurements and histology. Magn Reson Imaging 2011;29(3):324-34.

49.   Stubendorff JJ, Lammentausta E, Struglics A, Lindberg L, Heinegård D, Dahlberg LE. Is cartilage sGAG content related to early changes in cartilage disease? Implications for interpretation of dGEMRIC. Osteoarthritis Cartilage 2012;20(5):396-404.

50.   Joseph GB, Baum T, Alizai H, Carballido-Gamio J, Nardo L, Virayavanich W, et al. Baseline mean and heterogeneity MR cartilage T2 are associated with morphologic degeneration of cartilage, meniscus, and bone marrow over 3 years--data from the Osteoarthritis Initiative. Osteoarthritis Cartilage 2012;20(7):727-35.

51.   Li X, Schooler J, Liang F, Vishnudas KS, Chen W, Banerjee S, et al. Simultaneous acquisition of T1rho and T2 quantification in cartilage – reproducibility and diurnal variation.(Abstact) Proc Intl Soc Mag Reson Med 2011;19:506.

52.   Pakin SK, Schweitzer ME, Regatte RR. 3D-T1rho quantitation of patellar cartilage at 3.0T. J Magn Reson Imaging 2006;24(6):1357-63.

53.   Herzog W, Diet S, Suter E, Mayzus P, Leonard TR, Müller C, et al. Material and functional properties of articular cartilage and patellofemoral contact mechanics in an experimental model of osteoarthritis. J Biomech 1998;31(12):1137-45.

54.   Pelletier JP, Troncy E, Bertaim T, Thibaud D, Goulet AC, Abram F, et al. Treatment with tiludronic acid helps reduce the development of experimental osteoarthritis lesions in dogs with anterior cruciate ligament transection followed by reconstructive surgery: a 1-year study with quantitative magnetic resonance imaging. J Rheumatol 2011;38(1):118-28.

55.   Froimson MI, Ratcliffe A, Gardner TR, Mow VC. Differences in patellofemoral joint cartilage material properties and their significance to the etiology of cartilage surface fibrillation. Osteoarthritis Cartilage 1997;5(6):377–86.

56.   Lozano J, Saadat E, Li X, Majumdar S, Ma CB. Magnetic resonance T(1 rho) imaging of osteoarthritis: a rabbit ACL transection model. Magn Reson Imaging 2009;27(5):611-6.

57.   Boileau C, Martel-Pelletier J, Abram F, Raynauld JP, Troncy E, D'Anjou MA, et al. Magnetic resonance imaging can accurately assess the long-term progression of knee structural changes in experimental dog osteoarthritis. Ann Rheum Dis 2008;67(7):926-32.

58.   Yeow CH, Cheong CH, Ng KS, Lee PV, Goh JC. Anterior cruciate ligament failure and cartilage damage during knee joint compression: a preliminary study based on the porcine model. Am J Sports Med 2008;36(5):934-42.

59.   Frobell RB, Roos HP, Roos EM, Hellio Le Graverand MP, Buck R, Tamez-Pena J, et al. The acutely ACL injured knee assessed by MRI: are large volume traumatic bone marrow lesions a sign of severe compression injury? Osteoarthritis Cartilage 2008;16(7):829-36.

60.   Rogers BA, Murphy CL, Cannon SR, Briggs TW. Topographical variation in glycosaminoglycan content in human articular cartilage. J Bone Joint Surg Br 2006;88(12):1670-4.

61.   Kannus P, Järvinen M. Posttraumatic anterior cruciate ligament insufficiency as a cause of osteoarthritis in a knee joint. Clin Rheumatol 1989;8(2):251-60.

62.   Aglietti P, Zaccherotti G, De Biase P, Taddei I. A comparison between medial meniscus repair, partial meniscectomy, and normal meniscus in anterior cruciate ligament reconstructed knees. Clin Orthop Relat Res 1994;307:165-73.

63.   Li RT, Lorenz S, Xu Y, Harner CD, Fu FH, Irrgang JJ. Predictors of radiographic knee osteoarthritis after anterior cruciate ligament reconstruction. Am J Sports Med 2011;39(12):2595-603.

64.   Seon JK, Song EK, Park SJ. Osteoarthritis after anterior cruciate ligament reconstruction using a patellar tendon allograft. Int Orthop 2006;30(2):94-8.

65.   Neuman P, Kostogiannis I, Friden T, Roos H, Dahlberg LE, Englund M. Patellofemoral osteoarthritis 15 years after anterior cruciate ligament injury--a prospective cohort study. Osteoarthritis Cartilage 2009;17:284-90.

66.   Culvenor AG, Cook JL, Collins NJ, Crossley KM. Is patellofemoral joint osteoarthritis an under-recognised outcome of anterior cruciate ligament reconstruction? A narrative literature review. Br J Sports Med 2013 Jan;47(2):66-70.

67.   Oiestad BE, Holm I, Engebretsen L, Aune AK, Gunderson R, Risberg MA. The prevalence of patellofemoral osteoarthritis 12 years after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 2013;21(4):942-9.

68.   Shin CS, Carpenter RD, Majumdar S, Ma CB. Three-dimensional in vivo patellofemoral kinematics and contact area of anterior cruciate ligament-deficient and -reconstructed subjects using magnetic resonance imaging. Arthroscopy 2009;25(11):1214-23.

69.   Van de Velde SK, Gill TJ, DeFrate LE, Papannagari R, Li G. The effect of anterior cruciate ligament deficiency and reconstruction on the patellofemoral joint. Am J Sports Med 2008;36 (6):1150-9.

70.   Cicuttini FM, Forbes A, Yuanyuan W, Rush G, Stuckey SL. Rate of knee cartilage loss after partial meniscectomy. J Rheumatol 2002;29(9):1954-6.

71.   Magnussen RA, Mansour AA, Carey JL, Spindler KP. Meniscus status at anterior cruciate ligament reconstruction associated with radiographic signs of osteoarthritis at 5- to 10-year follow-up: a systematic review. J Knee Surg 2009;22:347-57.

72.   Keays S, Newcombe P, Bullock-Saxton J, Bullock M, Keays A. Factors involved in the development of osteoarthritis after isolated anterior cruciate ligament surgery. Am J Sports Med 2010;38(3):455-63.

73.   Louboutin H, Debarge R, Richou J, Selmi TA, Donell ST, Neyret P, et al. Osteoarthritis in patients with anterior cruciate ligament rupture: a review of risk factors. Knee 2009; 16: 239-44.

74.   Driban JB, Lo GH, Lee JY, Ward RJ, Miller E, Pang J,et al. Quantitative bone marrow lesion size in osteoarthritic knees correlates with cartilage damage and predicts longitudinal cartilage loss. BMC Musculoskelet Disord 2011;12:217.

75.   Wirth W, Larroque S, Davies RY, Nevitt M, Gimona A, Baribaud F, et al. Comparison of 1-year vs 2-year change in regional cartilage thickness in osteoarthritis results from 346 participants from the Osteoarthritis Initiative. Osteoarthritis Cartilage 2011;19(1):74-83.

76.   Gao B, Cordova ML, Zheng NN. Three-dimensional joint kinematics of ACL-deficient and ACL-reconstructed knees during stair ascent and descent. Hum Mov Sci 2012;31(1):222-35.

77.   Tsivgoulis SD, Tzagarakis GN, Papagelopoulos PJ, Koulalis D, Sakellariou VI, Kampanis NA, et al. Pre-operative versus post-operative gait variability in patients with acute anterior cruciate ligament deficiency. J Int Med Res

2011;39(2):580-93.

78. Chaudhari AM, Briant PL, Bevill SL, Koo S, Andriacchi TP. Knee kinematics, cartilage morphology, and osteoarthritis after ACL injury. Med Sci Sports Exerc 2008;40(2):215-22.

79. Shiomi T, Nishii T, Tamura S, Tanaka H, Murase K, Yoshikawa H, et al. Influence of medial meniscectomy on stress distribution of the femoral cartilage in porcine knees: a 3D reconstructed T2 mapping study. Osteoarthritis Cartilage 2012;20(11):1383-90.

80. Song Y, Greve JM, Carter DR, Koo S, Giori NJ. Articular cartilage MR imaging and thickness mapping of a loaded knee joint before and after meniscectomy. Osteoarthritis Cartilage 2006;14(8):728-37.

81. Kazemi M, Li LP, Buschmann MD, Savard P. Partial meniscectomy changes fluid pressurization in articular cartilage in human knees. J Biomech Eng 2012;134(2):021001.

82. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP,et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum 2006;54(5):1529-35.

83. Riordan EA, Frobell RB, Roemer FW, Hunter DJ. The health and structural consequences of acute injuries involving rupture of the anterior cruciate ligament. Rheum Dis Clin N Am 2013;39:107-122.

84. Eckstein F, Wirth W, Hudelmaier MI, Maschek S, Hitzl W, Wyman BT, et al. Relationship of compartment-specific structural knee status at baseline with change in cartilage morphology: a prospective observational study using data from the osteoarthritis initiative. Arthritis Res Ther. 2009;11(3):R90.

85. Hunter DJ, Zhang W, Conaghan PG, Hirko K, Menashe L, Li L,et al. Systematic review of the concurrent and predictive validity of MRI biomarkers in OA. Osteoarthritis Cartilage. 2011;19(5):557-88.

86. Owman H, Tiderius CJ, Neuman P, Nyquist F, Dahlberg LE. Association between findings on delayed gadolinium enhanced magnetic resonance imaging of cartilage and future knee osteoarthritis. Arthritis Rheum 2008;58(6):1727-30.

87. Pan J, Pialat JB, Joseph T, Kuo D, Joseph GB, Nevitt MC, et al. Knee cartilage T2 characteristics and evolution in relation to morphologic abnormalities detected at 3-T MR imaging: a longitudinal study of the normal control cohort from the Osteoarthritis Initiative. Radiology 2011;261(2):507-15.

88. Hunter DJ. Lower extremity osteoarthritis management needs a paradigm shift. Br J Sports Med 2011;45(4):283-8.

89. Kon E, Filardo G, Drobnic M, Madry H, Jelic M, van Dijk N, et al. Non-surgical management of early knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2012;20(3):436-49.

90. Li YL, Ning GZ, Wu Q, Wu QL, Li Y, Hao Y, et al. Single-bundle or double-bundle for anterior cruciate ligament reconstruction: A meta-analysis. Knee 2013; http://dx.doi.org/ 10.1016/ j.knee. 2012.12.004.

91. Gomoll AH, Filardo G, de Girolamo L, Espregueira-Mendes J, Marcacci M, Rodkey WG, et al. Surgical treatment for early osteoarthritis. Part I: cartilage repair procedures. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):450-66.

92. Gomoll AH, Filardo G, Almqvist FK, Bugbee WD, Jelic M, Monllau JC, et al. Surgical treatment for early osteoarthritis. Part II: allografts and concurrent procedures. Knee Surg Sports Traumatol Arthrosc 2012;20(3):468-86.

93. Webster KE, Feller JA, Wittwer JE. Longitudinal changes in knee joint biomechanics during level walking following anterior cruciate ligament reconstruction surgery. Gait Posture 2012;36(2):167-71.

94. Winiarski S, Czamara A. Evaluation of gait kinematics and symmetry during the first two stages of physiotherapy after anterior cruciate ligament reconstruction. Acta Bioeng Biomech 2012;14(2): 91-100.

95. Risberg MA, Moksnes H, Storevold A, Holm I, Snyder-Mackler L. Rehabilitation after anterior cruciate ligament injury influences joint loading during walking but not hopping. Br J Sports Med 2009;43(6):423-8.

96. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum 2005;52(11):3507-14.

97. Decker MJ, Torry MR, Noonan TJ, Sterett WI, Steadman JR. Gait retraining after anterior cruciate ligament reconstruction. Arch Phys Med Rehabil 2004;85(5):848-56.

98. Bennell KL, Kean CO, Wrigley TV, Hinman RS. Effects of a modified shoe on knee load in people with and without knee osteoarthritis. Arthritis Rheum 2013;65(3):701-9.

99. J. Bedson, P.R. Croft. The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. BMC Musculoskelet Disord 2008;9:116.

SUPPLEMENTARY MATERIAL

Appendix 1 - Search Strategies

Pubmed All terms were searched in [All Fields], next to –if applicable- [MeSh] **MeSH terms**	(**Osteoarthritis, knee** OR **knee osteoarthritis** OR **knee osteoarthritides** OR **chronic disease** OR **chronic diseases** OR **disease progression** OR **disease progressions** OR gonarthrosis OR osteoarthrosis OR degenerative arthrosis OR post-traumatic osteoarthritis OR secondary osteoarthritis OR **cartilage** OR **cartilage, articular** OR cartilage degeneration OR cartilage deterioration OR cartilage defect OR cartilage defects OR **joint disease** OR **joint diseases** OR **cartilage disease** OR **cartilage diseases**) AND (**anterior cruciate ligament reconstruction** OR **anterior cruciate ligament/surgery** OR anterior cruciate ligament repair OR anterior cruciate ligament operation OR anterior cruciate ligament plasty OR **anterior cruciate ligament/injuries** OR ACL injury OR ACL injuries OR ACL reconstruction OR ACL repair OR ACL surgery OR ACL operation OR ACL plasty)
SportDiscus – CINAHL – Biomedical Reference Collection: comprehensive (EbscoHost-version) Biomed Central Scirus	(Osteoarthr* knee OR knee osteoarthr* OR chronic disease* OR disease progression* OR gonarthr* OR osteoarthr* OR degenerative arthr* OR post-traumatic osteoarthr* OR secondary osteoarthr* OR cartilage OR cartilage, articular OR cartilage degeneration OR cartilage deterioration OR cartilage defect* OR joint disease* OR cartilage disease*) AND (anterior cruciate ligament reconstruction OR anterior cruciate ligament surgery OR anterior cruciate ligament repair OR anterior cruciate ligament operation OR anterior cruciate ligament plasty OR anterior cruciate ligament injur* OR ACL injur* OR ACL reconstruction OR ACL repair OR ACL surgery OR ACL operation OR ACL plasty)
Web of Science	1. Focus on cartilage quality TS=(Osteoarthr* knee OR "knee osteoarthr*" OR "chronic disease*" OR "disease progression*" OR gonarthr* OR osteoarthr* OR "degenerative arthr*" OR "post-traumatic osteoarthr*" OR "secondary osteoarthr*" OR cartilage OR cartilage, articular OR "cartilage degeneration" OR "cartilage deterioration" OR "cartilage defect* "OR "joint disease*" OR "cartilage disease*") AND TI=(osteoarthr* knee OR "knee osteoarthr*" OR gonarthr* OR "degenerative arthr*" OR "post-traumatic osteoarthr*" OR "secondary osteoarthr*" OR cartilage OR cartilage, articular OR "cartilage degeneration" OR "cartilage deterioration" OR "cartilage defect* "OR "joint disease*"OR "cartilage disease*") 2. Focus on ACL reconstruction TS=("anterior cruciate ligament reconstruction" OR "anterior cruciate ligament surgery" OR "anterior cruciate ligament repair" OR "anterior cruciate ligament operation" OR "anterior cruciate ligament plasty" OR "anterior cruciate ligament injur*" OR "ACL injur*" OR "ACL reconstruction" OR "ACL repair" OR "ACL surgery" OR "ACL operation" OR "ACL plasty") AND TI=("anterior cruciate ligament reconstruction" OR "anterior cruciate ligament

	surgery" OR "anterior cruciate ligament repair" OR "anterior cruciate ligament operation" OR "anterior cruciate ligament plasty" OR "anterior cruciate ligament injur*" OR "ACL injur*" OR "ACL reconstruction" OR "ACL repair" OR "ACL surgery" OR "ACL operation" OR "ACL plasty")

Appendix 2. Criteria quality appraisal: three composites of Total Quality Score (TQS).

Criteria	Answer	Remarks criteria qualifications
General: Reporting outcomes, external validity, internal validity[1]		
Reporting		
1. Is the hypothesis/aim/objective clearly described?	Y/N	
2. Are the main outcomes to be measured clearly described in the introduction or methods section?	Y/N	
3. Are the main characteristics of the patients included in the study clearly described?	Y/P/N	Should at least include: number of patients, gender, age, BMI. If *all* are described "Yes", if *none* are described "No", if *some but not all* are described "Partially".
4. Are the distributions of principal confounders clearly described?	Y/P/N	Age, gender, BMI, physical activity level, concomitant injuries (if applicable) , different grafts used (if applicable).
5. Are the main findings of the study clearly described?	Y/N	
6. Does the study provide numerical estimates of random variability in the data for the main outcomes?	Y/N	E.g., inter-quartile range, standard error, standard deviation, confidence interval.
7. Have the characteristics of the patients lost to follow-up been described?	Y/N	Should be answered "Yes" where there were no losses to follow-up or losses to follow-up were so small findings would be unaffected by their inclusion (i.e., response rate ≥80%). Should be answered "No" where study did not report losses to follow-up.
8. Have actual probability values been reported for the main outcomes except where probability is less than 0.001?	Y/N	
External validity		
9. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	Y/N/U	Must identify source of patient population and describe how patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample.
Internal validity		

No.	Question	Answer	Notes
10.	Do the analysis adjust for different lengths of follow-up of patients?	Y/N/U	When follow-up was the same for all study patients, or different lengths were adjusted for, answer "Yes". Studies where differences in follow-up are ignored should be answered "No".
11.	Were the statistical tests used to assess the main outcomes appropriate?	Y/N/U	
12.	Were main outcome measures used accurate (valid and reliable)?	Y/N/U	
13.	Were all study subjects recruited from the same population?	Y/N/U	E.g., comparison of groups recruited from the same hospital
14.	Were study subjects recruited over the same period of time?	Y/N/U	
15.	Was there adequate adjustment for confounding in the analysis from which main findings were drawn?	Y/N/U	If the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses, the question should be answered as "No".
16.	Were losses of patients to follow-up taken into account?	Y/N/U	If no loss to follow-up reported, the question should be answered as "Unable to determine". If the proportion loss to follow up was too small to affect the main findings, the question should be answered as "Yes".

Power

No.	Question	Answer	Notes
17.	Did the study perform a power analysis to have sufficient power to detect a clinically important effect where the probability value for a difference being due to change is less than 5%?	Y/N/U	

Field-specific methodological features - MRI acquisition and image-analysis: Reporting and internal validity$

Reporting

No.	Question	Answer	Notes
18.	In case of quantitative morphological or compositional imaging, were loading conditions of the knee during or prior to imaging described?	Y/N	e.g., period of rest or unloading, traction.
19.	Were magnetic field strength, scanner and coil type clearly described and appropriate?	Y/P/N	If adequately described and appropriate, the questions should be answered "Yes", if partially described, answer "Partially", if not reported or inadequate, answer "No". Appropriate= at least 1,0T with consistent use of field strength at follow-up.
20.	Were imaging sequence and parameters/technique clearly described and appropriate?	Y/P/N	Appropriate=appropriate choice of sequence and consistent between consecutive evaluation time points
21.	Were anatomic regions/sub-regions clearly described?	Y/N	
22.	Was a detailed, clear and appropriate description provided on how quantitative parameters were calculated? In case of semi-	Y/P/N	In case of adequate referral, the question should also be answered "Yes".

121

qualitative scoring systems, were different grades clearly reported?

Internal validity

23.	Were longitudinal data read in pairs and were readers blinded to sequence acquisition in view of follow-up? OR Were longitudinal data read ad random and blinded to subject ID?	Y/P/N/U — If the article does not provide information to answer, choose "unable to determine".
24.	Were measures of precision or reproducibility for image acquisition and/or post-processing analysis mentioned?	Y/N — E.g., CV or RMS CV
25.	Was number of readers, level of experience and measure of reliability of reader intervention described?	Y/P/N

Field-specific methodological features – Clinical considerations:
Reporting$^¥$

Reporting

26.	Was rehabilitation clearly described?	Y/P/N — "Partially" means that rehabilitation is only mentioned without without time-bound and/or exercise prescription mentioned nor referred to.
27.	Was graft use and surgical technique clearly described?	Y/P/N — "Partially" means that only graft use or name of technique is mentioned without more detailed description of surgical description.
28.	Was number of surgeons involved clearly described?	Y/N
29.	Was management of concomitant injuries described?	Y/N

¶ Criteria and qualifications adapted from Black and Downs [22] $ Criteria based on Eckstein et al. [23] (2006). ¥ Criteria 26,27, 29 derived/adapted from the Coleman methodology [24]. Questions 1-2,5-8,18,21,24, 28-29: "Y/Yes"= score 1, "N/No"=score 0. Questions 3-4,25-27: "Y/Yes"=score 2, "P/Partially"=score 1, "N/No"=score 0. Questions 9-17: "Y/Yes"= score 1, "N/No"=score 0, "U/Unable to determine"=score 0. Questions 19-20, 22-23: "Y/Yes"=score 2, "P/Partially"=score 1, "N/No"=score 0, "U/Unable to determine"=score 0.

Appendix 3. Quality appraisal of included eligible studies (n = 12)

CRITERIA	Weninger (2008)	Li (2011)	Lee (2013)	Costa-Paz(2001)	Faber (1999)	Frobell (2009)	Frobell (2011)	Potter (2012)	Neuman (2011)	Hosseini (2012)	Theologis (2011)	Arnoldi (2011)
Aim/hypothesis/objective	1	1	1	1	1	1	1	1	1	1	1	1
Main outcomes	1	1	1	1	1	1	1	1	1	1	1	1
Patient charactistics	1	1	1	1	1	1	2	1	2	1	2	1
Distribtuion principal confounders	1	2	1	1	1	1	2	1	2	1	1	0
Main findings	1	1	1	1	1	1	1	1	1	1	1	1
Numerical estimates random variability	1	1	0	0	1	1	1	1	1	1	0	1
Patient characteristics lost to follow-up	1	1	1	1	1	1	1	1	1	1	1	1
Actual P-values	0	1	0	0	1	0	1	1	0	1	0	0
Representative subjects	1	0	0	0	0	0	1	0	0	0	0	0
Adjusted analysis for lenght follow-up	0	1	0	0	1	1	1	1	1	1	1	0
Appropriate statistics	0	1	0	0	1	1	1	1	1	1	1	0
Accuracy methods	1	1	0	0	0	1	1	1	1	1	1	1
Recruited from same population	1	0	1	0	0	0	0	0	0	1	1	0
Recruited within same time period	1	1	1	0	0	1	1	1	1	0	0	0
Confounder-adjusted analysis	0	1	0	0	0	1	1	1	1	0	0	0

123

Loss to follow-up accounted for	1	1	1	1	1	1	1	1	1	1	1	1
Power analysis	0	0	0	0	0	0	0	0	0	0	0	0
Sub-score general(%)	63,2	78,9	52,6	36,8	52,6	68,4	89,5	73,7	84,2	68,4	63,2	42,1
Pre-imaging loading conditions	NA	0	NA	0	0	0	0	0	0	0	0	1
MRI equipment	1	2	0	0	0	2	2	2	2	2	2	1
Imaging acquisition/technique	1	2	1	1	1	2	2	2	2	1	2	2
Anatomic regions	0	1	1	1	1	1	1	1	1	1	1	1
Methodology derivation of MRI parameters	2	2	2	0	0	2	2	2	2	2	2	1
Blinding	0	0	0	0	0	0	0	1	0	0	0	0
Precision MRI measures	0	0	1	0	0	0	0	0	1	0	0	1
Reader number, experience, consistency	0	2	1	1	1	1	1	1	0	0	0	1
Sub-score MRI(%)	33,3	69,2	50	23,1	15,4	61,5	61,5	69,2	61,5	46,2	53,8	69,2
Rehabilitation	2	2	0	0	2	2	1	1	1	0	1	0
Graft use/surgical technique	1	1	2	1	1	1	1	1	0	2	0	0
Number surgeons	0	0	0	0	0	1	1	0	0	1	1	0
Management concomitant Injury	1	1	1	1	1	1	1	1	1	1	1	0
Sub-score clinical(%)	66,7	83,3	50	33,3	66,7	83,3	83,3	50	33,3	66,7	50	0
RELATIVE TQS (%)	54	76,3	51,3	31,6	44,7	68,4	78,9	68,4	68,4	61,5	57,9	44,7

Low quality (score < P50), High quality (score>P50), P50=59.7%.TQS:Total Quality Score

Appendix 4. Extracted data surgical characteristics and outcome.

Surgical characteristics reported in the included studies (n=12)

Authors	Average surgical delay (days)	Graft use (% reconstructions)	Single/multiple surgeon	Surgical technique
Faber (1999)	12	H (100)	NR	NR
Costa-Paz (2001)	60	BPTB (100)	NR	NR
Weninger (2008)	57.4	H (84.4) BPTB (13.3) Q (2.2)	NR	NR
Frobell (2009)	43	BPTB (44) H (56)	Multiple	NR
Arnoldi (2011)	NR	NR	NR	NR
Frobell (2011)	Early: 44.5 Late: 408	BPTB (50) H (50)	Multiple	NR
Li (2011)	NR	H (50) TP (33.3) A (16.7)	NR	NR
Neuman (2011)	144	NR	NR	NR

Study				
Potter (2012)	NR	BPTB (71,4)	NR	NR
		H (17.9)		
		A (10,7)		
Theologis (2011)	56	NR	Single	NR
Hosseini (2012)	135	BPTB (100)	Single	Trans-tibial
Lee (2013)	39	H (100)	NR	Anatomic double bundle

NR: Not Reported. H: Hamstrings;BPTB: Bone Patellar Tendon Bone; Q: Quadriceps tendon; TP: Tibialis Posterior; A: Achilles tendon.

Surgical outcomes after ACL-reconstruction reported in the included studies at baseline and follow-up compared to controls (n=12)

Authors	Laxity		Patient-reported		Peformance-based function		Rate return to sports	Comparison to Controls
	B	FU	B	FU	B	FU		
Faber (1999)	-	KT-1000	-	Mothadi Quality of Life measure	NR	NR	NR	ACL reconstructed un-injured cartilage KT-1000: = Mothadi Quality of Life: = -
Costa-Paz (2001)	-	KT-1000 Pivot shift	-	IKDC	NR	NR	NR	-
Weninger (2008)	-	Radiographic Lachman	-	Lysholm IKDC	-	1-legged hop	62% pre-injury level 16% restricted 4% no return	Contra-lateral intact knee Lachman: = Lysholm: = 1-legged hop: =
Frobell (2009)	NR	NR	Tegner	-	NR	NR	NR	Nonsurgical patients*
Arnoldi (2011)	-	KT-1000	-	Lysholm Tegner OAK	NR	NR	56% light labour/recreational sports	Contra-lateral intact knee KT-1000: ↑
Frobell (2011)	NR	NR	Tegner	Tegner↓	NR	NR	NR	Nonsurgical patients*
Li (2011)	NR	NR	NR	NR	NR	NR	NR	Healthy control subjects
Neuman (2011)	NR	NR	Activity Level	Activity Level =	NR	NR	NR	Nonsurgical patients Activity level: =
Potter (2012)	NR	NR	IKDC ADL SF-36 ARS NR	Lysholm IKDC↑ ADL= SF-36= ARS↓ NR	NR	NR	NR	Nonsurgical patients ARS: =
Theologis (2011)	NR	NR	NR	NR	NR	NR	NR	-
Hosseini (2012)	KT-1000	KT-1000↓	NR	NR	NR	NR	NR	Contra-lateral intact knee KT-1000: =
Lee (2013)	NR	NR	NR	NR	NR	NR	NR	-

B: Baseline. FU: Follow-up. IKDC: International Knee Documentation Committee; OAK: Orthopädische Arbeitsgruppe Knie; ARS: Activity Rating Scale; ADL: knee outcome score Activities of Daily Living; SF-36: Short Form 36-Item (RAND) questionnaire. NR: "Not Reported". "=" no (significant) difference when compared to baseline or controls. "↑/↓": (significant) increase/decrease when compared to baseline or controls. * Outcome not separately reported for ACL-reconstructed patients in this cohort.

CARTILAGE STATUS IN RELATION TO RETURN TO SPORTS AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

ABSTRACT

Background: Osteoarthritis after anterior cruciate ligament (ACL) reconstruction receives much attention in orthopaedic science. Anterior cruciate ligament reconstruction is related to increased joint fluid volumes, bone marrow edema, and cartilage biochemical and morphological changes believed to cause fragile joint conditions. These joint conditions may not be able to adequately counter the imposed loads during sports.

Hypothesis: At 6 months after surgery, knee cartilage displays inferior quality in ACL-reconstructed knees when compared with controls. This inferior quality is influenced by the time to return to sports and/or by the time to surgery.

Study Design: Cross-sectional study; Level of evidence, 3.

Methods: Fifteen patients treated with isolated ACL reconstruction were compared with 15 matched controls. In all participants, a 3-T magnetic resonance imaging cartilage evaluation was performed entailing quantitative morphological characteristics (3-dimensional volume/thickness), biochemical composition (T2/T2* mapping), and function (after a 30-minute run: *in vivo* deformation including recovery). Nonparametric statistics were executed reporting median (95% CI).

Results: No volume and thickness between-group differences existed. In patients, medial femur (FM) T2 was higher (45.44 ms [95% CI, 40.64-51.49] vs 37.19 ms [95%CI, 34.67-40.39]; *P* = .028), whereas T2* was lower in the FM (21.81 ms [95%CI, 19.89-22.74] vs 24.29 ms [95%CI, 22.70-26.26]; *P* = .004), medial tibia (TM) (13.81 ms [95%CI, 10.26-16.78] vs 17.98 ms [95% CI, 15.95-18.90]; *P* = .016), and lateral tibia (TL) (14.69 ms [95% CI, 11.71-16.72] vs 18.62 ms [95 % CI, 17.85-22.04]; *P*<.001). Patients showed diminished recovery at 30 minutes after a 30-minute run in the FM (−1.60%[95%CI, −4.82 to −0.13] vs 0.01% [95%CI, −0.34 to 1.23]; *P* = .040) and at 30 (−3.76%[95%CI, −9.29 to −1.78] vs 0.04% [95%CI, −1.52 to −0.72]; *P* = .004) and 45 minutes after exercise (−1.86% [95%CI, −4.66 to −0.40] vs 0.43%[95%CI, −0.91 to 0.77]; *P* = .024) in the TL. Eight patients returned to sports at 6 months or earlier. Return before 5 months (3/8 patients) was associated with increased cartilage thickness (in TM, TL, and lateral femur [FL]), deformation (in FL), and delayed recovery after running (in FL and FM). Median surgical delay was 10 weeks (range, 5-17 weeks). Surgery within 10 weeks (9/15 patients) was also associated with delayed cartilage recovery (in FL and FM). For the other parameters, no significant relationships could be established for either return to sports or surgical delay.

Conclusion: At 6 months after surgery, cartilage in patients with ACL reconstruction shows diminished quality and *in vivo* resiliency compared with controls. Caution is advised in an early return to sports especially when dealing with patients who received prompt surgery. Possibly, high impacts on this qualitatively diminished cartilage might play a role in the development of osteoarthritis in ACL reconstruction. Replication in larger samples and follow-up are warranted.

INTRODUCTION

Osteoarthritis (OA) after anterior cruciate ligament (ACL) reconstruction is a comorbidity receiving much attention in orthopaedic science. Pivoting sports are considered "high risk" for the incidence of ACL ruptures.[18] According to a review by Myklebust and Bahr,[18] treatment with ACL reconstruction effects a return to sports in 65% to 88% of athletes compared with 19% to 82% enrolled in nonoperative treatments.

Postoperative sports participation, however, might not be tailored to ongoing structural remodeling of the traumatized knee. Surgery within 6 weeks after injury showed increased joint fluid volumes combined with slowly resorbing bone marrow lesions (BMLs) and cartilage morphological changes during the first year. Hence, these signs depict precarious joint conditions that might not be able to counter the excessive torsional loads that the knee would be subjected to when returning to strenuous activities.[8,9] Interestingly, in a 6-year prospective study, Keays et al[14] found that type or level of postoperative sports played was not a predictor for the incidence of radiographic OA after ACL reconstruction. In cases of no further sustained chondral or meniscal damage requiring meniscectomy, pursuing athletic activities was suggested as not accelerating joint deterioration. Surgical delay (mean, 40.59 months), however, was in this particular study much longer and related to OA development, whereas the time to return to play was not reported or accounted for in the analysis. In cases of an average 3-month surgical delay, Hoffelner et al[11] concluded that patients, who all returned to competitive sports within 8 months after isolated ACL surgery, showed no increased OA risk at 10 years' follow-up. Osteoarthritis was detected in 36% of operated knees; however, no significant differences could be attained after cross-sectional within-patient comparison.

To date, it is agreed that surgical delay determines the presence of concomitant injury on arthroscopic evaluation, affecting the risk for OA progression.[10] Surgery is proposed to be performed within the first year, whereas a delay of over 6 months was even put forward as a risk factor for OA development.[5,10,23] In athletes wishing to return to pivoting sports, reconstruction is advised to be performed within 4 to 8 weeks in cases of absent joint swelling and full range of motion.[18] Also, in cases of surgery within 6 weeks, ACL-reconstructed knees were suggested to benefit from a longer recovery in view of the morphological sequellae that the joint seemed to endure after surgery.[8,9] Consequently, the impact of return to sports and surgical delay on future joint deterioration remains enigmatic.

In clinical practice, most patients are usually discharged from rehabilitation and attempt a full return to leisure or athletic activities at an average of 6 months after surgery.[16] Therefore, the purpose of this study was 2-fold. First, a comprehensive evaluation of cartilage status was undertaken in ACL-reconstructed patients 6 months after surgery and compared with matched control patients. Using 3-T magnetic resonance imaging (MRI), the cartilage evaluation entailed

in vivo quantitative morphological characteristics, biochemical composition, and function (ie, tissue resiliency). Second, within patients, the role of time to return to sports and/or surgical delay on cartilage status was explored. It was hypothesized that knee cartilage would display inferior quality when compared with controls. An early return to sports and/or surgery (ie, too soon[8,9]) was hypothesized to contribute to a worse cartilage outcome at 6 months after surgery. These results may have implications on fine-tuning rehabilitation strategies and surgical planning.

MATERIALS AND METHODS

Patients

Fifteen patients (8 men, 7 women) who underwent ACL reconstructive arthroscopic surgery were recruited from an experienced orthopaedic surgeon's practice. All patients received surgery between April 2010 and April 2011. Eligibility to participate was evaluated based on arthroscopic evaluation and standard questionnaires. Inclusion criteria were (1) 20 to 40 years of age to reduce the risk of cartilage degeneration associated with ageing, (2) body mass index (BMI) of 20 to 30, (3) regular sports participation before injury, (4) isolated ACL reconstruction using hamstring tendon autografts, and (5) being able to run 30 minutes. Exclusion criteria were (1) any cartilage lesion present on arthroscopic evaluation or diagnosed before injury as the latter might have evolved into degenerative changes regardless of the incident, (2) concomitant meniscectomy or meniscal sutures, (3) concomitant collateral ligament injuries ≥grade 2, (4) history of knee injury and/or surgery, (5) MRI contraindications, and (6) other joint and/or bone lesions. In all patients, anatomic ACL reconstruction was performed using a double semitendinosus and gracilis autograft with a cortical suspension system (Endobutton CL, Smith & Nephew, Andover, Massachusetts) on the femoral side and use of a metal interference screw (RCI, Smith & Nephew) and staple on the tibial side. Briefly, anatomic position was achieved with the tibial tunnel in the center of the ACL remnant and through the anteromedial portal for the femoral tunnel. The grafts were fixed with the knee in 30° of flexion. After surgery, all patients enrolled in a structured 4- to 6-month rehabilitation program.

Fifteen control participants were recruited from the community or university campus. Eligibility was verified using standard questionnaires. Control participants were matched to the patients by sex, age, BMI, sports participation (ie, type of sports and training volume as determined by the Baecke sport index[2]), and dominance of the limb to be investigated. Additionally, all controls were able to run 30 minutes. Exclusion criteria were (1) a history of knee injury including cartilage defects and/or surgery, (2) known bone and/or joint pathological abnormality including clinical or MRI-related signs of OA at the time of the study, and (3) MRI contraindications.

Informed consent forms ratified by the local ethics committee were obtained from all patients. Patient demographics are listed in Table 1.

Experimental Procedures

As the patients were evaluated 6 months after surgery, experimental procedures took place from October 2010 until November 2011. All patients were instructed not to practice sports the day before testing or on the testing day and to avoid running, lifting heavy weights, and taking stairs 4 hours preceding the actual experimental procedures. The procedures were performed on campus and occurred at the same time of day for all patients.[27] The protocol comprised (1) MRI evaluation of cartilage status including *in vivo* quantitative morphological characteristics (ie, 3-dimensional [3D] volume/thickness), biochemical composition (ie, T2/T2* mapping), and function (ie, deformational behavior including recovery); (2) evaluation of lower limb function and knee laxity; and (3) questionnaires.

MRI Evaluation of Cartilage Status: 3D Morphology, Biochemical Composition, and Function

High-resolution images of knee cartilage morphology (ie, sagittal 3D double echo steady state sequence with fat suppression by water excitation [DESS WE]) and baseline biochemical T2/T2* maps (T2/T2* MapIt, Siemens Medical Solutions, Erlangen, Germany) were obtained using a dedicated 8-channel knee coil on a 3-T Trio Tim magnet (Siemens Medical Solutions). Cartilage deformation and recovery were registered by means of monitoring the changes in cartilage morphological characteristics before and after an *in vivo* weightbearing exercise.[27] Upon diagnosis of the ACL rupture and at 6 months after surgery, an Intermediate (IM)-weighted fat-saturated turbo spin echo (TSE) sequence was included to determine the presence of BMLs.

The sagittal 3D DESS WE sequence implemented a partition thickness of 0.69 mm, echo time/repetition time [TE/TR] of 4.7/16.44 milliseconds, flip angle [FA] of 25°, field of view [FOV] of 140 mm, matrix of 384 pixels (in-plane resolution: 0.36x0.36), and acquisition time (TA) of 6'16". The T2 map, a sagittal multiecho spin echo sequence, centered on the medial and lateral knee compartment implemented a partition thickness of 3 mm; TE/TR of 13.8,27.6, 41.4, 55.2, and 69.0/1000 milliseconds; FA of 180°; FOV of 159 mm; matrix of 384 pixels (in-plane resolution:0.42x0.42); and TA of 3'28". The T2* map, a sagittal multiecho gradient echo sequence, centered on the medial and lateral knee compartment implemented a partition thickness of 3 mm; TE/TR of 4.18, 11.32, 18.46, 25.60, and 32.47/422 milliseconds; FA of 60°; FOV of 159 mm; matrix of 384 (interpolated 768x768) pixels (in-plane resolution: 0.21x0.21);

and TA of 2'42". The IM-weighted TSE sequence implemented a partition thickness of 3 mm, TE/TR of 44/3140 milliseconds, FA of 160°, FOV of 134x179 mm, matrix of 448x218 pixels (in-plane resolution: 0.40x0.40), and TA of 2'34".

To reduce interference from residual deformation preceding the experiment, the MRI protocol started with 1 hour of physical rest during which the participants were positioned supine.[15,27] After the patient had rested, baseline scans (ie, tpre: sagittal 3D DESS WE, T2 and T2* maps, IM-weighted TSE) were performed followed by the weightbearing exercise under study. Within a maximum of 2 minutes after exercise cessation,[15] the first postscans (ie, tpostt0: sagittal 3D DESS WE) were started and repeated with 15-minute intervals up to 45 minutes after the exercise (ie, tpostt15, tpostt30, and tpostt45: sagittal 3D DESS WE) (total of 4 scans).[27] "Deformation" is expressed as the morphological change measured at tpostt0 relative to baseline (ie, [(3D morphology tpostt0 – 3D morphology tpre)/3D morphology tpre 3 100]). Morphological changes measured at tpostt15, tpostt30, and tpostt45 relative to baseline are attributed to "recovery."[27] The sequence of events is displayed in Figure 1.
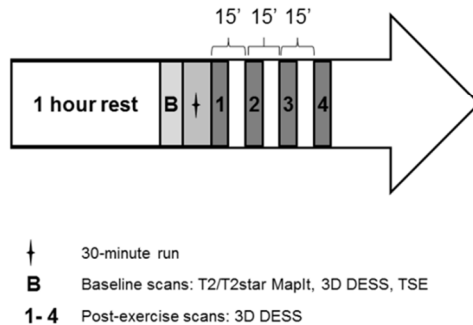

Figure 1. Schematic overview of the sequence of events during the magnetic resonance imaging experimental protocol. Adapted from Van Ginckel et al.27 B, baseline scans (tpre); 1-4, postexercise scans (tpostt0, tpostt15, tpostt30, and tpostt45: postexercise scans started within a maximum of 2 minutes after cessation of exercise [tpostt0] and repeated at 15, 30, and 45 minutes after onset of tpostt0, respectively).

Table 1. Group characteristics. Demographics, Symptoms and Function of ACL-reconstructed patients versus controls[a]

	Patients (N=15)	Controls (N=15)	P-value
Demographics			
BMI (kg/m²)	25.06(23.05;25.53)	23.48(21.91;25.62)	0.49
Age (yrs)	26.75(23.37;31.70)	27.32(22.10;33.30)	0.95
Symptoms and Function			
LSI (%)	86.91(74.78;98.84)	103.21(89.90;110.08)	0.010*
KOOS pain	58(47.22;69.44)	75(75;75)	<0.001*
KOOS other symptoms	50(46.43;64.29)	71.43(67.86;75)	<0.001*
KOOS quality of life	31.25(25;43.75)	75(75;75)	<0.001*
KOOS sports and recreation	40(25;65)	75(65;75)	<0.001*
KOOS ADL	69.12(66.18;73.53)	75(75;75)	<0.001*
Lysholm	82(75;87)	99(90;100)	<0.001*
VAS pain last week (/10)	1(0;3)	0(0;0)	<0.001*
FORRS	36(28;52)	34(26;52)	0.83
Baecke work index	2.25(2;3.13)	2(1.63;2.63)	0.08
Baecke sports index	3(2.5;3.75)	3.5(2.75;4)	0.15
Baecke leisure index	3(2.5;3.5)	3(2.25;3.5)	0.69
RAND-36 physical function	85(75;90)	100(100;100)	<0.001*
RAND-36 social function	100(87.5;100)	100(100;100)	0.05
RAND-36 role limitations phys. health	100(75;100)	100(100;100)	0.07
RAND-36 role limitations em. health	100(100;100)	100(100;100)	0.55
RAND-36 em. well-being	84(76;84)	88(80;92)	0.06
RAND-36 energy/fatigue	75(65;85)	75(65;85)	0.96
RAND-36 pain	89.75(77.55;89.80)	89.90(83.67;100)	0.040*
RAND-36 general health	80(70;90)	85(75;90)	0.50

[a] Values are presented as medians (95% Confidence Interval). ACL: anterior cruciate ligament BMI: Body Mass Index, LSI: Lower Limb Symmetry Index = [(average score operated leg- average score nonoperated leg)/100] corresponding legs in controls, KOOS: Knee Outcome Osteoarthritis Score, FORSS: Factor Occupational Rating System Scale, VAS: Visual Analogue Scale, RAND-36 role limitations phys. health: RAND-36 role limitations physical health, RAND-36 role limitations em. health: RAND-36 role limitations emotional health, RAND-36 em. well-being: RAND-36 emotional well-being. *Significant at α<0.05.

The weightbearing exercise consisted of a 30-minute run on a predefined track on campus. Participants ran at a self-selected comfortable pace during which running speed (km/h), distance (km), and step count were recorded. To standardize the cushioning properties of footwear, all patients wore the same type of neutral running shoe during the experiment (Ekiden 50; Kalenji, Villeneuve D'ascq, France). After running, all patients provided a visual analog scale (VAS) score for knee pain experienced during the exercise (on a 10-cm scale: 0 cm representing ''no pain at all'' and 10 cm representing ''extremely painful''). In view of the active patient population under study, running was preferred as it is a basic component in (late) postoperative rehabilitation and in many sports activities. A 30-minute run was adequate to allow for cartilage changes to be detected using MRI.[21,25] Tibial plates have been shown to recover within 60 minutes after a 20-km run, providing a rationale to repeat postscans up to $t_{postt}45$.[15]

Evaluation of Lower Limb Function and Knee Laxity

Functional performance was evaluated using the single-legged hop test for distance, and lower limb symmetry index (LSI) values were calculated. Anteroposterior knee laxity and side-to-side differences were quantified using the Genourob device (Genourob, Laval, France) with increasing loads (67, 89, 134, 150, and 250 N).

Questionnaires

All patients completed a Baecke questionnaire quantifying general physical activity scores based on a work index, sport index, and leisure index[2]; Tegner scale for current activity level; Factor Occupational Rating System Scale (FORSS)[19]; Knee injury and Osteoarthritis Outcome Score (KOOS); Lysholm knee score; and RAND 36-Item Health Survey. Within the patient group, additional data were collected concerning the injury and operation date, injury event (ie, activity during incident), VAS score for the amount of pain experienced during the last week (0 representing ''no pain at all'' and 10 representing ''extremely painful''), and return to sports at 6 months after surgery (If ''yes'': [1] What kind of sports is practiced at what weekly frequency? [2] When did you return to sports?). According to Keays et al,[14] postsurgery sports were allocated to 3 categories: ''safe,'' ''low risk,'' and ''high risk.'' Reasons for no return were also collected.

Data Analysis

Image Analysis: 3D Morphology and T2/T2 Quantification*

Three-dimensional reconstruction, volume calculation, surface area calculation, and model registration were performed using a commercial modeling software package (Mimics, version 14.0, Materialise NV, Leuven, Belgium).[27]

Three-dimensional DESS image stacks were subsequently segmented to generate a 3D reconstruction of lateral/medial femoral/tibial cartilage (lateral femur [FL], medial femur [FM], lateral tibia [TL], medial tibia [TM]). A semiautomatic segmentation procedure was implemented based on a 3D LiveWire algorithm and a slice-by-slice manual correction to digitize cartilage plates by masking. Manual correction was preceded by a region growing algorithm to dispose of abundant voxels. Subsequently, 3D cartilage plates were reconstructed, and absolute 3D volumes (in mm^3) were calculated for baseline and postscans.[27] Normalized 3D cartilage volumes (ie, normalized to cartilage-bone interface area: [absolute 3D volume (mm^3)/cartilage-bone interface area (mm^2)]) were determined. Cartilage-bone interface area was defined as the area of the underlying bone surface in contact with the cartilage plate. By means of eroding morphological operations, surface areas were extracted from 3D reconstructions of the underlying bone and determined by means of surface triangulation.[27] Normalized 3D volumes are considered an equivalent measurement of cartilage thickness for the cartilage areas under study[13] and are referred to as ''thickness (in mm)'' in this article.

The T2/T2* relaxation times were derived from online reconstructed maps centered on the medial/lateral compartment using a pixel-wise, mono-exponential, nonnegative least squares fit analysis (MapIt, Siemens Medical Solutions). Regions of interest covering full-thickness cartilage were segmented, delineating the entire layers to calculate the global T2/T2*. Next to cartilage tissue, posterior horns of the menisci were segmented. Although cases treated with meniscal procedures were excluded, subclinical meniscal degeneration was considered important in view of cartilage health outcomes.[28,29] The 3D DESS WE images served as visual guidance.

All image analyses were performed by a single researcher with 3 years of experience at the time of analysis and who was blinded to the time sequence of scanning. Intratester reliability, precision errors for volume, and T2/T2* quantification were determined (see the Appendix, available online at http://ajs.sagepub.com/supplemental/).

The Shapiro-Wilk test revealed an overall nonparametric distribution ($P < .05$). Hence, nonparametric statistics were executed, and descriptive statistics were reported as median (95% confidence interval [CI]). To investigate differences between groups, the Mann-Whitney U or Kruskal-Wallis test was applied. To test whether cartilage morphology for all plates changed over time, the Friedman test for repeated measures was implemented. Post hoc pairwise comparisons were conducted using Wilcoxon signed-rank tests. P values were adjusted for multiple comparisons of main effects (''time'' or ''cartilage plate'') by applying Bonferroni corrections. Spearman ρ coefficients were calculated to investigate all correlations. Significant correlation coefficients of ≥0.5 were considered. Level of significance was set at a < .05; SPSS (version 20, IBM Statistics, Armonk, New York) was used for all analyses.

RESULTS

Group Characteristics: Demographics, Symptoms, Function, Preinjury Sports Participation, and Injury Event

No significant between-group differences were present for BMI ($P = .49$); age ($P = .95$); Baecke work, sport, and leisure index ($P = .08$, P = .15, and $P = .69$, respectively); FORSS ($P = .83$); and all RAND 36 items except physical function and pain (Table 1). The latter were significantly decreased in the patients ($P < .001$ and $P = .04$, respectively). Additionally, in patients, Lysholm knee scores ($P < .001$), KOOS values (all subitems, $P < .001$), and LSI values ($P = .010$) were significantly decreased when compared with controls. On VAS scores, patients reported no to mild knee pain during the last week before the study (median, 1 [95% CI, 0-3]). Median (95% CI) and P values are tabulated in Table 1.

Sports practiced before injury were soccer (5/15), outdoor running (5/15), climbing (1/15), horseback riding (1/15), inline skating (1/15), rugby (1/15), and volleyball (1/15). Injury events were soccer (6/15), alpine skiing (4/15), rugby (1/15), volleyball (1/15), and other outdoor activities (3/15).

Side-to-side differences in knee laxity in patients ranged from 1.60 mm (95% CI, −0.24 to 2.10), 1.64 mm (95% CI, −0.74 to 2.30), 1.60 mm (95% CI, −0.46 to 2.70),and 1.70 mm (95% CI, −0.57 to 2.90) to 2.36 mm (95% CI,−0.93 to 3.51) with increasing loads (67, 89, 134, 150, and 250 N, respectively).

Cartilage Status: 3D Morphological Characteristics, Biochemical Composition, and Function

No significant between-group differences could be established for baseline 3D volume and thickness in all plates (Table 2). Global T2 relaxation times in the FM were significantly higher in patients when compared with controls (45.44 ms [95% CI, 40.64-51.49] vs 37.19 ms [95% CI, 34.67-40.39], respectively; P = .028). No other significant between-group differences could be revealed. Global T2* relaxation times were significantly lower in patients when compared with controls in the FM (21.81 ms [95% CI, 19.89-22.74] vs 24.29 ms [95% CI, 22.70-26.26], respectively; P = .004), TM (13.81 ms [95% CI, 10.26-16.78] vs 17.98 ms [95% CI, 15.95-18.90], respectively; P = .016), and TL (14.69 ms [95% CI, 11.71-16.72] vs 18.62 ms [95% CI, 17.85-22.04], respectively; P < .001). No significant between-group differences could be shown for the FL. T2/T2* relaxation times did not differ between groups for the meniscal posterior horn in the medial and lateral compartment. Median (95% CI) T2 and T2* relaxation times and P values for all cartilage plates and menisci are displayed in Table 2.

Regarding cartilage function, none of the plates showed a significant between-group difference at deformation (tpostt0: P = .80, P = 1.00, P = 1.00, and P = 1.00 for FM, FL, TM, and TL, respectively). During recovery, the patient group's FM continued to exhibit significantly larger volume decreases at tpostt15 when compared with controls (−1.60% [95% CI, −4.82 to −0.13] vs 0.01% [95% CI, −0.34 to 1.23], respectively; P = .040). In the TL, the patients showed significantly larger volume decreases at tpostt30 (−3.76% [95% CI, −9.29 to −1.78] vs 0.04% [95% CI, −1.52 to −0.72], respectively; P = .004) and tpostt45 (−1.86% [95% CI, −4.66 to −0.40] vs 0.43% [95% CI,−0.91 to 0.77], respectively; P = .024). During recovery, no other significant between-group differences were found (Table 3).

Table 2. Cartilage and meniscal status. Baseline quantitative 3-dimensional morphology and biochemical composition in patients versus controls[a]

	Patients (N=15)	Controls (N=15)	*P*-value
Volume (mm³)			
FM	5025.76(4310.52;5963.46)	4669.68(3624.92;5694.33)	1.00
FL	5565.77(4632.14;6890.84)	5285.34(4144.82;6832.41)	1.00
TM	1991.77(1687;2126.26)	1906.99(1455.70;2295.19)	1.00
TL	2598.78(1778.36;2877.70)	2236.29(1865.06;2744.33)	1.00
Thickness (mm)			
FM	1.94(1.6;2.13)	1.76(1.6;1.93)	0.49
FL	1.96(1.65;2.17)	1.96(1.56;2.16)	1.00
TM	1.90(1.57;1.94)	1.78(1.48;1.9)	1.00
TL	2.24(2.14;2.41)	2.09(1.95;2.24)	0.80
Cartilage T2 (msec)			
FM	45.44(40.64;51.49)	37.19(34.67;40.39)	0.028*
FL	35.54(33.19;42.56)	38.63(35.83;41.58)	1.00
TM	28.65(26.10;36.88)	29.74(27.08;33.16)	1.00
TL	31.30(25.57;41.73)	30.95(25.93;36.60)	1.00
Cartilage T2star (msec)	21.81(19.89;22.74)	24.29(22.7;26.26)	0.004*
FM	21.65(20.62;23.63)	23.38(20.87;24.81)	0.94
FL	13.81(10.26;16.78)	17.98(15.95;18.9)	0.016*
TM	14.69(11.71;16.72)	18.62(17.85;22.04)	<0.001*
TL			
Meniscus T2 (msec)			
Medial compartment	19.41(16.34;26.07)	23.08(18.32;28.36)	0.28
Lateral compartment	18.83(17.46;24.58)	17.93(15.23;21.8)	0.23
Meniscus T2star (msec)			
Medial compartment	7.95(7;8.33)	7.45(6.98;9.31)	0.72
Lateral compartment	7.56(5.8;7.75)	7.48(6.33;7.74)	0.80

[a] Values are presented as medians (95% confidence interval). FM: medial femur, FL: lateral femur, TM: medial tibia, TL: lateral tibia.* Significant at α<0.05.

138

Within controls, none of the morphological changes differed significantly in the FM ($P = .73$, $P = 1.00$, $P = .27$, and $P = .27$, respectively) and TM ($P = .10$, $P = .28$, $P = 1.00$, and $P = 1.00$, respectively) when compared with baseline. In the FL ($P = .020$), only changes at tpostt0 differed significantly from baseline. In the TL, decreases at both tpostt0 ($P = .005$) and tpostt15 ($P = .035$) were significant.

Table 3. Cartilage function: deformation and recovery. 3-dimensional morphological changes after exercise relative to baseline within and between groups

	Patients	Controls	*P*-value
FM			
Change 1 (at tpostt0)	-3.37(-9.97;-1.6)[¶]	-1.69(-4.1;-0.88)	0.80
Change 2 (at tpostt15)	-1.60(-4.82;-0.13)[¶]	0.01(-0.34;1.23)	0.040*
Change 3 (at tpostt30)	-0.40(-3.77;0.99)	0.49(0;1.23)	0.42
Change 4 (at tpostt45)	-0.06(-2.03;1.06)	0.49(0.01;1.23)	0.93
FL			
Change 1 (at tpostt0)	-3.74(-6.70;-2.06)[¶]	-3.28(-6.59;-0.55)[¶]	1.00
Change 2 (at tpostt15)	-1.83(-3.82;-0.09)[¶]	0.28(-1.21;1.30)	0.11
Change 3 (at tpostt30)	-0.44(-2.45;0.39)	0.49(0.12;1.53)	0.92
Change 4 (at tpostt45)	0.25(-0.74;0.76)	0.49(0.15;1.53)	0.93
TM			
Change 1 (at tpostt0)	-8.61(-10.96;-2.90)	-6.20(-9.52;-0.42)	1.00
Change 2 (at tpostt15)	-6.53(-9.25;-1.59)	-1.82(-4.54;0.54)	0.50
Change 3 (at tpostt30)	-4.63(-8.34;0.78)	0.26(-1.94;1.16)	0.35
Change 4 (at tpostt45)	-1.45(-5.81;0.78)	0.54(-1.64;1.61)	0.64
TL			
Change 1 (at tpostt0)	-6.63(-11.07;-4.32)[¶]	-5.95(-8.70;-2.48)[¶]	1.00
Change 2 (at tpostt15)	-6.53(-11.07;-2.65)[¶]	-2.37(-5.50;0)[¶]	0.06
Change 3 (at tpostt30)	-3.76(-9.29;-1.78)[¶]	0.036(-1.52;0.72)	0.004*
Change 4 (at tpostt45)	-1.86(-4.66;-0.40)[¶]	0.43(-0.91;0.77)	0.024*

FM: medial femur, FL: lateral femur, TM: medial tibia, TL: lateral tibia. tpostt0-15-30-45: morphology measured within maximum 2 minutes following cessation of exercise (tpostt0) and at 15-30-45 minutes after onset of tpostt0 respectively. *Significant difference *between* groups at α<0.05. [¶]Significant difference relative to baseline *within* groups at α<0.05. Descriptives are presented as medians (95% CI: lower bound;upper bound).

Within patients, morphological changes measured at both tpostt0 and tpostt15 differed significantly in the FM ($P = .005$ and $P = .030$, respectively) and FL ($P = .005$ and $P = .030$, respectively) when compared with baseline. In the TL, decreases at tpostt0 ($P = .005$), tpostt15 ($P = .005$), tpostt30 ($P = .005$), and tpostt45 ($P = .025$) remained significant when compared with baseline. In the TM, none of the changes differed significantly from baseline ($P = .15$, $P = .26$, $P = .46$, and $P = .82$, respectively).

No significant between-group differences in speed (P = .72), distance (P = .62), and step count (P = .51) were exposed during running. In patients, median speed was 10.00 km/h (95% CI, 7.20-11), distance was 4.82 km (95% CI, 3.75-5.38), and step count was 4680 (95% CI, 4577-4835). In controls, median speed was 9.80 km/h (95% CI, 9.00-10.70), distance was 4.94 km (95% CI, 4.64-5.30),and step count was 4742 (95% CI, 4737-4753). The patients reported no to mild knee pain during running (VAS score: median, 1.60 [95% CI, 0.00-2.90]). For all plates at all time points, percentage changes and P values are tabulated in Table 3 for both groups.

Upon diagnosis, BMLs were present on MRI in 10 of 15 cases (1 or multiple locations/patient: 12.5% posterior TM, 12.5% weight-bearing third FL, 25% anterolateral FL, 50% posterior TL), there was no BML present in 2 of 15, and there were no baseline MRI scans available in 3 of 15. At 6 months after surgery, in all but 1 MRI scan, no BML was detected.

Return to Sports and Surgical Delay

At 6 months after surgery, 8 of 15 patients had returned to sports. In these cases of return to sports, only 1 patient practiced the preinjury sport again (ie, outdoor running). The remainder engaged in another safe or low-risk sport:outdoor running (1/8), fitness (4/8), cycling (1/8), and swimming (1/8). Median weekly frequency was 2.5 h/wk (95% CI, 1.5-4.5). Tegner scores were significantly lower in patients when compared with controls (3 [95% CI, 2-4] vs 6 [95% CI, 3-8], respectively; P = .025). Whether a patient returned to sports or not did not correlate with symptomatic and/or functional status in this sample (for relevant variables in Table 1, all $r_s < 0.4$ and P > .05). Reasons for a restrained return were intrinsic motivation (3/7), withdrawing from facility (2/7), fear of reinjury (1/7), and "not yet allowed" (1/7).

Comparison between controls and either patients who returned to sports or those who did not revealed no significant differences for all cartilage status parameters except for function of the TL during recovery. More specifically, at tpostt30, the TL showed significantly larger volume decreases in those who returned to sports compared with those who did not and compared with controls (−6.15% [95% CI, −12.10 to −0.96], −2.96% [95% CI, −12.11 to 0.22], and 0.04% [95% CI, −1.52 to 0.72], respectively; P = .012 between "returned to sports" and "controls").

Among patients who returned to sports, timing of return was recorded the latest in the sixth month and the soonest in the fourth month after surgery. Spearman ρ revealed significant and strong positive correlations (ie,the sooner the return, the larger the baseline morphological characteristics) between time to return to sports and baseline volumes for the TM and TL (both r_s = 0.76, P = .049) and thickness for the FL (r_s = 0.76, P = .046) and TM (r_s = 0.76, P = .049). With regard to function, significant and strong negative correlations (ie, the sooner the return, the larger the morphological decrease) were shown for deformation in the FL (tpostt0: r_s = −0.95,

P = .001) and for recovery at tpostt15 in the FL (r_s = –0.79, P = .033) and FM (r_s = –0.78, P = .041) (Figure 2). All other parameters investigating cartilage status did not correlate with time to return to sports (all r_s<0.5, P > .05). Median surgical delay was 10 weeks (95% CI, 5-17). The shortest delay recorded was 3 weeks, and the longest was 24 weeks. With regard to recovery, significant and moderate to strong positive correlations (ie, the shorter the delay, the larger the morphological decrease) were found in the FM at tpostt30 (r_s = 0.60, P = .019) and in the FL at tpostt45 (r_s = 0.58, P = .025) (Figure 3). All other parameters investigating cartilage status did not correlate with surgical delay (all rs<0.4, P > .05). No correlation existed between time to return to sports and surgical delay (r_s = –0.03, P = .93).
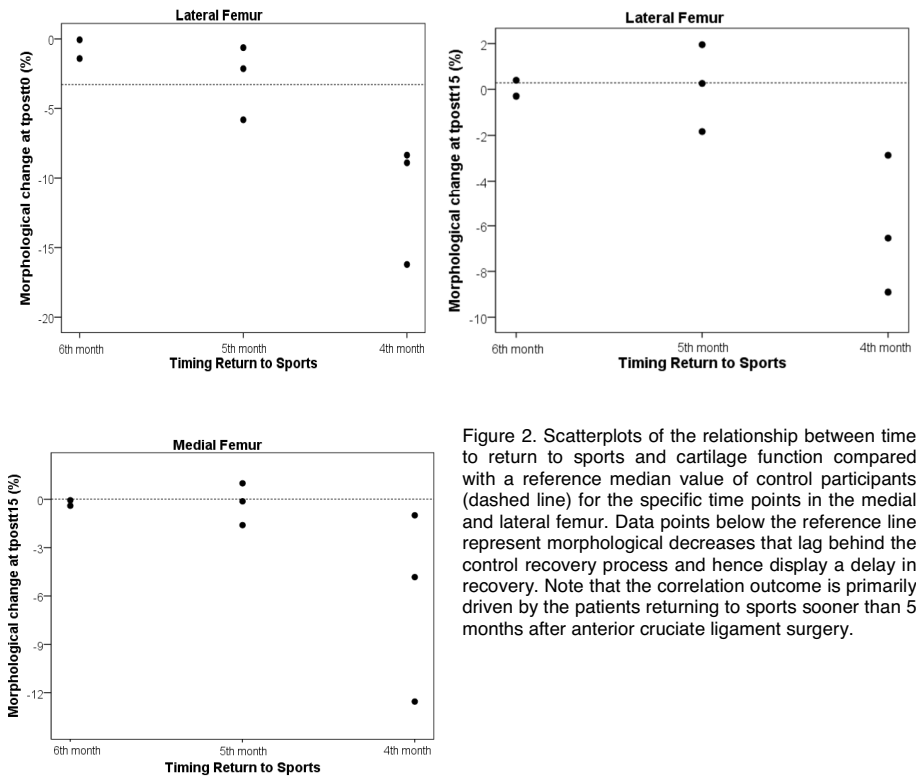
Figure 2. Scatterplots of the relationship between time to return to sports and cartilage function compared with a reference median value of control participants (dashed line) for the specific time points in the medial and lateral femur. Data points below the reference line represent morphological decreases that lag behind the control recovery process and hence display a delay in recovery. Note that the correlation outcome is primarily driven by the patients returning to sports sooner than 5 months after anterior cruciate ligament surgery.
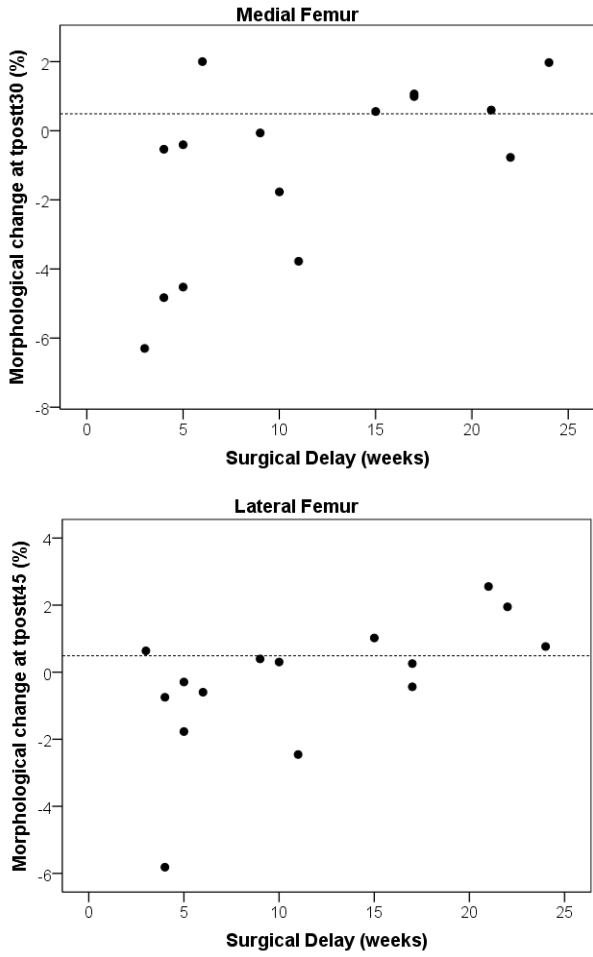
Figure 3. Scatterplots of the relationship between timing of surgical delay and cartilage function compared with a reference median value of control participants (dashed line) for the specific time points in the medial and lateral femur. Data points below the reference line represent morphological decreases that lag behind the control recovery process and hence display a delay in recovery. Note that the correlation outcome is primarily driven by the patients receiving surgery less than 10 weeks after anterior cruciate ligament injury.

DISCUSSION

The most important finding of this study revealed that although no differences in cartilage volume and thickness could be shown between ACL-reconstructed knees and healthy matched controls, differences in biochemical composition were apparent at 6 months after surgery. Based on 4 measurements up to 45 minutes after exercise cessation, equal cartilage deformation after a 30-minute run among patients and controls was noted. Equal deformation between groups preceded significantly slower recovery of cartilage morphological characteristics in patients. Additionally, early postoperative sports participation was related to trends toward increased cartilage volume, thickness, and deformation and to slower recovery of cartilage morphological characteristics after running. Similarly, although all patients were operated on before 6 months after injury, a shorter surgical delay was associated with slower cartilage recovery after running.

Knee cartilage volume and thickness did not differ between patients and controls. In this regard, interindividual differences in cartilage morphology are suggested as being larger than side-to-side differences, potentially accounting for the present lack of significance.[7] The rationale for including distinct control patients, however, was 3-fold. (1) After injury and during rehabilitation, overstraining of the nonreconstructed knee might occur as a compensatory overuse phenomenon to guard the operated side.[11] (2) Biochemical marker concentrations were reported to be elevated also in the contralateral joint possibly because of altered gait patterns and/or release of matrix fragments or cytokines into circulation.[6,26] (3) Cartilage defects, meniscal injuries, and previous ACL injuries are possibly sustained in the opposite limb. Excluding those patients would have further impeded recruitment. Nonetheless, cross-sectional designs do not adequately reflect cartilage adaptations over time. Frobell et al[9] monitored cartilage changes after surgery, revealing overall small changes that could only attain significance after 2 years. Hence, substantial changes might not yet have occurred at 6 months' follow-up.

When compared with controls, T2 prolongation was detected in patients' FM, and T2* decreases were registered in the FM, TM, and TL. T2/T2* mapping techniques are reported to target water content and collagen matrix. As opposed to increasing T2, T2* tends to decrease in case of degeneration.[3] To the best of our knowledge, this is the first study reporting T2/T2* values in ACL reconstructed knees as soon as 6 months after surgery. One year after reconstruction, Li et al[17] reported T2 elevations of 1% to 12.2%. Potter et al[22] substantiated progressive prolongation of T2 in the FL from 1 year onward. Although the capacity of T2* in early disease detection warrants further investigation,[3] overall, biochemical mapping results suggest diminished cartilage quality in ACLreconstructed knees at 6 months after surgery.

Regarding cartilage function, deformation appeared equal among patients and controls. However, between-group differences revealed significantly slower recovery of cartilage deformation after running in patients for the FM and TL. Additionally, differences in significant time effects within groups suggest potential differences between groups that could not be statistically proven because of sample size. As such, a tendency is noted toward slower recovery in the FL and more deformation in the FM. Our results are in agreement with those of Hosseini et al,[12] who reported, during a single-legged quasistatic lunge at lower flexion angles (0°-15°), increased contact deformation in overlapping tibiofemoral cartilage layers in both medial and lateral compartments of isolated ACLreconstructed knees at 6 months after surgery. Investigation of *in vivo* cartilage deformational behavior provides a means to encompass tissue resiliency, determining in part its vulnerability to degeneration.[27] Collagen disruption, endorsed by the aberrant T2 values, causes loss of collagen tensile strength, which possibly accounts for the delayed recovery observed. Delayed recovery might induce a state of maintained deformation and dehydration as compared with healthy joints. Enduring dehydration may have deleterious effects on chondrocyte metabolism.[24] In this respect, because of the fast and repetitive (high) impact loads to be encountered during sports, delayed cartilage recovery may be potentially deleterious, eliciting a negative vicious circle toward degeneration.

The results of our study are the first to associate an early return to sports with trends toward increased cartilage deformation and diminished cartilage function at 6 months after surgery. Although not significantly different from controls, a tendency toward increasing volume/thickness in the patients may be suggestive signs of OA onset. During early OA, collagen disruption causes water accumulation, resulting in swollen tissue recognized by the present abnormal T2. In support, in early OA, next to thinning, cartilage thickening associated with swelling was previously described.[4,8] Frobell et al[8,9] similarly reported central FM volume increases up to 2 years after ACL surgery. Notwithstanding the limited sample (Figure 2), return to sports from 5 months onward (ie, 5/8 returners) seemed to be associated with deformation and recovery similar to the control group. As such, postponing sports this far may be more suitable for knee cartilage to counter excessive repetitive loads. One might argue that baseline measurements are needed to attribute the worse cartilage outcomes to timing of return to sports. This study did not include baseline testing because current rehabilitation guidelines advise against 30-minute runs being implemented in the acute postinjury or postreconstruction phase.[16] Recent longitudinal MRI studies in ACL-reconstructed knees, however, describe baseline increases in T2/T1ρ along with decreases in delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), which were attributed to blunt trauma. Subsequently, these markers of decreased tissue quality were described to monitor an attempt of restitution during the first year(s).[17,20] Hence, in support of the current correlation outcomes, it is likely to suggest that the earlier the return to sport during the first year, the more fragile the cartilage and thus the more deleterious the effect of resuming sports. In agreement with the recent epidemiological literature[1] and the

rehabilitation guidelines,[16] the return rate at 6 months was relatively low. Fifty-three percent practiced some sort of sports, of which 12.5% reported a return to the preinjury sport. Actual return to the same sport does not affect the main outcome of this study. In fact, the current notion of cartilage fragility supports the advice to consider a delayed return to sports. In this regard, investigation of later time points would be an interesting lead for future research.

Interestingly, the sooner surgery was performed, the more cartilaginous functional decline was apparent at 6 months after surgery. In view of the body of literature,[5,10,23] in this sample, with a median surgical delay of 2.5 months, all patients received early surgery, which accounted for the absence of substantial collateral damage on baseline arthroscopic surgery (as intended). Considering limited sample sizes, the data in Figure 3 suggest that surgery before approximately 10 weeks (ie, 9/15 patients) was associated with delayed cartilage recovery after running. Surgery performed within a 2.5- to 6-month window led to recovery comparable with the control group, suggesting adequate tissue resiliency. During an ACL rupture, considerable impact is cushioned, accounting for inflammatory processes and posttraumatic BMLs to present. On top of primary-phase BMLs, secondary-phase BMLs are introduced by surgery itself, accompanied by prolonged knee effusion.[9] Surgery performed promptly potentially endangers the joint's possibility to allow the primary-phase effect to be adequately processed. Hence, potential biological side effects of surgery on cartilage homeostasis[26] might be amplified when patients enroll in rehabilitation. In case of the need for surgery, early reconstruction (ie, within the first 6 months or 1 year at the latest[5,10,23]) is encouraged to avoid progressive cartilage and/or meniscal damage. However, based on the results of this study, proper balance with return to sports is suggested for consideration.

The nature of the protocol led to a relatively limited sample size. Although suggested outcomes agree with results from longitudinal studies using larger samples[8,9,17,20] and significance is attained for the main results, replication remains warranted for the sake of generalizability. Second, selection based on "clear arthroscopic evaluation" (ie, absence of concomitant visible cartilage lesions, meniscus injuries, or other grade 2 ligament injuries) was pursued to include a homogeneous sample, which facilitates the investigation into the effect of the reconstruction itself. It should be mentioned that arthroscopic surgery cannot preclude the presence of ultrastructural damage in the cartilage or menisci at baseline. With regard to the latter, next to the exclusion of cases treated with concomitant meniscal procedures at baseline, it is interesting to note that biochemical mapping did not reveal between-group differences for the menisci in the present analysis. Third, comparison with nonoperatively treated ACL tears and/or patient baseline conditions would be an interesting expansion of the protocol. However, healthy matched controls were our first option as the former 2 groups cannot serve as healthy references. Fourth, as this is a retrospective case control design, prospects in view of long-term outcome cannot be drawn.

CONCLUSION

This experimental study reports MRI signs suggesting cartilage fragility at 6 months after isolated ACL reconstruction in a young, active population compared with matched control patients. Although no macromorphological differences existed, ultrastructural MRI changes suggested early degeneration, corresponding with declining *in vivo* tissue resiliency. Diminished cartilage cushioning properties are proposed to be a potential danger in view of a full return to sports. Based on the results of this study, caution is advised for an early return to sports (ie, before 5 months after surgery), especially when dealing with patients who received early surgery (ie, within 10 weeks after injury).

ACKNOWLEDGMENTS

REFERENCES

1. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to the pre-injury level of competitive sport after anterior cruciate ligament reconstruction surgery: two-thirds of patients have not returned by 12 months after surgery. Am J Sports Med. 2011;39(3):538-543.
2. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982;36(5):936-942.
3. Bittersohl B, Miese FR, Hosalkar HS, et al. T2* mapping of hip joint cartilage in various histological grades of degeneration. Osteoarthritis Cartilage. 2012;20(7):653-660.
4. Buck RJ, Wyman BT, Le Graverand MP, et al. Osteoarthritis may not be a one-way-road of cartilage loss: comparison of spatial patterns of cartilage change between osteoarthritic and healthy knees. Osteoarthritis Cartilage. 2010;18(3):329-335.
5. Church S, Keating JF. Reconstruction of the anterior cruciate ligament: timing of surgery and the incidence of meniscal tears and degenerative change. J Bone Joint Surg Br. 2005;87(12):1639-1642.
6. Dahlberg L, Roos H, Saxne T, et al. Cartilage metabolism in the injured and uninjured knee of the same patient. Ann Rheum Dis. 1994;53(12):823-827.
7. Eckstein F, Mü̈ller S, Faber SC, Englmeier KH, Reiser M, Putz R. Side differences of knee joint cartilage volume, thickness, and surface area, and correlation with lower limb dominance: an MRI-based study. Osteoarthritis Cartilage. 2002;10(12):914-921.
8. Frobell RB. Change in cartilage thickness, posttraumatic bone marrow lesions and joint fluid volumes after acute ACL disruption: a two-year prospective MRI study of sixty-one subjects. J Bone Joint Surg Am. 2011;93:1096-1103.
9. Frobell RB, Le Graverand MP, Buck R, et al. The acutely ACL injured knee assessed by MRI: changes in joint fluid, bone marrow lesions, and cartilage during the first year. Osteoarthritis Cartilage. 2009;17:161-167.
10. Granan LP, Bahr R, Lie SA, Engebretsen L. Timing of anterior cruciate ligament reconstructive surgery and risk of cartilage lesions and meniscal tears: a cohort study based on the Norwegian National Knee Ligament Registry. Am J Sports Med. 2009;37(5):955-961.
11. Hoffelner T, Resch H, Moroder P, et al. No increased occurrence of osteoarthritis after anterior cruciate ligament reconstruction after isolated anterior cruciate ligament injury in athletes. Arthroscopy. 2012;28(4):517-525.
12. Hosseini A, Van de Velde S, Gill TJ, Li G. Tibiofemoral cartilage contact biomechanics in patients after reconstruction of a ruptured anterior cruciate ligament. J Orthop Res. 2012;30(11):1781-1788.
13. Hunter DJ, Li L, Zhang YQ, et al. Region of interest analysis: by selecting regions with denuded areas can we detect greater amounts of change? Osteoarthritis Cartilage. 2010;18(2):175-183.
14. Keays SL, Newcombe PA, Bullock-Saxton JE, Bullock ML, Keays AC. Factors involved in the development of osteoarthritis after anterior cruciate ligament surgery. Am J Sports Med. 2010;38(3):455-463.

15. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. Am J Sports Med. 2008;36(5):966-970.

16. Kvist J. Rehabilitation following anterior cruciate ligament injury: current recommendations for sports participation. Sports Med. 2004;34(4):269-280.

17. Li X, Kuo D, Theologis A, et al. Cartilage in anterior cruciate ligamentreconstructed knees: MR imaging T1r and T2-initial experience with 1-year follow-up. Radiology. 2011;258(2):505-514.

18. Myklebust G, Bahr R. Return to play guidelines after anterior cruciate ligament surgery. Br J Sports Med. 2005;39(3):127-131.

19. Neeb TB, Aufdemkampe G, Wagener JHD, Mastenbroek L. Assessing anterior cruciate ligament injuries: the association and differential value of questionnaires, clinical tests and functional tests. J Orthop Sports Phys Ther. 1997;26(6):324-331.

20. Neuman P, Tjörnstrand J, Svensson J, et al. Longitudinal assessment of femoral knee cartilage quality using contrast enhanced MRI (dGEMRIC) in patients with anterior cruciate ligament injury: comparison with asymptomatic volunteers. Osteoarthritis Cartilage. 2011; 19(8):977-983.

21. Niefhoff A, Müller M, Bru¨ ggeman L, et al. Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. Osteoarthritis Cartilage. 2011;19(8):1003-1010.

22. Potter HG, Jain SK, Ma Y, Black BR, Fung S, Lyman S. Cartilage injury after acute, isolated anterior cruciate ligament tear: immediate and longitudinal effect with clinical/MRI follow-up. Am J Sports Med. 2012;40(2):276-285.

23. Seon JK, Song EK, Park SJ. Osteoarthritis after anterior cruciate ligament reconstruction using a patellar tendon autograft. Int Orthop. 2006;30(2):94-98.

24. Song Y, Greve JM, Carter DR, Giori NJ. Meniscectomy alters the dynamic deformational behavior and cumulative strain of tibial articular cartilage in knee joints subjected to cyclic loads. Osteoarthritis Cartilage. 2008;16(12):1545-1554.

25. Subburaj K, Kumar D, Souza RB, et al. The acute effects of running on knee articular cartilage and meniscus magnetic resonance relaxation times in young healthy adults. Am J Sports Med. 2012;40(9):2134-2141.

26. Taskiran E, Taskiran D, Duran T, Lo¨ k V. Articular cartilage homeostasis after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc. 1998;6:93-98.

27. Van Ginckel A, Roosen P, Almqvist KF, Verstraete K, Witvrouw E. Effects of in vivo exercise on ankle cartilage deformation and recovery in healthy volunteers: an experimental study. Osteoarthritis Cartilage. 2011;19(9):1123-1131.

28. Williams A, Qian Y, Golla S, Chu CR. UTE-T2* mapping detects subclinical meniscus injury after anterior cruciate ligament tear. Osteoarthritis Cartilage. 2012;20(6):486-494.

29. Zarins ZA, Bolbos RI, Pialat JB, et al. Cartilage and meniscus assessment using T1rho and T2 measurements in healthy subjects and patients with osteoarthritis. Osteoarthritis Cartilage. 2010;18(11):1408-1416.

SUPPLEMENTARY MATERIAL

Intra-tester reliability (ICC) and precision error (RMS CV) for volume and T2/T2star quantification based on 3 repeats for each variable.

	Patients (N=3)		Controls (N=3)	
	ICC	RMS CV	ICC	RMS CV
Volume quantification				
TL	0.99	0,01	1.00	0,01
TM	0.99	0,08	1.00	0,03
FL	0.91	0,03	0.99	0,01
FM	0.99	0,03	0.99	0,01
T2 quantification				
TL	0.91	0,05	0.97	0,03
TM	0.96	0,08	0.98	0,08
FL	0.76	0,05	0.99	0,01
FM	0.99	0,04	0.92	0,04
Medial meniscus	0.97	0,12	0.99	0,07
Lateral meniscus	0.94	0,11	0.97	0,04
T2star quantification				
TL	0.98	0,03	0.82	0,04
TM	0.98	0,02	0.99	0,03
FL	0.91	0,04	0.90	0,04
FM	0.96	0,01	0.97	0,01
Medial meniscus	0.99	0,02	0.70	0,07
Lateral meniscus	0.95	0,05	0.94	0,06

FM: medial femur, FL: lateral femur, TM: medial tibia, TL: lateral tibia. ICC: intra-class correlation coefficient, RMS CV: root mean square coefficient variation.

ACUTE CARTILAGE LOADING RESPONSES AFTER AN *IN VIVO* SQUAT
EXERCISE IN DOUBTFUL OR MILD KNEE OSTEOARTHRITIS
A CASE CONTROL STUDY

ABSTRACT

Background: Effects of exercise on osteoarthritic cartilage remain elusive.

Objective: To investigate the effect of dynamic *in vivo* squatting exercise on the magnitude and spatial pattern of acute cartilage responses in tibiofemoral osteoarthritis (i.e., Kellgren-Lawrence (K/L) grade 1-2).

Design: Case-control

Methods: Eighteen patients with medial doubtful-to-mild radiographic signs of tibiofemoral osteoarthritis were compared to 18 middle-aged controls. Using Three-dimensional (3D) Magnetic Resonance Imaging, deformation and recovery was monitored based on 3D cartilage volume calculation (i.e., total volume and anterior-central-posterior sub-regions) before and after a 30-repetition squat exercise. 3D volumes were estimated following semi-automatical segmentation and were calculated at 4 time points (1 pre- and 3 post-scans). Post-scans ran immediately after the exercise repeated according to a 15-minute interval.

Results: In both groups, significant deformation was noted in the medial compartment (Femur Medial (FM):-3.4%, *P*=0.02; Tibia Medial (TM) :-3.2%, *P*=0.01 versus FM:-2.8%, *P*=0.04; TM:-3.8%, *P*=0.01 in patients and controls respectively). Only patients showed significant deformation in Femur Lateral ((FL):-3.9%, *P*=0.001), and a trend towards significance in Tibia Lateral ((TL):-3.1%, *P*=0.05)). From 15 minutes after exercise cessation onward, volume changes were no longer significantly different from baseline. At all time-points, no significant between-group differences could be revealed for volume changes. In patients, a tendency was noted towards slower recovery preceded by larger deformations in entire cartilage plates and sub-regions. The spatial sub-regional pattern of deformation, however, was similar between groups.

Limitations: Generalizability is limited to people with doubtful/mild OA and low levels of pain.

Conclusions: Following the exercise, tibiofemoral cartilage deformation appeared similar in magnitude and spatial pattern between middle-aged subjects with and without tibiofemoral osteoarthritis (i.e., K/L grade 1-2). Restitution of volumes warranted a 15 minute-recovery, especially in case of osteoarthritic cartilage degeneration.

INTRODUCTION

Clinical guidelines for OA management depict exercise as an important component of first-line treatment strategies because of its potential to diminish pain and improve physical function.[1-6] A weak correlation, however, exists between clinical presentation and structural joint health, especially in the early stages of the OA disease process (i.e., Kellgren-Lawrence grade (K/L) 1,2).[7, 8] As the majority of trials focused on symptom-related outcomes, effects of exercise on structural outcome in the OA joint, however, remain subject to disparity. While in young healthy adults, exercise appears to beneficially influence cartilage integrity, with increasing age, protective effects may persist in case of light-to-moderate (therapeutic) exercise in those individuals without radiographic signs of OA or at risk for progressive radiographic OA (e.g., K/L 1, previous knee injury or surgery, occasional knee symptoms).[9-17] In individuals with established radiographic OA (i.e., K/L 2-4), single-event as well as long-term intervention trials (alone or combined with diet or glucosamine supplementation) reported beneficial changes or stability in cartilaginous biomarkers concerning ultra-structural compounds or anti-inflammatory responses.[18-22] In contrast, Woollard et al.[23] presented small cartilage volume changes up to a loss of 3.8% in the central medial femur after treatment including aerobic, strengthening and flexibility exercises only or added with agility and perturbation. While disparity in treatment effects might be due to grouping of patients with varying radiographic disease stages, subject characteristics (e.g., BMI, lower limb alignment) as well as differences in cartilage measures or exercise modes,[2, 23] a concern is that weight-bearing exercise may lead to acceleration of cartilage degradation instead of a deceleration.[19] Degraded cartilage shows proteoglycan loss and disruption of the collagen fiber network.[24-26] These ultra-structural changes affect the mechanical behavior of cartilage. Fibrillation of the collagen network induces loss of tensile strength and causes decreased cartilage compressive stiffness and increased tissue permeability.[25-27] In fact, in subjects at risk for radiographic OA development displaying ultra-structural cartilage degeneration (i.e., collagen disruption and water accumulation) cartilage showed delayed recovery of volumes after an *in vivo* running event.[28] Maintained deformation and dehydration of cartilage tissue after loading was suggested to increase its vulnerability towards accelerated degeneration when repetitive (high) impact loads are to be encountered.[28, 29]

While moderate (therapeutic) exercise including weight-bearing neuromuscular control and strength exercises such as squatting exercise, showed to beneficially affect physical functioning and cartilage integrity in subjects in the early stages of OA development (i.e., K/L 1-2),[10, 30] these subjects, in turn, are also at increased risk for accelerated OA progression.[31] Therefore, insight into required recovery times after an *in vivo* weight-bearing exercise in these patients, may be a first step towards appropriate design of treatment programs to positively influence cartilage structural integrity and retard disease progression.

Therefore, the purpose of this study was to investigate the effect of a dynamic *in vivo* weight-bearing squatting exercise on acute cartilage responses in individuals presenting with K/L 1-2. To this end, this study evaluated *in vivo* cartilage deformation (i.e., magnitude and spatial pattern) and time to recovery in both subjects diagnosed with doubtful-to-mild radiographic OA (i.e., K/L 1-2), and in middle-aged healthy referents. Although spatial patterns were expected to be similar between groups, It was hypothesized that knee cartilage in doubtful-to-mild radiographic OA would exhibit increased deformation[27, 32] followed by slower recovery after the exercise.[28, 29]

METHODS

Study design overview

This case-control study compares i*n vivo* cartilage deformation including recovery after a squatting exercise between subjects diagnosed with radiographic signs of OA (i.e., K/L 1-2 and with cartilage defects on MRI) and middle-aged referents (i.e., K/L 0 and without cartilage defects).

Participants

Eighteen patients (12 men, 6 women) were recruited from the university hospital's department of Physical Medicine and Orthopedic Surgery. Eligibility to participate was based on clinical assessment, medical imaging as well as standard questionnaires. Inclusion criteria were clinical and radiographic diagnosis of doubtful-to-mild medial tibiofemoral OA (i.e., K/L 1-2),[33, 34] and medial tibiofemoral cartilage defects on MRI (i.e., Whole-Organ Magnetic Resonance Imaging Score (WORMS) grade ≥ 2).[35] All patients presented with degenerative meniscal tears on MRI. Additionally, patients should be able to perform the exercise at the time of study correctly without substantial discomfort (i.e., Visual Analogue Scale (VAS) for pain during the exercise <5 cm and active knee flexion Range Of Motion (ROM) ≥90°). Exclusion criteria were prior history of knee surgery including meniscal procedures and/or arthroplasty, corticosteroid or hyaluronan injections within 3 months prior to the study, MRI contra-indications, other known joint and/or bone pathologies. In case of unilateral disease, the affected knee was investigated. In case of bilateral radiographic disease, the worst case (within K/L 1-2) was included or, when both knees were affected to a similar extent, the dominant leg was investigated. Leg dominance was defined as the limb the subject would choose to kick a ball.[36-38]

151

Eighteen middle-aged reference subjects were recruited from the community or university campus. Eligibility was verified using medical imaging and standard questionnaires. Inclusion criteria were no radiographic signs of OA and no cartilage defects on MRI. Additionally, control subjects were selected with similar physical activity levels (i.e., Baecke score[28, 37-39]), and according to a similar proportion in view of gender and limb dominance. Exclusion criteria were a history of knee pain and/or injury including previous diagnosis of cartilage defects, previous knee surgery, Body Mass Index (BMI) > 30, and age younger than 40 and older than 60 years old. In this way the risk for cartilage abnormalities on MRI with increasing age (even in the presence of normal radiographic appearances)[40] is reduced. Final exclusion criteria were known bone and/or joint pathologies (i.e., presence of bone marrow lesions or meniscal displaced tears or complete degeneration on MRI[41]) and MRI contra-indications.

Informed consents ratified were obtained from all subjects. Subject demographics are listed in Table 1.

Setting and Experimental procedures

All experimental procedures were performed during 1 test appointment. All subjects were instructed to not practice sports the day before testing or on the testing day and to avoid running, lifting heavy weights and taking stairs 4 hours preceding the actual experimental procedures.[28, 36-38, 42] The procedures were performed on hospital campus and occurred at the same time of day for all subjects.[28, 36, 38] The protocol comprised (1) MRI evaluation for *in vivo* deformation including recovery, (2) evaluation of lower limb function and knee alignment, and (3) questionnaires.

<u>MRI evaluation of cartilage</u>

Image acquisition and parameters

Cartilage deformation and recovery was registered by means of monitoring the changes in cartilage quantitative morphology (i.e., 3D volume) before and after an *in vivo* weight-bearing exercise.[28, 36, 38] High-resolution images of cartilage morphology were acquired by means of a sagittal 3D double echo steady state sequence with water excitation (3D DESS WE). Additionally, to determine eligibility for inclusion, next to the 3D DESS WE sequence, an Intermediate-weighted fat-saturated turbo-spin echo (TSE) sequence was included at baseline allowing for cartilage WORMS grading.[35] Finally, a T2 map (MapIt, Siemens medical solutions, Erlangen, Germany) was included. T2 relaxation times depict ultra-structural changes in the

collagen/water content of the cartilage matrix. Increases in T2 value are associated with early degeneration before macroscopic changes present and were added to estimate the presence of insidious cartilage disease next to the macro-mophological appearance of the cartilage surface.[43]

T2 maps were centred on the tibiofemoral compartments and are reconstructed on-line using a pixel-wise, mono-exponential, nonnegative least square fit analysis (MapIt, Siemens medical solutions, Erlangen, Germany) enabling instant T2 quantification after image acquisition. All images were obtained using a dedicated 8-channel knee coil on a 3T Trio Tim magnet (Siemens medical solutions, Erlangen, Germany). Knee joints were scanned in extension and neutral rotation was assured for by rigid foam placed around the lower leg. Patient supine positioning was standardized using the position of the knee joint according to the reference points on the knee coil.[37]

The sequence parameters for the 3D DESS WE, Intermediate-weighted TSE sequence and T2 maps were implemented as previously described. [28]

Table 1. Group characteristics.Demographics, symptoms and function of patients with doubtful or mild OA vs. controls

	Patients (N=18)	Controls (N=18)	P-value[a,b]
Demographics			
BMI (kg/m^2)	27.1 (3.7)	24.0 (3.5)	0.02 [b,*]
Age (yrs)	54.5 (49.8;64.3;14.3)	43.0 (40.0;45.0;5.0)	<0.001[a,*]
Knee alignment: absolute IC/IM distance (mm)[¶]	-2.6 (27.1)	6.4 (19.6)	0.26 [b]
Symptoms and Function			
Baecke Physical Activity Level	8.4 (1.5)	8.3 (1.4)	0.93 [b]
FORRS	49.6 (18.3)	38.8 (19.8)	0.10 [b]
FTSTS best time (sec)	7.8 (6.8;9.0;2.2)	7.1 (6.1;7.7;1.6)	0.06[a]
FTSTS mean time (sec)	8.1 (6.9;9.8;2.9)	7.3 (6.9;8.1;1.2)	0.12 [a]
RAND-36 physical function	55.0 (32.5;82.5;50.0)	100.0 (90.0;100.0;10.0)	<0.001 [a]
RAND-36 social function	87.5 (62.5;100;37.5)	100.0 (100.0;100.0;0.0)	0.001 [a,*]
RAND-36 role limitations phys. health	100.0 (50.0;100.0;50.0)	100.0 (100.0;100.0;0.0)	0.03 [a]
RAND-36 role limitations em. health	100.0 (100.0;100.0;0.0)	100.0 (100.0;100.0;0.0)	0.32 [a]
RAND-36 em. well-being	78.0 (72.0;88.0;16.0)	88.0 (80.0;93.0;13.0)	0.03 [a,*]
RAND-36 energy/fatigue	70.0 (65.0;76.3;11.3)	80.0 (73.8;86.3;7.5)	0.01 [a,*]
RAND-36 pain	67.4 (53.0;79.6;26.6)	100.0 (79.6;100.0;20.4)	0.002 [a,*]
RAND-36 general health	72.5 (65.0;85.0;30.0)	82.5 (70.0;90.0;20.0)	0.22 [a]
RAND-36 health change	50.0 (50.0;50.0;0.0)	50.0 (50.0;50.0;0.0)	0.44 [a]
WOMAC standardized total score (/100)	80.2 (62.8;95.8;33.0)	100 (100;100;0)	<0.001 [a,*]
VAS pain last week (/10)	2.8 (0.0;5.0;5.0)	0.0 (0.0;0.0;0.0)	0.001 [a,*]

BMI: Body Mass Index, FORSS: Factor Occupational Rating System Scale, FTSTS: Five Times Sit To Stand test, RAND-36 role limitations phys. health: RAND-36 role limitations physical health, RAND-36 role limitations em. health: RAND-36 role limitations emotional health, RAND-36 em. well-being: RAND-36 emotional well-being, WOMAC: Western Ontario and McMaster Universities Arthritis Index, total standardized sum-scores were calculated (i.e., the higher the score, the less the disease impact) according to the following formula: [((96- total sum-score)x100)/96], 96 being the maximum score. VAS: Visual Analogue Scale. [¶]Positive values represent tendencies towards varus alignment, negative values represent tendencies towards valgus alignment. *Significant difference between groups at α<0.05.[a]P-values are the result of the Mann-Whitney U test. Descriptives are presented as median (P25;P75;inter-quartile range).[b]P-values are the result of the t-test for independent samples. Descriptives are presented are presented as mean (S.D.).

Deformation and recovery after in vivo exercise

To reduce interference from residual deformation preceding the experiment, the MRI protocol started with 1 hour of physical rest during which the participants were positioned supine.[28, 36, 38, 44] After resting, baseline scans (i.e., tpre: baseline sag 3D DESS WE, T2 map, Intermediate-weighted TSE) were performed followed by the weight-bearing exercise under study. Within maximum 90 sec after exercise cessation,[36, 38] first post-scans (i.e., tpostt0: sag 3D DESS WE) were started and repeated according to a 15-minute interval up to 30 minutes after the exercise (i.e., tpostt15-30: sag 3D DESS WE). "Deformation" is expressed as the 3D volume change measured at tpostt0 relative to baseline (i.e., [(3D volume tpostt0-3D volume tpre)/3D volume tpre)x100]. Morphology changes measured at tpostt15-30 relative to baseline are attributed to "recovery".[28, 38] The sequence of events is displayed in Figure 1.
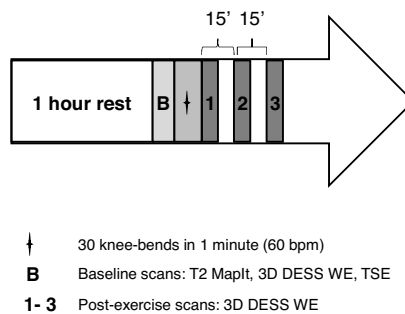
Figure 1. Schematic overview of the sequence of events during the MRI experimental protocol. Adapted from Van Ginckel et al.[28,38] B: baseline scans (tpre),1-3: post-exercise scans (tpostt0-15-30: onset post-exercise scans started within maximum 90 sec following cessation of exercise (tpostt0) and repeated at 15-30 minutes after onset of tpostt0 respectively).

The exercise consisted of 30 bilateral knee bends until the upper leg was lowered horizontally (referenced by the seat of a chair) in 1 min. To control for a correct and standardized performance, the exercise was carried out under a researcher's supervision and performed barefoot next to the scanner magnet.[36, 38, 44] Exercise speed was set to the pace of a metronome (i.e., 60 bpm). VAS scores were collected for the extent of knee pain experienced during the exercise (on a 10 cm scale: "0 cm" representing "no pain at all" and "10 cm" representing

"extremely painful"). The effect of 30 knee-bends on cartilage changes was previously evaluated in adults using MRI.[44-46]

Evaluation of lower limb function and knee alignment

Functional lower limb performance was evaluated using the Five-times Sit-to-Stand (FTSTS) test.[47] FTSTS was performed twice of which both "mean" and "best times" were used for analysis.

Knee alignment (i.e., genu varum/valgum) was determined by measuring the inter-condylar (IC) or inter-malleolar (IM) distance with an inside caliper as previously described.[48] IC and IM distances were subtracted, and this value was considered the absolute IC/IM distance. Quantification of absolute IC/IM distance attained a high inter- and intra-tester reliability (respectively ICC = 0.95 and ICC = 0.96)[48] and showed to be valid when compared to full-limb radiographs (Bland-Altman plot: R2=0.98, P<0.001, no correlation between BMI and IC/IM distance (r=-0.03, P=0.85)).

Questionnaires

All subjects completed a Baecke questionnaire quantifying general physical activity level based on a work-, sports- and leisure-index,[39] Factor Occupational Rating System Scale (FORSS) rating knee joint load during work situations in particular,[49] Likert-scale version of the Western Ontario and McMaster Universities Arthritis Index (WOMAC) quantifying pain, stiffness and ADL physical functioning[50], and RAND 36-Item Health Survey measuring quality of life.[51] VAS-scores (/10) for the amount of pain experienced during the last week were collected (i.e.,"0" representing "no pain at all" and "10" representing "extremely painful") and self-reported duration of knee complaints (i.e., in months).

Data-analysis

Image analysis: 3D volume calculation

3D reconstruction, volume calculation, and model registration were performed using a commercial modeling software package (Mimics, version 14.0, Materialise NV, Leuven, Belgium).[28, 36, 38]

3D DESS image stacks were subsequently segmented to generate a 3D reconstruction of lateral/medial femoral/tibial cartilage (FL, FM, TL, TM). A semi-automatical segmentation procedure was implemented based on a 3D LiveWire algorithm[52] and a slice-by-slice manual correction to digitize cartilage plates by masking. A region-growing algorithm to dispose of abundant voxels preceded manual correction. 3D cartilage plates were reconstructed and absolute 3D volumes (in mm³) were calculated for baseline and post-scans.[28, 36, 38] Next to the calculation of total volumes at all time-points, sub-regional tibiofemoral volumes were determined to investigate spatial deformational patterns (i.e., at tpostt0).[27] As defined in the cartilage WORMS scoring system,[35] femoral and tibial cartilage plates were subdivided in an anterior, central and posterior sub-region (FMA: antero-medial femur, FMC: centro-medial femur, FMP: postero-medial femur. FLA: antero-lateral femur, FLC: centro-lateral femur, FLP: postero-lateral femur. TMA: antero-medial tibia, TMC: centro-medial tibia, TMP: postero-medial tibia. TLA: antero-lateral tibia, TLC: centro-lateral tibia, TLP: postero-lateral tibia). An illustration of the division into sub-regions is displayed in Figure 2.
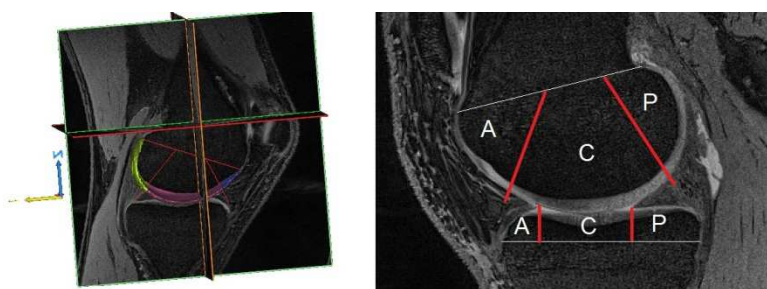

Figure 2. Illustration of the sub-regions applied in this study as originally defined in the WORMS scoring system.[35] The femoral and tibial surfaces are further subdivided into anterior (A), central (C) and posterior (P) regions. Region A of the femur corresponds to the patellofemoral articulation; region C the weight bearing surface, and region P the posterior convexity that articulates only in deeper flexion. Region C of the tibial surface corresponds to the uncovered portion between the anterior and posterior horns of the meniscus centrally and the portion covered by the body of the meniscus peripherally.[35] Images of the 3D reconstructions are screen shots taken from the Mimics software interface.

All image analyses were performed by a single researcher with 4 years of experience at the time of analysis and who was blinded to the time sequence of scanning.[28, 36, 38, 53] Based on 3 repeats for all cartilage plates, 3D volumetric measurements' intra-tester reliability (i.e., Intra-Class Correlation Coefficient (ICC)) and precision errors (Root Mean Square (RMS CV)) attained an ICC ranging from 0.96-0.99 in 3 controls and from 0.92-0.99 in 3 patients and a RMS CV ranging from 0.02-0.03 in both patients and controls.

Within subjects with varying K/L grades and ultra-structural cartilage degeneration, mean (S.D.) morphological changes after an in vivo load range from -1.8 (3.0)% to -7.9 (11.0)%.[27, 28, 32] To attain the smallest difference with a statistical significance of α<0.05 and standard power, one needs to include at least 24 subjects in the entire group. However, expected between-group differences range from 0.1% to ~4.5%.[27, 28, 32] In view of our precision errors (that comply with precision errors reported in the relevant literature[27, 54]), between-group differences need to attain ~3% to be relevant in this study. To be able to detect this difference, one needs to include at least 16 subjects in each group. The power analyses were performed using GPower (version 3.1.5., Universität Kiel, Germany).

Statistical analysis

The Shapiro-Wilk test revealed a parametric distribution (*P*>0.05) for all included variables except age, total WOMAC score, all RAND 36-Items, VAS score on last week's pain and pain during the study exercise, FTSTS best and mean time. Parametric and nonparametric statistics were executed and descriptives were presented as means (S.D.) or medians (P25, P75, inter-quartile range) respectively. To investigate baseline differences in group characteristics, the t-test for independent samples or Mann-Whitney U test were applied. To test the hypothesis that cartilage morphology for all plates changed significantly over time within and between groups, a General Linear Model for repeated measures was implemented applying within-subject factors "time" and "cartilage plate" and as a between-subject factor the "patient-control" group allocation. The model corrected for the main confounding factors BMI and age addressed as co-variates. Bonferroni corrections adjusted *P*-values for multiple comparisons of main effects. Level of significance was set at α<0.05 and SPSS (version 21, IBM Statistics) was used for all analyses.

RESULTS

Group characteristics: demographics, symptoms and function

The group characteristics are tabulated in Table 1. No significant between-groups differences were present for the Baecke Physical Activity Score (*P*= 0.93), FORSS (*P*= 0.10), FTSTS mean (*P*= 0.12) and best time (*P*=0.06), and knee alignment (*P*= 0.26). The total standardized WOMAC score was significantly decreased in patients when compared to controls (*P*<0.001) as were all RAND-36 Items except for Role Limitations Emotional Health (*P*=0.32), General Health (*P*=0.21) and Health Change (*P*=0.44). Controls were younger and had a decreased BMI when

compared to the patient group (*P*<0.001 and *P*=0.02 respectively). In patients, VAS-scores on last week's pain revealed mild-to-moderate discomfort (median (P25;P75;inter-quartile range) score 2.8 (0.0;5.0;5.0)). Duration of self-reported knee complaints equaled a mean (S.D.) of 40.36 (31.8) months.

***In vivo* cartilage deformation and recovery: percentage 3D volume changes at tpostt0 and tpostt15-30 respectively**

In the entire sample (N=36), the squatting exercise effected significant deformation (i.e., mean (S.D.) reduction in 3D volumes at tpostt0) when compared to baseline, in FL (-3.3 (3.6)%, *P*<0.001), FM (-3.1 (4.0)%, *P*<0.001), TL (-2.2 (4.5)% , *P*=0.02), and TM (-3.5 (3.6)%, *P*<0.001). None of the plates showed significant volume decreases at the recovery time points (i.e., tpostt15 and tpostt30).

Within controls (N=18), when compared to baseline, none of the morphological changes at all post-exercise time-points differed significantly in FL (*P*=0.10, *P*=1.00, *P*=1.00 respectively) and TL (*P*=0.73, *P*=1.00, *P*=1.00 respectively). In FM and TM, only changes measured at tpostt0 (i.e., deformation) were significantly different from baseline (-2.8 (4.6)%, *P*= 0.04; -3.2 (3.9)%, *P*=0.01 respectively).

Within patients (N=18), in all plates, changes measured at tpostt0 differed significantly from baseline (-3.9 (3.5)%, *P*=0.001; -3.4 (3.2)%, *P*=0.02; -3.8 (3.3)%, *P*=0.01) for FL, FM, and TM respectively), next to a tendency towards significance in TL (-3.1 (4.6)%, *P*=0.05).

After completion of the squatting exercise, the patients reported no-to-mild knee pain (median (P25;P75;inter-quartile range) VAS score (cm): 1.0 (0.4;3.3;2.9)).

No significant between-groups differences could be revealed.

For all plates, at all time points, percentage changes, confounder-adjusted *P*-values are displayed in Table 2.

***In vivo* cartilage spatial deformational patterns: sub-regional analysis of 3D percentage changes at tpostt0**

Within both groups, 3D volumes were significantly decreased in all sub-regions at tpostt0 when compared to baseline with the highest deformation noted in the posterior femoral condyles and anterior tibial plateaus. Based on the magnitude of the mean sub-regional volume decreases,

similar spatial deformational patterns were observed in both groups (FMP>FMA=FMC; FLP>FLC>FLA; TMA>TMP>TMC; TLA>TLP>TLC and FMP>FMA=FMC; FLP=FLC>FLA; TMA>TMP>TMC; TLA>TLP>TLC in patients and controls respectively). For all plates, sub-regional percentage changes and confounder-adjusted *P*-values are displayed in Table 3.

Table 2. Cartilage *in vivo* deformation and recovery. 3D volume changes post-exercise relative to baseline within and between groups[$]

	All knees (N=36)	Patients (N=18)	Controls (N=18)	*P*-value Between Groups
FM				
Change 1 (at tpostt0)	-3.1 (4.0)[¶]	-3.4 (3.2)[¶]	-2.8 (4.6)[¶]	1.00
Change 2 (at tpostt15)	-0.3 (3.7)	-0.7 (3.6)	0.2 (3.8)	1.00
Change 3 (at tpostt30)	0.4 (3.5)	0.5 (3.4)	0.3 (3.7)	1.00
FL				
Change 1 (at tpostt0)	-3.3 (3.6)[¶]	-3.9 (3.5)[¶]	-2.8 (3.7)	1.00
Change 2 (at tpostt15)	-1.4 (3.2)	-2.6 (3.0)	-0.3 (3.0)	0.12
Change 3 (at tpostt30)	-0.6 (3.7)	-1.6 (3.7)	0.3 (3.5)	0.42
TM				
Change 1 (at tpostt0)	-3.5 (3.6)[¶]	-3.2 (3.9)[¶]	-3.8 (3.3)[¶]	1.00
Change 2 (at tpostt15)	0.0 (4.8)	0.8 (4.2)	-0.7 (5.5)	1.00
Change 3 (at tpostt30	0.5 (4.9)	1.5 (4.0)	-0.5 (5.5)	0.76
TL				
Change 1 (at tpostt0)	-2.2 (4.5)[¶]	-3.1 (4.6)	-1.4 (4.4)	0.92
Change 2 (at tpostt15)	-1.0 (3.8)	-1.5 (2.3)	-0.5 (4.8)	1.00
Change 3 (at tpostt30)	-0.6 (3.0)	-1.1 (2.5)	-0.1 (3.4)	0.94

Descriptives are presented as means (S.D.). FM: medial femur, FL: lateral femur, TM: medial tibia, TL: lateral tibia. tpostt0-15-30: morphology measured within maximum 90 sec following cessation of exercise (tpostt0) and at 15-30minutes after onset of tpostt0 respectively. [¶]Significant difference relative to baseline *within* groups at α<0.05. P-values are adjusted for multiple comparisons of main effects and confounding of age and BMI. [$] Co-variates appearing in the model are evaluated at the following values: Age=50.0, BMI=25.6.

Table 3. *In vivo* cartilage deformational patterns. Sub-regional analysis of 3D volume changes relative to baseline at tpostt0 within groups[$]

Sub-region	Change at tpostt0 in controls (N=18)	P-value within controls	Change at tpostt0 in patients (N=18)	*P*-value within patients
FMA	-7.1 (3.6)	<0.001[¶]	-7.1 (3.0)	<0.001[¶]
FMC	-7.0 (3.0)	<0.001[¶]	-7.1 (3.6)	<0.001[¶]
FMP	-10.6 (8.6)	0.001[¶]	-13.8 (11.6)	0.014[¶]
FLA	-2.7 (3.4)	0.028[¶]	-3.0 (3.4)	0.001[¶]
FLC	-5.1 (3.4)	<0.001[¶]	-5.4 (3.4)	<0.001[¶]
FLP	-5.0 (3.6)	<0.001[¶]	-5.7 (3.4)	<0.001[¶]
TMA	-20.2 (2.7)	<0.001[¶]	-20.5 (2.4)	<0.001[¶]
TMC	-5.5 (3.6)	<0.001[¶]	-5.8 (3.2)	<0.001[¶]
TMP	-12.0 (3.4)	<0.001[¶]	-12.3 (3.0)	<0.001[¶]
TLA	-11.5 (1.8)	<0.001[¶]	-12.5 (1.9)	<0.001[¶]
TLC	-3.5 (2.0)	<0.001[¶]	-4.6 (1.2)	<0.001[¶]
TLP	-6.2 (2.4)	0.003[¶]	-7.9 (2.2)	0.004[¶]

Descriptives are presented as means (S.D.). FMA: antero-medial femur, FMC: centro-medial femur, FMP: postero-medial femur. FLA: antero-lateral femur, FLC: centro-lateral femur, FLP: postero-lateral femur. TMA: antero-medial tibia, TMC: centro-medial tibia, TMP: postero-medial tibia. TLA: antero-lateral tibia, TLC: centro-lateral tibia, TLP: postero-lateral tibia. tpostt0: morphology measured within maximum 90 sec following cessation of exercise. [¶]Significant difference relative to baseline at α<0.05. *P*-values are adjusted for multiple comparisons of main effects and confounding of age and BMI. [$] Co-variates appearing in the model are evaluated at the following values: Age=50.0, BMI=25.6.

DISCUSSION

The main purpose of this study was to investigate tibiofemoral cartilage deformational responses including recovery after a 30-repetition squat exercise in subjects with osteoarthritic cartilage degeneration (i.e., up to radiographic signs of mild OA, K/L 1-2) compared with middle-aged referents (i.e., no radiographic signs of OA and no cartilage defects on MRI). The principal finding was that, despite a tendency towards more deformation in the patient group, no significant differences in volume decrease immediately after the exercise could be revealed between the groups. Additionally, both groups displayed similar spatial deformational patterns. Interestingly, recovery tended to occur slower in patients, requiring at least 15 minutes after exercise cessation for all cartilage plates to restitute to baseline volumes.

In the present study, mean cartilage deformation in patient tibiofemoral compartments ranged from -3.1% up to -3.9%. To the best of our knowledge, this is the first report on the effects of an *in vivo* weight-bearing dynamic exercise on deformational behavior of human osteoarthritic cartilage. Two previous studies including a patient population with K/L 2 to 4[27] and K/L 2 and 3[32], addressed tibiofemoral morphological changes after a static load applied onto a 20° flexed knee. Relative changes ranged from +1.92% to -7.85%. Static loading is described to convey more deformation than dynamic loading which might explain the broader range in outcomes observed in the static experiments. Gradually applied static loads allow cartilage deformational responses to more easily adapt to the imposed load which leads to larger deformations of the tissue without a considerable pressure surge within its matrix.[36, 44, 45, 55, 56] *In vitro* experiments in healthy and osteoarthritic cartilage revealed that dynamic intermittent loading protocols might up-regulated matrix synthesis, while in contrast, static and injurious impacts tended to decrease the production of matrix compounds and to stimulate protease activity, exerting a deleterious effect on cartilage quality.[56-58] Therefore, in view of clinical practice, the present study preferred the investigation of dynamic exercise.

In the middle-aged reference group, mean 3D volume decreases of -1.4% to -3.2% were observed. In young adults, a similar exercise yielded mean 3D volume changes of +0.1% up to -3.9% in tibiofemoral compartments.[44-46] Interestingly, the deformational outcomes of both controls and patients lie within the ranges established in young adults. In fact, current mean differences between both groups at deformation attained 1.7% at the most which does not meet the required difference of ~3%. However, a tendency was noted towards more deformation in the patients, especially in FL and TL. Although not involved on radiography, baseline biochemical T2 maps showed increased T2 values in FL and TL (i.e. respectively 37.4 (4.0) msec versus 40.1 (5.9) msec, and 27.4 (4.8) msec versus 32.3 (6.2) msec in controls versus

patients). Increases in T2 value are associated with early degeneration even before macroscopic changes are present.[43] An *ex vivo* study in uni-

compartmental OA confirmed that cartilage in unaffected compartments is mechanically inferior to normal cartilage despite sound clinical, radiological and morphological appearances.[59] Hence, tendencies towards ultra-structural deterioration in these compartments possibly brought about the larger volume decreases immediately after the exercise. Interestingly, although this study included patients with medial compartmental radiographic signs of OA, this study did not reveal between-groups differences for the medial cartilage plates either. In contrast, in previous static *in vivo* loading experiments, medial compartments in the OA patients were driving the larger morphology decreases.[27, 32] However, those particular studies included patients with K/L-grades of at least 2 as opposed to a K/L-grade of maximum 2 in the present study, and thus, displayed more advanced disease possibly enabling more evident differences to be established between groups.

In agreement with Cotofana et al.,[27] sub-regional spatial deformational patterns were similar for the healthy and diseased knees,[27] exhibiting the highest deformation in posterior femoral condyles and anterior tibial plateaus. Kinematic analyses showed that during increasing knee flexion, tibiofemoral contact areas shifted to the posterior femur.[60, 61] While this observation might explain the current spatial femoral patterns, the anteriorly directed deformational patterns on the tibial plateaus may result from altered tibial rotation during knee flexion in increasing age and OA.[62] In healthy subjects, next to femoral roll-and-glide motion and tibial valgus, coupled tibial internal rotation accounts for increased anterior and posterior load on the medial and lateral tibial cartilage respectively. In older OA subjects, decreased axial rotation was described with more apparent diminished rollback of the lateral femur over the tibial plateau.[62] Therefore, one may hypothesize that tibiofemoral contact may have occurred more anteriorly during flexion movements increasing the load on the anterior regions of both the tibial cartilage plates.

Early recovery encompasses the most important and critical changes after pressure release.[38, 63] Recovery appeared similar in both groups (i.e., mean between-group differences of 0.2% to 2.3% do not reach or exceed the ~3% precision error). However, the course of volume changes as presented in Table 2 suggests a tendency towards delayed recovery in the OA group when compared to controls. Recovery required at least 15 minutes for all tibiofemoral cartilage plates to restitute to baseline morphological status, including the lateral knee compartment. Delayed recovery might induce a state of maintained deformation and dehydration which may have deleterious effects on chondrocyte metabolism.[28, 29] As such, hasty load repetitions may potentially induce a negative vicious circle towards progressive degeneration.

The results of this study should be interpreted in view of the relatively limited sample size and limited generalizability of the findings. Individuals were recruited with radiographic signs of doubtful-to-mild OA, with low levels of pain and the majority being male.

The current study intentionally did not recruit subjects with moderate and severe OA (i.e., K/L 3,4). Due to the heterogeneous symptomatic and structural OA presentation, effects of exercise are advised to be investigated in sub-groups rather than the aggregate OA group.[64] While it was suggested to shift the focus of OA management also to individuals at increased risk for OA development or progression rather than only to those with established disease,[65] experimentally induced OA rat models showed that exercise initially led to suppression of inflammation and promotion of matrix synthesis. When OA was more progressed over time, exercise effects appeared similar to those in unexercised joints or appeared to aggravate catabolic responses promoting joint deterioration.[66, 67] As endorsed by the sub-regional analysis, the current squatting exercise induced general dynamic joint loading which may facilitate matrix synthesis.[56-58] While from a clinical point of view this exercise is usually not included in exercise programs for patients with advanced and severe OA, unstable and/or malaligned knees, and/or patellofemoral arthritis because of pain aggravation, it is commonly incorporated in therapeutic programs to rehabilitate neuromuscular control and functional strength in patients with meniscal degeneration (e.g., SCOPEX trial including patients with K/L grade 1-2) - as also recruited in this study - or following partial meniscectomy.[10, 30, 68, 69] Whereas in these particular patient populations with doubtful-to-mild OA, weight-bearing exercises such as squatting showed to improve physical function and potentially cartilage integrity,[10, 30, 69, 70] these subjects, in turn, are at higher risk for accelerated OA progression.[31]

At the time of study, however, patients did not exhibit considerable levels of pain. In view of the clinical presentation of patients that seek treatment, one may question the relevance of the currently investigated population. As disease perception does not correlate well with joint health status,[7, 8] symptoms are known to fluctuate over time, and to display large inter-individual variations.[71, 72] In fact, current constructs of pain intensity (i.e., VAS and WOMAC) were based on a 1-week history at the most and were collected at the time of study. Hence, the pain *intensity* measures do not cover the patients' entire history of symptomatic knee OA. While the patients were recruited from an outpatient setting at our university hospital - and thus did seek treatment for their condition - and mean duration of self-reported knee symptoms equaled 40 months, the clinical relevance of the present patient population is supported.

The clinical relevance of this study entails that in case weight-bearing exercises such as squatting are considered clinically feasible and applied in a middle-aged patient with doubtful-to-mild OA as described above, awareness should be raised amongst clinicians for the discordance between symptomatic responses and potentially disproportionate cartilage deformational behavior that may incite a downward spiral towards accelerated cartilage

degeneration. Hence, the present results may have implications on exercise therapy concerning the design of treatment programs in these particular patient groups. Ideally, after a full weight-bearing 30-repetition squat, middle-aged individuals should calculate in ~15 minutes for tibiofemoral cartilage volumes to recover, especially in case of doubtful to mild radiographic OA. Hence, cartilage recovery can sufficiently proceed suggested to protect against (progressive) deterioration.[28, 29] Translation of these findings into clinical practice may entail (1) shorter exercise sessions but dispersed with a higher frequency over the course of day, (2) alternation between weight-bearing and non weight-bearing exercise, (3) (alternated) use of assisted weight-bearing exercises (i.e., seated leg-press, assisted weight-bearing squat under vertical pulley apparatus, aquatic exercise, etc.). Nonetheless, future research should continue to investigate the effect of structured therapeutic exercise regimen on cartilage structural integrity in the longer term.

Finally, the present gender distribution does not concur with the typical presentation of OA in the community where higher prevalences are recorded amongst women as opposed to men.[73] In the younger age ranges (<63 years old) as is the case in the present study (i.e., 42-65 years of age), epidemiological reports conversely describe higher prevalences and/or incidences in men supporting the validity of the current study population in this developmental OA phase.[73-75] Nonetheless, present analyses took gender distribution into account and the direction of the main results is in agreement with studies including all-women OA samples.[27]

CONCLUSION

Following a 30-repetition squat exercise, tibiofemoral cartilage deformation appeared similar in magnitude (within the measurement error) and spatial pattern between middle-aged subjects with and without doubtful-to-mild radiographic tibiofemoral osteoarthritis (i.e., K/L grade 1-2). Restitution of volumes warranted a 15 minute-recovery after the exercise, especially in the subjects with osteoarthritic cartilage degeneration. In terms of prevention of accelerated OA progression, these results may have implications on dosing and grading in exercise therapy in individuals with doubtful-to-mild OA for whom weight-bearing exercise is considered clinically feasible.

ACKNOWLEDGMENTS

REFERENCES

1. Bennell K, Hinman R. Exercise as a treatment for osteoarthritis. Curr Opin Rheumatol. 2005;17(5):634-640.
2. Bennell KL, Hinman RS. A review of the clinical evidence for exercise in osteoarthritis of the hip and knee. J Sci Med Sport. 2011;14(1):4-9.
3. Fitzgerald GK, Oatis C. Role of physical therapy in management of knee osteoarthritis. Curr Opin Rheumatol. 2004;16(2):143-147.
4. Baker K, McAlindon T. Exercise for knee osteoarthritis. Curr Opin Rheumatol. 2000;12(5):456-463.
5. Fransen M, McConnell S. Exercise for osteoarthritis of the knee. Cochrane Database Syst Rev. 2008(4):CD004376.
6. Bischoff HA, Roos EM. Effectiveness and safety of strengthening, aerobic, and coordination exercises for patients with osteoarthritis. Curr Opin Rheumatol. 2003;15(2):141-144.
7. Lane NE, Brandt K, Hawker G, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarthritis Cartilage. 2011;19(5):478-482.
8. Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol. 2000;27(6):1513-1517.
9. Tiderius CJ, Svensson J, Leander P, Ola T, Dahlberg L. dGEMRIC (delayed gadolinium-enhanced MRI of cartilage) indicates adaptive capacity of human knee cartilage. Magn Reson Med. 2004;51(2):286-290.
10. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum. 2005;52(11):3507-3514.
11. Hovis KK, Stehling C, Souza RB, et al. Physical activity is associated with magnetic resonance imaging-based knee cartilage T2 measurements in asymptomatic subjects with and those without osteoarthritis risk factors. Arthritis Rheum. 2011;63(8):2248-2256.
12. Stahl R, Luke A, Li X, et al. T1rho, T2 and focal knee cartilage abnormalities in physically active and sedentary healthy subjects versus early OA patients--a 3.0-Tesla MRI study. Eur Radiol. 2009;19(1):132-143.
13. Hanna F, Teichtahl AJ, Bell R, et al. The cross-sectional relationship between fortnightly exercise and knee cartilage properties in healthy adult women in midlife. Menopause. 2007;14(5):830-834.
14. Racunica TL, Teichtahl AJ, Wang Y, et al. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum. 2007;57(7):1261-1268.
15. Teichtahl AJ, Wluka AE, Forbes A, et al. Longitudinal effect of vigorous physical activity on patella cartilage morphology in people without clinical knee disease. Arthritis Rheum. 2009;61(8):1095-1102.
16. Teichtahl AJ, Wluka AE, Wang Y, et al. Effect of long-term vigorous physical activity on healthy adult knee cartilage. Med Sci Sports Exerc. 2012;44(6):985-992.
17. Doré DA, Winzenberg T, Ding C, et al. The association between objectively measured physical activity and knee structural change using MRI. Ann Rheum Dis. 2012.
18. Helmark IC, Mikkelsen UR, Borglum J, et al. Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. Arthritis Res Ther. 2010;12(4):R126.
19. Helmark IC, Petersen MC, Christensen HE, Kjaer M, Langberg H. Moderate loading of the human osteoarthritic knee joint leads to lowering of intraarticular cartilage oligomeric matrix protein. Rheumatol Int. 2012;32(4):1009-1014.
20. Chua SD, Jr., Messier SP, Legault C, Lenz ME, Thonar EJ, Loeser RF. Effect of an exercise and dietary intervention on serum biomarkers in overweight and obese adults with osteoarthritis of the knee. Osteoarthritis Cartilage. 2008;16(9):1047-1053.
21. Petersen SG, Saxne T, Heinegard D, et al. Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training. Osteoarthritis Cartilage. 2010;18(1):34-40.
22. Bautch JC, Clayton MK, Chu Q, Johnson KA. Synovial fluid chondroitin sulphate epitopes 3B3 and 7D4, and glycosaminoglycan in human knee osteoarthritis after exercise. Ann Rheum Dis. 2000;59(11):887-891.
23. Woollard JD, Gil AB, Sparto P, et al. Change in knee cartilage volume in individuals completing a therapeutic exercise program for knee osteoarthritis. J Orthop Sports Phys Ther. 2011;41(10):708-722.
24. Loeser RF, Jr. Aging and the etiopathogenesis and treatment of osteoarthritis. Rheum Dis Clin North Am. 2000;26(3):547-567.
25. Silver FH, Bradica G, Tria A. Viscoelastic behavior of osteoarthritic cartilage. Connect Tissue Res. 2001;42(3):223-233.
26. Bank RA, Soudry M, Maroudas A, Mizrahi J, TeKoppele JM. The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation. Arthritis Rheum. 2000;43(10):2202-2210.
27. Cotofana S, Eckstein F, Wirth W, et al. In vivo measures of cartilage deformation: patterns in healthy and osteoarthritic female knees using 3T MR imaging. Eur Radiol. 2011;21(6):1127-1135.
28. Van Ginckel A, Verdonk P, Victor J, Witvrouw E. Cartilage Status in Relation to Return to Sports After Anterior Cruciate Ligament Reconstruction. Am J Sports Med. 2013.

29. Song Y, Greve JM, Carter DR, Giori NJ. Meniscectomy alters the dynamic deformational behavior and cumulative strain of tibial articular cartilage in knee joints subjected to cyclic loads. Osteoarthritis Cartilage. 2008;16(12):1545-1554.

30. Stensrud S, Roos EM, Risberg MA. A 12-Week Exercise Therapy Program in Middle-Aged Patients With Degenerative Meniscus Tears: A Case Series With 1-Year Follow-up. J Orthop Sport Phys. 2012;42(11):919-931.

31. Cibere J, Sayre EC, Guermazi A, et al. Natural history of cartilage damage and osteoarthritis progression on magnetic resonance imaging in a population-based cohort with knee pain. Osteoarthritis Cartilage. 2011;19(6):683-688.

32. Subburaj K, Souza RB, Stehling C, et al. Association of MR relaxation and cartilage deformation in knee osteoarthritis. J Orthop Res. 2012;30(6):919-926.

33. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986;29(8):1039-1049.

34. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16(4):494-502.

35. Peterfy CG, Guermazi A, Zaim S, et al. Whole-organ magnetic resonance imaging score (WORMS) of the knee in osteoarthritis. Osteoarthritis Cartilage. 2004;12(3):14.

36. Van Ginckel A, Almqvist F, Verstraete K, Roosen P, Witvrouw E. Human ankle cartilage deformation after different in vivo impact conditions. Knee Surg Sports Traumatol Arthrosc. 2011;19(1):137-143.

37. Van Ginckel A, Baelde N, Almqvist KF, Roosen P, McNair P, Witvrouw E. Functional adaptation of knee cartilage in asymptomatic female novice runners compared to sedentary controls. A longitudinal analysis using delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC). Osteoarthritis Cartilage. 2010;18(12):1564-1569.

38. Van Ginckel A, Roosen P, Almqvist KF, Verstraete K, Witvrouw E. Effects of in vivo exercise on ankle cartilage deformation and recovery in healthy volunteers: an experimental study. Osteoarthritis Cartilage. 2011;19(9):1123-1131.

39. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982;36(5):936-942.

40. Guermazi A, Niu J, Hayashi D, et al. Prevalence of abnormalities in knees detected by MRI in adults without knee osteoarthritis: population based observational study (Framingham Osteoarthritis Study). BMJ. 2012;345:e5339.

41. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):401-406.

42. Bingham JT, Papannagari R, Van de Velde SK, et al. In vivo cartilage contact deformation in the healthy human tibiofemoral joint. Rheumatology. 2008;47(11):1622-1627.

43. Apprich S, Mamisch TC, Welsch GH, et al. Quantitative T2 mapping of the patella at 3.0T is sensitive to early cartilage degeneration, but also to loading of the knee. Eur J Radiol. 2012;81(4):e438-443.

44. Eckstein F, Lemberger B, Gratzke C, et al. In vivo cartilage deformation after different types of activity and its dependence on physical training status. Ann Rheum Dis. 2005;64(2):291-295.

45. Eckstein F, Lemberger B, Stammberger T, Englmeier KH, Reiser M. Patellar cartilage deformation in vivo after static versus dynamic loading. J Biomech. 2000;33(7):819-825.

46. Hudelmaier M, Glaser C, Hohe J, et al. Age-related changes in the morphology and deformational behavior of knee joint cartilage. Arthritis Rheum. 2001;44(11):2556-2561.

47. Sled EA, Khoja L, Deluzio KJ, Olney SJ, Culham EG. Effect of a home program of hip abductor exercises on knee joint loading, strength, function, and pain in people with knee osteoarthritis: a clinical trial. Phys Ther. 2010;90(6):895-904.

48. Witvrouw E, Danneels L, Thijs Y, Cambier D, Bellemans J. Does soccer participation lead to genu varum? Knee Surg Sports Traumatol Arthrosc. 2009;17(4):422-427.

49. Neeb TB, Aufdemkampe G, Wagener JH, Mastenbroek L. Assessing anterior cruciate ligament injuries: the association and differential value of questionnaires, clinical tests, and functional tests. J Orthop Sports Phys Ther. 1997;26(6):324-331.

50. Roorda LD, Jones CA, Waltz M, et al. Satisfactory cross cultural equivalence of the Dutch WOMAC in patients with hip osteoarthritis waiting for arthroplasty. Ann Rheum Dis. 2004;63(1):36-42.

51. Hayes RD, Shelbourne CD, Mazel RM. The RAND 36-Item Health survey 1.0. Health Econ. 1993;2:217-227.

52. Bowers ME, Trinh N, Tung GA, Crisco JJ, Kimia BB, Fleming BC. Quantitative MR imaging using "LiveWire" to measure tibiofemoral articular cartilage thickness. Osteoarthritis Cartilage. 2008;16(10):1167-1173.

53. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage. 2006;14 Suppl A:A46-75.

54. Wirth W, Eckstein F. A technique for regional analysis of femorotibial cartilage thickness based on quantitative magnetic resonance imaging. IEEE Trans Med Imaging. 2008;27(6):737-744.

55. Herberhold C, Faber S, Stammberger T, et al. In situ measurement of articular cartilage deformation in intact femoropatellar joints under static loading. J Biomech. 1999;32(12):1287-1295.

56. Suh JK, Li Z, Woo SL. Dynamic behavior of a biphasic cartilage model under cyclic compressive loading. J Biomech. 1995;28(4):357-364.

57. Ramage L, Nuki G, Salter DM. Signalling cascades in mechanotransduction: cell-matrix interactions and mechanical loading. Scand J Med Sci Sports. 2009;19(4):457-469.

58. Jeon J, Schrobback K, Hutmacher D, Klein T. Dynamic compression improves biosynthesis of human zonal chondrocytes from osteoarthritis patients. J Tissue Eng Regen Med. 2012;6:70-70.

59. Obeid EM, Adams MA, Newman JH. Mechanical properties of articular cartilage in knees with unicompartmental osteoarthritis. J Bone Joint Surg Br. 1994;76(2):315-319.

60. von Eisenhart-Rothe R, Siebert M, Bringmann C, Vogl T, Englmeier KH, H G. A new in vivo technique for determination of 3D kinematics and contact areas of the patello-femoral and tibio-femoral joint. J Biomech. 2004;37(6):927-937.

61. Patel VV, Hall K, Ries M, et al. A three-dimensional MRI analysis of knee kinematics. J Orthop Res. 2004;22(2):283-292.

62. Scarvell JM, Smith PN, Refshauge KM, Galloway HR. Magnetic resonance imaging analysis of kinematics in osteoarthritic knees. J Arthroplasty. 2007;22(3):383-393.

63. Rubenstein JD, Kim JK, Henkelman RM. Effects of compression and recovery on bovine articular cartilage: appearance on MR images. Radiology. 1996;201(3):843-850.

64. Minor MA. Physical activity and knee osteoarthritis: answers and questions. Arthritis Rheum. 2007;57(1):1-2.

65. Hunter DJ. Lower extremity osteoarthritis management needs a paradigm shift. Br J Sports Med. 2011;45(4):283-288.

66. Nam J, Perera P, Liu J, Butterfield T, Agarwal S. Effects of exercise on progression of OA in knee joints. Osteoarthritis Cartilage. 2010;18(S2):27.

67. Perera P, Nam J, Friezner S, Agarwal S. Exercise or not to exercise: a genome wide analysis of effects of exercise on early and late OA. Osteoarthritis Cartilage. 2009;17(S1):21.

68. Hall M, Hinman RS, Wrigley TV, et al. The effects of neuromuscular exercise on medial knee joint load post-arthroscopic partial medial meniscectomy: 'SCOPEX' a randomised control trial protocol. BMC Musculoskelet Disord. 2012;13:233.

69. Thorstensson CA, Henriksson M, von Porat A, Sjodahl C, Roos EM. The effect of eight weeks of exercise on knee adduction moment in early knee osteoarthritis--a pilot study. Osteoarthritis Cartilage. 2007;15(10):1163-1170.

70. Coleman EA, Buchner DM, Cress ME, Chan BK, de Lateur BJ. The relationship of joint symptoms with exercise performance in older adults. J Am Geriatr Soc. 1996;44(1):14-21.

71. Paradowski PT, Englund M, Roos EM, Lohmander LS. Similar group mean scores, but large individual variations, in patient-relevant outcomes over 2 years in meniscectomized subjects with and without radiographic knee osteoarthritis. Health Qual Life Outcomes. 2004;2:38.

72. Paradowski PT, Englund M, Lohmander LS, Roos EM. The effect of patient characteristics on variability in pain and function over two years in early knee osteoarthritis. Health Qual Life Outcomes. 2005;3:59.

73. Felson DT, Zhang YQ. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum. 1998;41(8):1343-1355.

74. Felson DT. The Epidemiology of Knee Osteoarthritis - Results from the Framingham Osteoarthritis Study. Semin Arthritis Rheum. 1990;20(3):42-50.

75. Bijlsma JW, Knahr K. Strategies for the prevention and management of osteoarthritis of the hip and knee. Best Pract Res Clin Rheumatol. 2007;21(1):59-76.
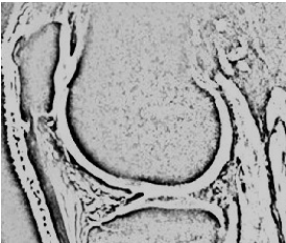
GENERAL DISCUSSION

1 Summary of the findings, implications and reflections

The main goal of this dissertation was to contribute to the understanding of how *in vivo* exercise or exercise implementation may affect protection of articular cartilage. To this end, the effect of *in vivo* exercise on cartilage deformation and functional adaptation was addressed in healthy volunteers (Part 1), and in individuals at increased risk for – or diagnosed with signs of early radiographic OA (i.e., K/L grade of maximum 2) (Part 2). In this way, 3 important phases in the OA developmental process were investigated.

1.1 Part 1 – Exercise and chondroprotection: a fundamental approach

From the intriguing observation of the upper ankle being rarely afflicted with idiopathic OA when compared to the knee, in **chapter 1**, ankle cartilage deformational behavior was investigated after different *in vivo* impact conditions. In general, cartilage biphasic behavior was apparent in terms of more deformation following a static load and a tendency towards larger volume decreases after repetitive high impact loads.[1] Although duration of the load was not uniform across exercises, deformational outcomes are primarily driven by type of loading activity rather than "absolute duration" (data not published: partial correlation between duration of the activity and deformational outcome (i.e., volume percentage change) controlled for type of activity (i.e., exercise 1 to 4): r=0.081 and *P*=0.59). Interestingly, unlike the reported ankle (talar) cartilage *in vitro* increased compressive stiffness,[2] the monitored mean talar volume decreases of 7.2-14.6% in chapters 1 and 2 suggested considerable deformation when compared to the available reports on *in vivo* tibiofemoral cartilage deformation in healthy adults.[1, 3, 4] In agreement, in chapter 6, a similar exercise yielded smaller deformational outcomes in the tibiofemoral compartments of healthy referents (i.e., 2.8-3.8%) when compared to the tibiotalar layers. The extent of loaded surface areas was proposed to mainly drive the volumetric changes noted. This suggestion was corroborated in **chapter 2** by means of a moderate-to-strong positive correlation between "degree of deformation" and "surface areas involved". Additionally, whereas in chapter 6 tibiofemoral cartilage plates recovered within 15 minutes after a similar exercise, the relatively slow (i.e., 30 min) recovery process observed in the talar cartilage layers supported the role of surface area rather than local thickness change in the deformational outcomes (as is also illustrated in Figure 3 of chapter 2). In view of optimal chondrocyte metabolism as put forward in the outline of this dissertation, one might argue that the talar cartilage 30-min recovery may be disadvantageous when compared to the ~15-min recovery noted in the knee joints in chapter 6. In this respect, it is important to mention that – as was observed in chapter 2 – in healthy cartilage plates recovery proceeds gradually approximately linear in time and is suggested proportionate to the degree of *in vivo* deformation.[5] In contrast, in chapter 6, the patients presenting with early, or doubtful-to-mild, radiographic OA showed disproportionate recovery patterns when compared to the healthy referents. In fact, while our data

and the available literature[2, 6] more likely point towards the role of surface area in the measured deformational outcomes, during deformation and potentially slower recovery, the upper ankle matrix constituents are protected against excessive strain.

Based on the results of chapters 1 and 2, from a preventative point of view, one might propose that generalized joint load directed by surface areas involved is in part responsible for tissue conditioning that aids in protection from progressive cartilage deterioration. Hence, *in vivo* exercise may need to encompass this feature when cartilage quality preservation is pursued. Ankle cartilage behavior under load and matrix composition, however, only partly guard the joint from idiopathic OA.[2] Hence, in view of the role of generalized joint load in cartilage health, circumspection is advised when translating these features directly onto the knee. Additionally, the studies in chapter 1 and 2 were cross-sectional in nature, hampering cause-effect relationships to be drawn.

Therefore, in a longitudinal analysis, **chapter 3** addressed the fundamental question whether mature knee cartilage is able to functionally adapt to a longer-term regimen of *in vivo* load. Hence, relative PG turn-over was estimated using dGEMRIC (i.e., dGEMRIC index) to evaluate cartilage conditioning in response to a supervised and standardized 10-week STR novice runner program. A chondroprotective effect of the running scheme was suggested in that an increase in dGEMRIC index was registered in the runners when compared to sedentary controls over the 10-week period, endorsed by a positive correlation between dGEMRIC index change and physical activity change. While the effect was measured in a clinically relevant femoral cartilage region[7-9] and population, the running scheme proves potentially valuable in strategies aiming at matrix quality maintenance and may support the promotion of *regular* exercise in our - often sedentary - Western lifestyles.[7] In Western society, many people exercise in short sharp bursts, often undertaking or trying out different sports, interspersed with (long) periods of inactivity.[7] The resulting decreased dGEMRIC indices are associated with decreased compressive stiffness[10] not adequately suited to cushion nonprevalent increased stress and may predispose to degenerative changes and eventually OA.[7] As a beneficial response is possible, the question arises whether clinicians or health-care professionals should recommend *everybody* to take up running or enroll in a novice runner program. The conclusions in chapter 3 are confined to relatively young women without a history of knee surgery or internal derangements and inexperienced in (running) sports. Although participation in vigorous sports (including running) may initially slow down cartilage volume loss or development and worsening of cartilage defects,[11,12, 13] it remains unclear how the STR scheme would affect structural outcomes in inexperienced novice runners with existing cartilage or OA disease. Whereas older aged knees may exhibit limited functional adaptive capacity with similar to (tendencies of) decreased deformation during running compared to younger adults,[14, 15] the impacts imposed upon the joint during running may be hypothesized to accelerate cartilage deterioration when tissue homeostasis failure precludes adequate up-regulation of PG synthesis. Overall, the STR may be

implemented in view of primary OA prevention in the "healthy and inexperienced" aged below 40 years old. Although prolonged recreational running may come along with a limited risk for future radiographic OA development,[16, 17] the preventative value of the STR requires confirmation in larger samples including longer training periods. In this respect, the results of chapter 3 cannot discern the PG up-regulation as a sign of prolonged protection from an omen of tissue homeostasis failure. Future research should also implement markers able to detect matrix deterioration prior to actual GAG depletion[18] and should aim at identifying those individuals susceptible to accelerated cartilage deterioration. Risk factor identification may include intrinsic factors (e.g., muscle strength and co-activation, limb alignment, hormonal status, BMI, etc. ; see "General methodological strengths and limitations") as well as extrinsic factors (e.g., running surface and shoes). While in chapter 3 environmental factors such as shoe en running surface were controlled for (i.e., all runners wore the same type of neutral running shoe and during the group sessions ran on the same surface), it remains to be investigated how different running surfaces and running shoes may affect lower limb kinematics and kinetics, and hence, may influence cartilage integrity over time.[19-22] Additionally, it should be mentioned that the STR program is organized in several track and field clubs across the region of Flanders and is accessible to the entire public. Based on past research experiences in this population, participants are usually of normal weight or overweight.[23, 24] As increased BMI is a risk factor for OA onset and progression both from a biomechanical (i.e., overload of the (malaligned) joint) and biochemical (i.e., adipose tissue releases adipokines contributing to the inflammatory component of OA) point of view,[25-29] one would not recommend overweight or obese individuals to participate in such a program. Apart from 1 runner, all runners, however, showed a positive increase in dGEMRIC index after the 10-week running program and BMI did not differ between and within groups. Hence, one may suggest that BMI was no confounding factor in the present comparison. However, as listed below (i.e., "General methodological strengths and limitations"), the effect of BMI on longitudinal cartilage change in running remains to be investigated both in the short as well as in the longer term.

1.2   Part 2 – Exercise and chondroprotection: clinical implementation in individuals at increased risk for - or diagnosed with early radiographic OA

To gain more insight into the effect of *in vivo* exercise or exercise implementation on cartilage quality preservation in an at-risk population, the systematic literature review in **chapter 4** described the evolution of MRI-measured cartilage adaptation after ACL injury and reconstruction. Although few in number, the first high quality reports on this subject determined that progression of cartilage semi-quantitative and quantitative morphology required on average 2 years before clearly detectable by MRI. Whereas during the first year following injury, morphological changes showed more pronounced in ACL-reconstructed knees when compared to nonsurgical options, so far - apart from 1 study in isolated reconstructed knees - longer follow-up periods could not convincingly

assign a treatment option as superior over the other in terms of cartilage quality decline. In view of prevention of progressive cartilage deterioration, the importance of molecular MR imaging methods was underlined in the detection of ultra-structural change prior to or along with macroscopic lesions or morphometric cartilage change. In both lateral and medial knee compartments, ultra-structural decline was monitored following injury onwards. Although failing, the lateral compartment displayed initial attempts to recover where cartilage in the medial compartment remained aberrant in terms of biochemical composition, tissue resiliency and morphology. Although risk factors are not limited to this list, moderate-to-strong evidence was provided for meniscal lesions/meniscectomy, BML, persistent altered biomechanics and length of follow-up as affecting rate of cartilage change.

Although the epidemiological literature noted that not all patients necessarily resume pre-injury sports level after the recommended 3 to 9 month recovery periods,[30-33] the natural course of cartilage adaptation following injury and reconstruction suggests that cartilage structural integrity in the early year(s) following injury or surgery may not be suited to cushion the loads during sports.[34, 35] This assumption may explain why lateral compartment restitution fails, medial compartment degeneration continues and BMLs only slowly restitute or appear again in a 1 to 2 year follow-up after injury.[34] Depending upon type of sports resumed (i.e., low risk vs high risk), return to sports recommendations mainly rely on surgical and rehabilitation variables (i.e., graft type, graft fixation, laxity, symptoms and functional status) aiming to reduce the risk of graft failure, subsequent giving way episodes and/or re-injury.[36-39] Taking into account the evolution of cartilage adaptation, return to sports approvals are proposed to consider the structural integrity of the cartilage and joint as well especially when the identified risk factors are present. Although altered biomechanics (apart from passive knee laxity) was not evaluated, at 6 months from surgery, **chapter 5** revealed decreased cartilage resiliency (i.e., delayed recovery of baseline cartilage morphology) after a 30-min run combined with diminished cartilage quality in terms of biochemical composition in isolated ACL-reconstructed knees when compared to uninjured controls. While these cartilaginous abnormalities were apparent even in the absence of BML and considerable meniscal dysfunction at the time of study, deleterious effects of the running activity on deformational outcomes were aggravated in those patients who resumed sports before 5 months after surgery and those operated on within 10 weeks from injury. Additionally, an early return to sports (before 5 months) showed an association with increased cartilage volume and thickness at 6 months after surgery denoted as early arthritic changes.

Considering the functional and symptomatic status of the patient, generally, only safe and low-risk activities (e.g., running) are allowed in between 3 to 6 months timespan after surgery.[30, 40] In view of the results described in chapters 4 and 5, the suggestion to consider prolonged rehabilitation of ACL-reconstructed knees in terms of delayed implementation of sports-specific activities in the late post-operative phase may be supported,[33, 34, 41] even despite potential improvements in functional outcome. In fact, moderate-to-strong correlations were noted between higher LSI % and Tegner

levels on the one hand and the suggested deleterious cartilage outcomes on the other hand at the time of study in chapter 5 (data not published: LSI% and T2* TM: r_s=-0.56 P=0.03; Tegner level and TL volume: r_s=0.58 P=0.024; Tegner level and T2 TM: r_s=0.52 P=0.046; Tegner level and thickness FL: r_s=0.53 P=0.044; Tegner level and thickness FM: r_s=0.55 P=0.035). In agreement, in patients who were considered fully functional, without pivoting/giving-way episodes, normal clinical examination findings, and had not decreased their activity levels at 4 years follow-up, a recent cohort study revealed differences in patient-reported outcomes (i.e., more pain and decreased quality of life) and disadvantageous biomarker collagen cleavage-to synthesis ratios between healthy control participants and ACL-reconstructed patients with abnormal joint space widths (not present at baseline).[42] Although controlled therapeutic exercise may positively influence dGEMRIC indices in an at risk-population,[9] it remains to be investigated whether a gradually built-up running scheme as often implemented in late post-operative rehabilitation or return to sports in ACL injury and reconstruction, may contribute to or brake cartilage quality decline over time as described in chapters 4 and 5. In this respect, the outcomes of chapter 3 assume native knee biomechanics during running. Analysis of joint kinematics and contact paths during running activity revealed larger sliding motions in the medial knee compartment when compared to the lateral that were associated with the extent of tibial internal rotation.[43] As described in chapter 4, persistent altered biomechanics including rotational instability is reported amongst ACL-injured or reconstructed knees often accompanied by BML or meniscal lesions. Although the course of BML nor inflammatory parameters were monitored in between injury and the time of study in chapter 5, similar to delayed surgery, early surgery (within 10 weeks) combined with potential rotational instability may increase the joint's vulnerability furthering a deleterious cartilaginous response when engaging in sports within the considered 3 to 6-months period after surgery.[34, 35]

The choice for treatment (surgical or nonsurgical) after ACL injury is currently hotly debated and relies on many factors such as the patient's clinical status, willingness to engage in high-risk sports, non-coping with conservative treatment or the surgeon's preference.[44-47] While the study design in chapter 5 did not include nonsurgical patients for comparison, no conclusion regarding treatment effects can be drawn in terms of cartilage quality (and prevalence of subsequent meniscal lesions) and return to sports. Additionally, the number of studies in chapter 4 that compared cartilage adaptations in ACL reconstruction versus ACL deficiency is small, hence, providing limited evidence for a potential treatment effect. Consequently, the importance of larger scaled studies is highlighted to confirm the results.

In case of choice for surgery, the proposed delay in return to (safe and low-risk) sports after surgery (especially in case of surgery within 10 weeks) applies to young recreational active adults treated with anatomic isolated reconstruction using hamstring autografts whose pre-injury sports levels entailed either low- or high-risk pivoting sports. While at our institution, after functional evaluation, resuming high-risk pivoting sports can be commenced at 6 to 9 months after surgery,[40] in this

specific patient group, it remains to be investigated to what extent resuming (high-risk) sports after 6 months would affect cartilage outcomes. The appraised articles in chapter 4 did not consistently report on timing and return to sports rate. Nonetheless, the overall cartilage quality decline potentially accelerated by persistent rotational instability, may suggest that – in accordance with nonoperative treatment recommendations[41, 48] – those patients previously engaging in high-risk pivoting sports may be recommended to consider activity modification to safe or low-risk activities[41]. In terms of generalizability, chapter 5 does not cover the case of a professional athlete for whom delay of rehabilitation and return to sports may not be an option nor do statements apply to patients with concomitant baseline macroscopic cartilage defects or meniscal surgery that make up an important part of the ACL injured population and – of importance – that may experience even worse cartilage outcomes, especially those patients undergoing meniscectomy procedures.[41, 49, 50]

A success-full return to play may be considered as a return to the pre-injury level, without the increased risk for re-injury and OA development.[30] At this point in time, the rehabilitation team is advised to consider the structural longevity of the knee equally next to graft protection and functional improvement. While this may imply that return to activity equals delaying of and/or confinement to safe or low-risk activities in moderately active recreational athletes, the long-term effect and safety of revised return to play decisions, combined with biological, surgical or rehabilitation techniques aiming at restoring knee dynamic (rotational) stability and cartilage preservation (as discussed in chapter 4) needs confirmation in short- and long-term clinical trials. Last but not least, while – so far – no treatment option appears convincingly superior in protection from radiographic OA,[44, 45] ACL injury prevention strategies remain key.[41]

Based on chapters 1 and 2, generalized joint load directed by surface areas was suggested to contribute to cartilage quality preservation in congruent upper ankle joints. In chapter 3, it was shown that mature healthy knee cartilage is able to functionally adapt to some extent to an *in vivo* loading regimen. Corroborated by the positive effects of weight-bearing neuromuscular and strength exercise in terms of PG turn-over and symptoms or function, exercise was suggested beneficial also in those individuals exhibiting early stages of OA disease.[9, 51] Subjects in the early stage of the disease, however, were also assigned at higher risk for accelerated OA progression.[52] Considering the potential altered cartilage deformational responses to *in vivo* load in an OA joint,[53-55] **chapter 6** evaluated to what extent recovery is required to restitute baseline quantitative morphology in tibiofemoral cartilage plates after an *in vivo* squat exercise in individuals exhibiting early radiographic OA (i.e., K/L grade of maximum 2). Preceded by equal deformation between patients and controls, the results revealed a tendency towards slower recovery in the patients requiring approximately 15 minutes for all entire plates to recover to baseline status. Similar to the literature reporting on advanced stages of OA,[53-55] regional analysis of cartilage plates showed similar patterns in load transfer with a tendency towards more deformation in the patient group compared

to the control subjects. The participating patients were in a remission period symptom-wise and the exercise itself did not substantially exacerbate pain perception. While one may argue that between-group deformational differences are small, it is our contention that this is exactly where the danger for disease progression lies. Similar to chapter 5, delayed recovery preceded by equal deformation is suggested as a sign of impaired cartilage cushioning properties that may incite a downwards spiral towards degeneration as optimal chondrocyte metabolism may be compromised.[56] Hence, in the middle-aged knee patient, delayed recovery suggests the need for prolonged rest periods after a similar exercise that risks being neglected due to the low symptomatic response to the exercise. Although confirmation of this hypothesis is necessary in long-term follow-up, chapter 6 proposed options for translation into clinical practice (e.g., shorter exercise sessions, interspersed weight-bearing and non-weight-bearing compounds within a session, interspersed controlled loading exercise by means of partial body weight support). These results apply to those patients with a radiographic OA status of maximum 2 (in either knee), macroscopic cartilage defects, limited presence of BML and meniscal degeneration without partial meniscectomy.

1.3   General reflections

"X-Rays don't weep." (Liang, M.H. 2004)[57]

In pursuit of the proposal put forward by the OARSI-FDA initiative to separate treatment effects at the joint-level from those at the illness-level, this dissertation focused on structural outcomes.[58] In turn, one may argue that this distinction does not imply that strategies aiming at structural maintenance are relevant in view of the patient's clinical presentation.[59, 60] As this may be true for the early phases of OA development, disease status in terms of deteriorating K/L grades was denoted to reveal worse pain and/or function in studies of ACL reconstruction and OA.[52, 60-62] As such, in the patient at risk for (accelerated) development of radiographic OA such as dealt with in this work, rehabilitation treatment goals are proposed to be directed towards both symptom relief and functional improvement complementary to joint structural preservation.

"Early OA, what's in a name?"

Within the field, the interpretation of "early OA" is often mixed either pertaining to the radiographic definition most commonly applying (variable interpretations of) K/L grades or MRI morphological or biochemical presentation.[63] In view of early disease detection and prevention strategies, MRI has the advantage to image change before bony adaptations occur describing an arthritis knee as displaying cartilage defects, BML, effusion, meniscal degeneration, etc.[64] In fact, in knees graded K/L 1 and 2 denuded bone areas may already be prevalent on MRI.[65] Despite this advantage,

however, no clear cut-offs are available in the (morphological or ultra-structural) MRI definition of (early) OA and the radiographic definitions remain most commonly applied.[63, 66] In chapter 6, to distinguish cases from controls, early OA was defined as those patients exhibiting MRI-related signs of OA including cartilage defects and meniscal degeneration, however, limited to those knees presenting with early radiographic signs of OA up to a maximum K/L grade of 2 (i.e., indicated as doubtful or mild OA with grade 2 representing "definite osteophytes and doubtful joint space narrowing").[63, 67] Consequently, as intended, the patient population in chapter 6 did not represent *true* "established OA". Next to K/L grade 0, control subjects in chapter 6 were only included in case of absent MRI-measured cartilage defects, BML, meniscal displaced tears, (partial) resection, complete destruction and main OA risk factors.[66] To this day, even if the focus is shifted to "early OA", it remains to be established to what degree the human joint or cartilage may deteriorate for (multi-disciplinary) treatment strategies to prevent accelerated structural failure.

"The patellofemoral joint: the under-recognized compartment in (post-traumatic) OA?"

The radiological analyses of the knee joint as presented in this dissertation were restricted to the tibiofemoral compartments. However, in the epidemiological literature concerning OA as well as ACL injury and reconstruction (as touched upon in chapter 4), the role of PF OA has been brought to the attention as common and as an important source of symptoms and disability in the longer term.[68-71] Due to differences in cartilage material properties, joint geometry, patellar tracking and thus strain and shear stress accommodation, treatment or prevention strategies designed for tibiofemoral compartments may not be adequately suited for the PF joint.[69] Further research is required in this respect.

2   General methodological strengths and limitations

The main strength of this dissertation's methodology is the concept of *in vivo* evaluation of cartilaginous responses to exercise in humans. Basic science studies employing *in vitro*, *ex vivo* models already provided with important insights into the effects of loading on cartilage outcomes including chondrocyte biosynthetic activity. However, these outcomes often do not take into account the natural human physiological loading cycles and load distribution during dynamic activities, including joint lubrication, incongruent joint surfaces, mechanics of cartilage-cartilage contact, thickness variability over the joint surface etc..[72-74] While basic science studies are suggested to support the understanding of *in vivo* situations, *in vivo* outcomes as measured in humans incorporate the interaction of all these contributing factors. Hence, the possibilities of *in vivo* research outgrow the relatively limited view of *in vitro* or *ex vivo* approaches. To this end, this

dissertation applied advanced high-resolution morphological and ultra-structural MR imaging (dGEMRIC, T2 and T2*mapping) techniques and (3D) analysis techniques to provide with a comprehensive evaluation of cartilage properties. This particular field of study has been evolving since the late nineties and has mainly been focusing on the development of OA pertaining to rate of cartilage change and its contributing factors (e.g., the Osteoarthritis Initiative consortium; http://oai.epi-ucsf.org/datarelease/). Therefore, this approach is relatively novel in rehabilitation research and was used in this dissertation to address contemporary highly relevant questions in the field concerning 3 relevant phases of the OA developmental process (i.e., healthy, at risk, presenting with signs of early radiographic OA). With regard to the investigation of cartilage deformational behavior, this work's methodology was conform with the most commonly applied methods in the literature (i.e., the investigation of change within single cartilage plates) and their ranges of precision.[75] Whereas studies on *in vivo* deformation including time to recovery are few in number, this dissertation included recovery processes nonetheless to obtain a more complete view on tissue resiliency in view of its vulnerability when loaded. Despite the overall advantage of insight into *in vivo* effects of dynamic exercise, this methodology has 2 main limitations: (1) deformational responses are measured after the exercise due to MRI-related motion artifacts potentially missing out on deformational responses depending upon the cartilage plate and the activity, (2) depending upon the cartilage plate, activity and disease severity, inherent precision of the method may not be able to detect subtle between-groups differences.

In this dissertation, subjects were intentionally recruited according to specific inclusion and exclusion criteria with the aim to investigate homogenous groups. One may argue that in this way generalizability is limited. Due to the heterogeneous nature of the knee OA population and inter-individual differences in response to treatment and exercise, however, it was recommended to move beyond general statements of risk or efficacy in the aggregate OA group.[76] Hence, although there is a great deal of unexplained variation in all aspects of OA,[76] including the effects of exercise as was also shown in this work, this dissertation attempted to provide insights into the effects of exercise in specific sub-groups and discussed to whom the results may or may not apply.

Although, overall, statistical significance was achieved for the main research questions considering the detectable differences (conform with the relevant literature as discussed in the relevant papers), a general limitation throughout this entire work is the use of relatively limited sample sizes that warrant replication of the findings. The limited samples are the result of the experimental approach and its protocol issues (e.g., invasive contrast MRI technique, duration of the protocol, voluntary cooperation in view of investigated activity), MRI cost and availability of the MRI suite in view of project time and funding, analysis time. By means of specific inclusion and the statistical and sub-group approaches, important potential confounding factors in cartilage change were controlled for (e.g., gender, age, BMI, knee alignment, baseline joint status and physical activity level) in between-group comparisons in chapters 3, 5 and 6. However, in view of persisting variation in cartilage

outcomes within groups, the limited samples precluded investigation of potential factors affecting cartilage outcomes that could be either directing rehabilitation goals or facilitating the prediction of cartilage outcomes (e.g., dynamic knee stability including muscle co-contraction, muscle strength and function, presence of BML, extent of meniscal degeneration, history of physical activity experience, hormonal status in female populations, smoking behavior and, as discussed along with the implications of chapter 3, BMI and environmental factors etc.). Similarly, the systematic review in chapter 4 revealed only a relatively low number of high quality - and heterogeneous - studies that precluded a formal meta-analysis. A systematic instead of narrative approach remained preferred because the quality appraisal revealed methodological caveats to be considered in the interpretation of the results as well as in the direction of future research and allowed a best-evidence synthesis to be performed.

Knee joint loading is primarily driven by external ground reaction forces and inertia of the body and lower limbs. Next to intra-articular and articular structures such as bone, cartilage and ligaments, the muscles play a vital part in counteracting and attenuating impact.[77] Next to isolated quadriceps strength, other aspects such as muscle co-activation, co-contraction (e.g., Quadriceps:Hamstrings ratio), and proprioceptive acuity may influence knee joint load as well.[77-79] It is noteworthy that muscle co-contraction has already been argued to potentially increase axial compression contrarily leading to excessive and prolonged cartilage loading.[77, 80] Therefore, actual biomechanical measures of joint dynamic stability, joint contact forces, muscle strength, co-activations and coordinated timing (including calf, thigh and hip muscles), could have provided more insight into *actual* joint loading including asymmetric joint loading, and could have provided leads for muscle rehabilitative exercises.[4, 77-79] Although in ACL reconstruction and OA muscle weakness and/or altered muscle activation patterns including proprioceptive deficits are commonly observed or reported[77, 81, 82], in this dissertation these parameters were not implemented in the statistical analyses to explain any variation in cartilage deformational outcomes. Wherever possible (i.e., chapters 3 and 6) sample sizes were powered to detect between-group differences in cartilage outcomes, these studies were not powered to investigate the potential influence of an array of intrinsic or extrinsic risk factors as listed above. Functional tests (i.e., FTSTS and hop test), however, were included in the experimental protocol that unite important factors determining joint load such as muscle strength, timing and co-activation, proprioceptive acuity, and fear of loading.[77] In chapter 5, although median LSI% was >85 in the patients, a difference in LSI% was noted between the healthy subjects and the subjects treated with ACL reconstruction. In the entire sample, LSI% did not correlate with deformational outcomes nor was running distance, speed and step count (a measure for cumulative load) different between the two groups. In chapter 6, no differences between the FTSTS was observed between the two groups. Additionally, symptomatic responses to the exercise under study were absent-to-low reducing the risk of reflex inhibition of knee joint muscles during the exercise. While in both chapters 5 and 6 baseline FORSS score (i.e., measure of occupational knee joint load) did not differ between groups, outcomes of functional tests were not

included as a covariate in the statistical analyses processing deformational outcomes but were merely included as descriptors of the populations under study. In chapter 3, no measures of muscle strength, either isolated or functional, were incorporated due to the logistic challenges associated with the MR imaging protocol.

In the investigation of exercise effects on cartilage status, physical activity level, type and intensity are important to monitor as these parameters co-determine joint and cartilage load. In chapters 1, 2, 5 and 6, the physical activity itself was standardized in that the 30 knee bending exercises (chapters 1,2,6) were performed according to a pre-defined speed (60 bpm), constrained knee flexion and maximal ankle dorsal flexion angle. The running exercise in chapter 5 was controlled for as well in that duration of the activity was pre-defined. Parameters for intensity and cumulative joint load entailed running distance, speed and step count which did not significantly differ between the groups under study. With regard to Chapter 3, one could argue that, other than completion of the standardized start to run program and self-report, no actual objective measure was included to determine change in physical activity level in that particular study. Additionally, to assess baseline physical activity status, in all experimental studies self-reported questionnaires were administered with the Baecke questionnaire repeatedly implemented. Questionnaires in general and the Baecke questionnaire in particular, lack validity as they are considered less accountable as laboratory measurements and are subject to misinterpretation, intrinsic motivation and recall bias.[93-95] Although in this dissertation the Baecke questionnaire was not used to asses physical activity change but merely to compare or describe physical activity levels of groups under study, its interpretability into "high" versus "low" physical activity levels is unclear as is its minimal detectable difference or change.[93, 94] As the latter may have impeded between-group comparison, one should add that the pre-coding of categories in the analysis may have masked inter-individual variability within an occupation.[96] Therefore, one could suggest the use of pedometers or accelerometers to overcome the limitations of self-report.[93] Although both devices are considered suitable in all populations, pedometers may lack validity with physical activity energy expenditure and are only relevant in populations that generally perform walking activities. Additionally, for both measurement techniques, it remains uncertain how long a monitoring period should last to be considered valid.[93] Therefore, self-reported questionnaires remained the measure of choice in this dissertation to assess baseline physical activity level. Cross-comparison of the Baecke questionnaire versus other relevant and commonly used questionnaires in the field (e.g., activity rating scale (ARS), Tegner, Physical Activity Scale for the Elderly (PASE), International Physical Activity Questionnaire (IPAQ)), revealed an overall advantage of the Baecke questionnaire in terms of multi-dimensional approach of physical activity including measurement of frequency, intensity and duration, and limited risk for misinterpretation of translated questions as the questionnaire is originally published in Dutch.[94] Albeit limited, validity was shown in a healthy Flemish male population accompanied by acceptable reliability.[97, 98] Furthermore, its applicability in various populations improved coherence of questionnaire use throughout this dissertation[96], whereas previous expertise within our research

environment guaranteed adequate data collection and processing.[24, 97, 98] Finally, although the outcomes of the Baecke questionnaire may be prone to recall bias,[93, 94] in view of baseline cartilage status general weekly- and yearly-based report of sports participation was preferred.

In this dissertation cartilage is the core tissue of interest. As outlined in the consensus definition of OA (i.e., General background), the disease may be initiated by abnormalities in any of the synovial structures.[58] Although cartilage loss is the eventual hallmark of disease progression,[58, 83-85] the work in this dissertation did not address how *in vivo* load affects the properties of structures other than cartilage (e.g., meniscal deformation and recovery as the menisci substantially share in load distribution[3, 86]) and how these results could have been interpreted in view of cartilage and joint preservation. While the choice of MRI sequences in chapters 5 and 6 could have enabled these additional explorations, analyses were confined to the cartilage because of the considerable segmentation and post-processing time already required, especially with regard to the 3D MRI analysis methods requesting substantial manual interference. Hence, analysis features were specifically selected in view of the specific research question and cartilage plates to be evaluated with the aim to pursue consistency and precision within the ranges of the published literature.

The T1 and T2/T2* quantification methods (i.e., curve fitting algorithms) as applied in this dissertation are considered appropriate – at least in a setting using clinical MRI magnets.[87] One could argue that the MapIts employed a mono-exponential fit to calculate the T2/T2* relaxation times. However, the T2 MapIt MESE sequence was constructed starting with the first TE >10 ms potentially only capable of monitoring the signal decays of the longer T2 components represented by free, bulk water. Hence, the resulting signal intensity decay is likely to be appropriately fitted in a mono-exponential decay model.[88] While the T2* sequences also included a shorter first TE time potentially capturing signal intensity decays of short T2 components (i.e., water molecules bound to collagen has a signal decay of ~4ms), a bi-exponential fitting method may have been more appropriate to correctly calculate the T2* values.[88-90] Despite this potential source of noise, in chapter 5, similar imaging and analysis methods are used in both groups counterbalancing the influence of measurement errors in between-group comparison also including the potential error caused by magic angle artifacts.[87] The use of only global values (i.e., regions of interest encompassing full thickness cartilage) may be argued upon since additional layer or laminar analysis may enhance detection of early disease or change (i.e., superficial vs deep layers).[91] In biochemical mapping techniques "adequate signal intensity capture (i.e., the larger the pixel, the better)" and "possibility to adequately detect regional variations in signal intensity change (i.e., the smaller the pixel, the better)" balance each other out. For an optimal laminar analysis into superficial and deep layers, 6 pixel layers are proposed[87] which was not met with the use of Siemens MapIts and dGEMRIC images in this work. However, in chapters 3 and 5 where detection of early change was pursued, differences between groups could be achieved nonetheless. Additionally, T2* mapping is praised as an emerging marker with increased sensitivity to early change (i.e., also

responding to changes in water bound to PG or collagen before accumulation of bulk water) which may be endorsed in chapter 5 by the number of plates showing aberrant T2* values vs the number of plates showing aberrant T2 values. Increased sensitivity may also imply sensitivity to scanner imperfections rather than disease.[92] However, in view of the evolution of cartilage adaption after ACL injury and reconstruction as summarized in chapter 4, the measured T2* between-group differences at 6 months after surgery in chapter 5, are suggested to be likely due to early disease.

3   Future research directions

Based upon the results and considerations within this dissertation, 4 main research directions are proposed:

- Mature cartilage appears to functionally adapt to some extent to a gradually built-up running scheme. Future research should elaborate on the implementation – and definition – of "safe exercise" regimen in the primary prevention of cartilage degeneration. Next to the effect on cartilage outcomes, investigation into the long-term effects of exercise on other synovial structures such as the menisci is advisable. These investigations should encompass both short- and longer term studies including the identification of individuals at increased risk for accelerated cartilage quality decline. "Safe exercise" is proposed to imply a low risk for joint injury such as ACL injuries (bringing to the attention the need for injury prevention programs in those individuals participating in higher risk sports) and compliance to regular participation. In this respect, "safe load intensities" should be determined as well.
- More research is needed into the long(er)-term effects of exercise regimen on structural integrity in early radiographic OA including the identification of responders and nonresponders.
- In patient populations at increased risk for radiographic OA development such as ACL injured or reconstructed patients, both short- and long-term clinical trials are needed to evaluate the efficacy and safety of biological, surgical and rehabilitation techniques in mediating cartilage quality decline.
- Next to the ongoing research to identify suitable MRI markers in the early detection of cartilage disease, research into synovial fluid, urinary or serum biomarkers is another fast evolving and innovating field which could aid in the development of early disease detection methods feasible for implementation in clinical settings.

REFERENCES

1. Eckstein F, Lemberger B, Gratzke C, et al. In vivo cartilage deformation after different types of activity and its dependence on physical training status. Ann Rheum Dis. 2005;64(2):291-295.
2. Kuettner KE, Cole AA. Cartilage degeneration in different human joints. Osteoarthritis Cartilage. 2005;13(2):93-103.
3. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Volume changes in the menisci and articular cartilage of runners: an in vivo investigation based on 3-D magnetic resonance imaging. Am J Sports Med. 2006;34(5):832-836.
4. Boocock M, McNair P, Cicuttini F, Stuart A, Sinclair T. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. Osteoarthritis Cartilage. 2009;17(7):883-890.
5. Eckstein F, Tieschky M, Faber S, Englmeier KH, Reiser M. Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. Anat Embryol (Berl). 1999;200(4):419-424.
6. Hosseini A, Van de Velde SK, Kozanek M, et al. In-vivo time-dependent articular cartilage contact behavior of the tibiofemoral joint. Osteoarthritis Cartilage. 2010;18(7):909-916.
7. Seedhom BB. Conditioning of cartilage during normal activities is an important factor in the development of osteoarthritis. Rheumatology (Oxford). 2006;45(2):146-149.
8. Tiderius CJ, Tjornstrand J, Akeson P, Sodersten K, Dahlberg L, Leander P. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): intra- and interobserver variability in standardized drawing of regions of interest. Acta Radiol. 2004;45(6):628-634.
9. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum. 2005;52(11):3507-3514.
10. Kurkijarvi JE, Nissi MJ, Kiviranta I, Jurvelin JS, Nieminen MT. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) and T2 characteristics of human knee articular cartilage: topographical variation and relationships to mechanical properties. Magn Reson Med. 2004;52(1):41-46.
11. Teichtahl AJ, Wluka AE, Wang Y, et al. Effect of long-term vigorous physical activity on healthy adult knee cartilage. Med Sci Sports Exerc. 2012;44(6):985-992.
12. Racunica TL, Teichtahl AJ, Wang Y, et al. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum. 2007;57(7):1261-1268.
13. Teichtahl AJ, Wluka AE, Forbes A, et al. Longitudinal effect of vigorous physical activity on patella cartilage morphology in people without clinical knee disease. Arthritis Rheum. 2009;61(8):1095-1102.
14. Mosher TJ, Liu Y, Torok CM. Functional cartilage MRI T2 mapping: evaluating the effect of age and training on knee cartilage response to running. Osteoarthritis Cartilage. 2010;18(3):358-364.
15. Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis Cartilage. 2009;17(8):971-979.
16. Lane NE, Michel B, Bjorkengren A, et al. The risk of osteoarthritis with running and aging: a 5-year longitudinal study. J Rheumatol. 1993;20(3):461-468.
17. Lane NE, Oehlert JW, Bloch DA, Fries JF. The relationship of running to osteoarthritis of the knee and hip and bone mineral density of the lumbar spine: a 9 year longitudinal study. J Rheumatol. 1998;25(2):334-341.
18. Stubendorff JJ, Lammentausta E, Struglics A, Lindberg L, Heinegard D, Dahlberg LE. Is cartilage sGAG content related to early changes in cartilage disease? Implications for interpretation of dGEMRIC. Osteoarthritis Cartilage. 2012;20(5):396-404.
19. Ly QH, Alaoui A, Erlicher S, Baly L. Towards a footwear design tool: influence of shoe midsole properties and ground stiffness on the impact force during running. J Biomech. 2010;43(2):310-317.
20. Dixon SJ, Collop AC, Batt ME. Surface effects on ground reaction forces and lower extremity kinematics in running. Med Sci Sports Exerc. 2000;32(11):1919-1926.
21. Hackney JM, Wade MG, Larson C, Smith JP, Rakow J. Impairment in people with anterior cruciate ligament reconstruction in adjusting ground reaction force in running. Physiother Theory Pract. 2010;26(5):289-296.
22. Kutzner I, Stephan D, Dymke J, Bender A, Graichen F, Bergmann G. The influence of footwear on knee joint loading during walking--in vivo load measurements with instrumented knee implants. J Biomech. 2013;46(4):796-800.
23. Ghani Zadeh Hesar N, Van Ginckel A, Cools A, et al. A prospective study on gait-related intrinsic risk factors for lower leg overuse injuries. Br J Sports Med. 2009;43(13):1057-1061.
24. Van Ginckel A, Thijs Y, Hesar NG, et al. Intrinsic gait-related risk factors for Achilles tendinopathy in novice runners: a prospective study. Gait Posture. 2009;29(3):387-391.
25. Teichtahl AJ, Wluka AE, Wang Y, et al. Obesity and adiposity are associated with the rate of patella cartilage volume loss over 2 years in adults without knee osteoarthritis. Ann Rheum Dis. 2009;68(6):909-913.
26. Teichtahl AJ, Wang Y, Wluka AE, et al. The longitudinal relationship between body composition and patella cartilage in healthy adults. Obesity (Silver Spring). 2008;16(2):421-427.
27. de Boer TN, van Spil WE, Huisman AM, et al. Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. Osteoarthritis Cartilage. 2012;20(8):846-853.
28. Gunardi AJ, Brennan SL, Wang Y, et al. Associations between measures of adiposity over 10 years and patella cartilage in population-based asymptomatic women. Int J Obes (Lond). 2013 doi: 10.1038/ijo.2013.42.
29. Wang Y, Wluka AE, Simpson JA, et al. Body weight at early and middle adulthood, weight gain and persistent overweight from early adulthood are predictors of the risk of total knee and hip replacement for osteoarthritis. Rheumatology (Oxford). 2013;52(6):1033-1041.
30. Thomee R, Kaplan Y, Kvist J, et al. Muscle strength and hop performance criteria prior to return to sports after ACL reconstruction. Knee Surg Sports Traumatol Arthrosc. 2011;19(11):1798-1805.
31. Ardern CL, Taylor NF, Feller JA, Webster KE. Return-to-sport outcomes at 2 to 7 years after anterior cruciate ligament reconstruction surgery. Am J Sports Med. 2012;40(1):41-48.
32. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. Br J Sports Med. 2011;45(7):596-606.
33. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to the preinjury level of competitive sport after anterior cruciate ligament reconstruction surgery: two-thirds of patients have not returned by 12 months after surgery. Am J Sports Med. 2011;39(3):538-543.
34. Frobell RB. Change in Cartilage Thickness, Posttraumatic Bone Marrow Lesions, and Joint Fluid Volumes After Acute ACL Disruption: A Two-Year Prospective MRI Study of Sixty-one Subjects. J Bone Joint Surg Am. 2011;93(12):1096-1103.

35. Frobell RB, Le Graverand MP, Buck R, et al. The acutely ACL injured knee assessed by MRI: changes in joint fluid, bone marrow lesions, and cartilage during the first year. Osteoarthritis Cartilage. 2009;17(2):161-167.

36. Lentz TA, Zeppieri G, Jr., Tillman SM, et al. Return to preinjury sports participation following anterior cruciate ligament reconstruction: contributions of demographic, knee impairment, and self-report measures. J Orthop Sports Phys Ther. 2012;42(11):893-901.

37. Myer GD, Paterno MV, Ford KR, Quatman CE, Hewett TE. Rehabilitation after anterior cruciate ligament reconstruction: criteria-based progression through the return-to-sport phase. J Orthop Sports Phys Ther. 2006;36(6):385-402.

38. Barber-Westin SD, Noyes FR. Factors Used to Determine Return to Unrestricted Sports Activities After Anterior Cruciate Ligament Reconstruction. Arthroscopy. 2011;27(12):1697-1705.

39. Myklebust G, Bahr R. Return to play guidelines after anterior cruciate ligament surgery. Br J Sports Med. 2005;39(3):127-131.

40. Kvist J. Rehabilitation following anterior cruciate ligament injury: current recommendations for sports participation. Sports Med. 2004;34(4):269-280.

41. Renstrom PA. Eight clinical conundrums relating to anterior cruciate ligament (ACL) injury in sport: recent evidence and a personal reflection. Br J Sports Med. 2012.

42. Tourville T.W. JRJSJR, Naud S., Beynnon B.D. Relationship between markers of type II collagen metabolism and tibiofemoral joint space width changes after ACL injury and reconstruction. Am J Sports Med. 2013.

43. Hoshino Y, Tashman S. Internal tibial rotation during in vivo, dynamic activity induces greater sliding of tibio-femoral joint contact on the medial compartment. Knee Surg Sports Traumatol Arthrosc. 2012;20(7):1268-1275.

44. Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS. A randomized trial of treatment for acute anterior cruciate ligament tears. N Engl J Med. 2010;363(4):331-342.

45. Frobell RB, Roos HP, Roos EM, Roemer FW, Ranstam J, Lohmander LS. Treatment for acute anterior cruciate ligament tear: five year outcome of randomised trial. BMJ. 2013;346:f232.

46. Richmond JC, Lubowitz JH, Poehling GG. Prompt operative intervention reduces long-term osteoarthritis after knee anterior cruciate ligament tear. Arthroscopy. 2011;27(2):149-152.

47. Levy BA, Krych AJ, Dahm DL, Stuart MJ. Treating ACL injuries in young moderately active adults. BMJ. 2013;346:f963.

48. Grindem H, Eitzen I, Moksnes H, Snyder-Mackler L, Risberg MA. A pair-matched comparison of return to pivoting sports at 1 year in anterior cruciate ligament-injured patients after a nonoperative versus an operative treatment course. Am J Sports Med. 2012;40(11):2509-2516.

49. Claes S, Hermie L, Verdonk R, Bellemans J, Verdonk P. Is osteoarthritis an inevitable consequence of anterior cruciate ligament reconstruction? A meta-analysis. Knee Surg Sports Traumatol Arthrosc. 2012.

50. Oiestad BE, Holm I, Gunderson R, Myklebust G, Risberg MA. Quadriceps muscle weakness after anterior cruciate ligament reconstruction: a risk factor for knee osteoarthritis? Arthritis Care Res (Hoboken). 2010;62(12):1706-1714.

51. Stensrud S, Roos EM, Risberg MA. A 12-Week Exercise Therapy Program in Middle-Aged Patients With Degenerative Meniscus Tears: A Case Series With 1-Year Follow-up. J Orthop Sport Phys. 2012;42(11):919-931.

52. Cibere J, Zhang H, Thorne A, et al. Association of clinical findings with pre-radiographic and radiographic knee osteoarthritis in a population-based study. Arthritis Care Res (Hoboken). 2010;62(12):1691-1698.

53. Cotofana S, Eckstein F, Wirth W, et al. In vivo measures of cartilage deformation: patterns in healthy and osteoarthritic female knees using 3T MR imaging. Eur Radiol. 2011;21(6):1127-1135.

54. Shin CS, Souza RB, Kumar D, Link TM, Wyman BT, Majumdar S. In vivo tibiofemoral cartilage-to-cartilage contact area of females with medial osteoarthritis under acute loading using MRI. J Magn Reson Imaging. 2011;34(6):1405-1413.

55. Subburaj K, Souza RB, Stehling C, et al. Association of MR relaxation and cartilage deformation in knee osteoarthritis. J Orthop Res. 2012;30(6):919-926.

56. Song Y, Greve JM, Carter DR, Giori NJ. Meniscectomy alters the dynamic deformational behavior and cumulative strain of tibial articular cartilage in knee joints subjected to cyclic loads. Osteoarthritis Cartilage. 2008;16(12):1545-1554.

57. Liang MH. Pushing the limits of patient-oriented outcome measurements in the search for disease modifying treatments for osteoarthritis. J Rheumatol Suppl. 2004;70:61-65.

58. Lane NE, Brandt K, Hawker G, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarthritis Cartilage. 2011;19(5):478-482.

59. Baert IA, Staes F, Truijen S, et al. Weak associations between structural changes on MRI and symptoms, function and muscle strength in relation to knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2013.

60. Oiestad BE, Holm I, Engebretsen L, Risberg MA. The association between radiographic knee osteoarthritis and knee symptoms, function and quality of life 10-15 years after anterior cruciate ligament reconstruction. Br J Sports Med. 2011;45(7):583-588.

61. Javaid MK, Kiran A, Guermazi A, et al. Individual magnetic resonance imaging and radiographic features of knee osteoarthritis in subjects with unilateral knee pain: the health, aging, and body composition study. Arthritis Rheum. 2012;64(10):3246-3255.

62. Schiphof D, Kerkhof HJ, Damen J, et al. Factors for pain in patients with different grades of knee osteoarthritis. Arthritis Care Res (Hoboken). 2012.

63. Schiphof D, de Klerk BM, Kerkhof HJ, et al. Impact of different descriptions of the Kellgren and Lawrence classification criteria on the diagnosis of knee osteoarthritis. Ann Rheum Dis. 2011;70(8):1422-1427.

64. Guermazi A, Niu J, Hayashi D, et al. Prevalence of abnormalities in knees detected by MRI in adults without knee osteoarthritis: population based observational study (Framingham Osteoarthritis Study). BMJ. 2012;345:e5339.

65. Frobell RB, Wirth W, Nevitt M, et al. Presence, location, type and size of denuded areas of subchondral bone in the knee as a function of radiographic stage of OA - data from the OA initiative. Osteoarthritis Cartilage. 2010;18(5):668-676.

66. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):401-406.

67. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986;29(8):1039-1049.

68. Farrokhi S, Piva SR, Gil AB, Oddis CV, Brooks MM, Fitzgerald GK. Severity of coexisting patellofemoral disease is associated with increased impairments and functional limitations in patients with knee osteoarthritis. Arthritis Care Res (Hoboken). 2012.

69. Crossley KM, Hinman RS. The patellofemoral joint: the forgotten joint in knee osteoarthritis. Osteoarthritis Cartilage. 2011;19(7):765-767.

70. Hinman RS, Crossley KM. Patellofemoral joint osteoarthritis: an important subgroup of knee osteoarthritis. Rheumatology (Oxford). 2007;46(7):1057-1062.

71. Culvenor AG, Cook JL, Collins NJ, Crossley KM. Is patellofemoral joint osteoarthritis an under-recognised outcome of anterior cruciate ligament reconstruction? A narrative literature review. Br J Sports Med. 2013;47(2):66-70.

72. Van Ginckel A, Almqvist F, Verstraete K, Roosen P, Witvrouw E. Human ankle cartilage deformation after different in vivo impact conditions. Knee Surg Sports Traumatol Arthrosc. 2011;19(1):137-143.

73. Van Ginckel A, Roosen P, Almqvist KF, Verstraete K, Witvrouw E. Effects of in vivo exercise on ankle cartilage deformation and recovery in healthy volunteers: an experimental study. Osteoarthritis Cartilage. 2011;19(9):1123-1131.

74. Eckstein F, Hudelmaier M, Putz R. The effects of exercise on human articular cartilage. J Anat. 2006;208(4):491-512.

75. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage. 2006;14 Suppl A:A46-75.

76. Minor MA. Physical activity and knee osteoarthritis: answers and questions. Arthritis Rheum. 2007;57(1):1-2.

77. Bennell KL, Wrigley TV, Hunt MA, Lim BW, Hinman RS. Update on the role of muscle in the genesis and management of knee osteoarthritis. Rheum Dis Clin North Am. 2013;39(1):145-176.

78. Ericsson YB, Tjornstrand J, Tiderius CJ, Dahlberg LE. Relationship between cartilage glycosaminoglycan content (assessed with dGEMRIC) and OA risk factors in meniscectomized patients. Osteoarthritis Cartilage. 2009;17(5):565-570.

79. Kersting UG, Stubendorff JJ, Schmidt MC, Bruggemann GP. Changes in knee cartilage volume and serum COMP concentration after running exercise. Osteoarthritis Cartilage. 2005;13(10):925-934.

80. Tsai LC, McLean S, Colletti PM, Powers CM. Greater muscle co-contraction results in increased tibiofemoral compressive forces in females who have undergone anterior cruciate ligament reconstruction. J Orthop Res. 2012;30(12):2007-2014.

81. Strandberg S, Lindstrom M, Wretling ML, Aspelin P, Shalabi A. Muscle morphometric effect of anterior cruciate ligament injury measured by computed tomography: aspects on using non-injured leg as control. BMC Musculoskelet Disord. 2013;14:150.

82. Ingersoll CD, Grindstaff TL, Pietrosimone BG, Hart JM. Neuromuscular consequences of anterior cruciate ligament injury. Clin Sports Med. 2008;27(3):383-404, vii.

83. Eckstein F, Maschek S, Wirth W, et al. One year change of knee cartilage morphology in the first release of participants from the Osteoarthritis Initiative progression subcohort: association with sex, body mass index, symptoms and radiographic osteoarthritis status. Ann Rheum Dis. 2009;68(5):674-679.

84. Hunter DJ, Niu J, Zhang Y, et al. Change in cartilage morphometry: a sample of the progression cohort of the Osteoarthritis Initiative. Ann Rheum Dis. 2009;68(3):349-356.

85. Wluka AE, Forbes A, Wang Y, Hanna F, Jones G, Cicuttini FM. Knee cartilage loss in symptomatic knee osteoarthritis over 4.5 years. Arthritis Res Ther. 2006;8(4):R90.

86. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. Am J Sports Med. 2008;36(5):966-970.

87. Burstein D, Gray M, Mosher T, Dardzinski B. Measures of molecular composition and structure in osteoarthritis. Radiol Clin North Am. 2009;47(4):675-686.

88. Pauli C, Bae WC, Lee M, et al. Ultrashort-Echo Time MR Imaging of the Patella with Bicomponent Analysis: Correlation with Histopathologic and Polarized Light Microscopic Findings. Radiology. 2012;264(2):484-493.

89. Qian Y, Williams AA, Chu CR, Boada FE. Repeatability of ultrashort echo time-based two-component T(2) (*) measurements on cartilages in human knee at 3 T. Magn Reson Med. 2012.

90. Goto H, Fujii M, Iwama Y, Aoyama N, Ohno Y, Sugimura K. Magnetic resonance imaging (MRI) of articular cartilage of the knee using ultrashort echo time (uTE) sequences with spiral acquisition. J Med Imag Radiat On. 2012;56(3):318-323.

91. Li X, Kuo D, Theologis A, et al. Cartilage in anterior cruciate ligament-reconstructed knees: MR imaging T1{rho} and T2--initial experience with 1-year follow-up. Radiology. 2011;258(2):505-514.

92. Bittersohl B, Miese FR, Hosalkar HS, et al. T2* mapping of hip joint cartilage in various histological grades of degeneration. Osteoarthritis Cartilage. 2012;20(7):653-660.

93. Warren JM, Ekelund U, Besson H, et al. Assessment of physical activity - a review of methodologies with reference to epidemiological research: a report of the exercise physiology section of the European Association of Cardiovascular Prevention and Rehabilitation. Eur J Cardiovasc Prev Rehabil. 2010;17(2):127-139.

94. Terwee CB, Bouwmeester W, van Elsland SL, de Vet HC, Dekker J. Instruments to assess physical activity in patients with osteoarthritis of the hip or knee: a systematic review of measurement properties. Osteoarthritis Cartilage. 2011;19(6):620-633.

95. Ono R, Hirata S, Yamada M, Nishiyama T, Kurosaka M, Tamura Y. Reliability and validity of the Baecke physical activity questionnaire in adult women with hip disorders. BMC Musculoskelet Disord. 2007;8:61.

96. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982;36(5):936-942.

97. Philippaerts RM, Lefevre J. Reliability and validity of three physical activity questionnaires in Flemish males. Am J Epidemiol. 1998;147(10):982-990.

98. Philippaerts RM, Westerterp KR, Lefevre J. Doubly labelled water validation of three physical activity questionnaires. Int J Sports Med. 1999;20(5):284-289.
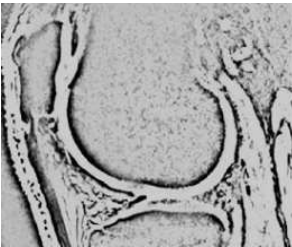
ENGLISH SUMMARY

Osteoarthritis (OA) of the knee is designated as one of the most important and prevalent disabling disease entities in Western Society. This "Whole-Organ disease" may initiate in or affect all synovial structures with cartilage loss as the main marker for its structural progression over time. Because research and treatment were traditionally targeting advanced stages of the disease directed at palliation, a paradigm shift was introduced. This paradigm shift proposed to focus on individuals at increased risk for accelerated cartilage degeneration and on individuals with early disease in which prevention (or reversal) of progressive cartilage deterioration may still be an option. In view of the weak correlation between symptoms and joint health especially in the early stages of OA disease, it was proposed for research strategies to separate treatment effects at the joint-level from those at the illness-level (i.e., the patient's perception of the symptoms and function). Although not straight forward, the body of literature suggests that in the presence of internal knee derangements or in individuals at risk for accelerated OA progression, light to moderate exercise may - at least initially - have a beneficial effect on cartilage structural integrity. While dedicated exercise programs have the potential to alleviate symptoms and improve physical function in knee OA, the effect of exercise on cartilage outcomes remains to be further explored especially since cartilage in OA joints appears to display altered deformational behavior under (*in vivo)* load .

The two-fold goal of this dissertation was to contribute to the understanding of how *in vivo* exercise may assist in chondroprotection in view of OA prevention in a first part, and, in a second part, how exercise may be implemented in those individuals at increased risk for radiographic OA development or accelerated OA progression (i.e., diagnosed with early radiographic OA, K/L grade ≤2).

The first part of this dissertation investigated *in vivo* cartilage deformation in the upper ankle, a joint rarely afflicted with idiopathic OA. In view of the importance of load-dependent matrix composition in cartilage viability, insight into this joint's *in vivo* load transfer may assist in identifying the features exercise needs to encompass in cartilage preservation. It was shown that tibiotalar cartilage deforms to a substantial extent when compared to the available reports on the knee joint. After a 30-repetition squat exercise, considerable deformation preceded a 30-min recovery in the talar layers that showed to be driven by surface area involvement. As such, it was suggested that, in pursuit of cartilage quality maintenance, exercise may need to encompass generalized load directed by surface areas. In order to translate exercise-related chondroprotection onto the knee joint, in a longitudinal analysis, the first part of this dissertation additionally investigated whether mature knee joint cartilage is able to functionally adapt to a gradually built-up novice runner program, the Start To Run program. It was shown that mature knee femoral cartilage in relatively young women inexperienced in running activity, is able to adapt to some extent to changes in physical activity. Although future research is needed in larger samples, different populations and over longer periods of time, the novice runner program was suggested to show promise in the primary prevention

against cartilage degeneration in relatively young and inexperienced adults (i.e., <40 years of age) and may support the promotion of regular physical activity in our Western society.

To comply with the proposed paradigm shift, the second part of this dissertation applied the effect of exercise implementation on cartilage outcomes in a group of patients at increased risk for OA development (i.e., ACL injury or reconstruction) and in a group of patients diagnosed with early radiographic OA (i.e., K/L grades of maximum 2). First, a systematic review was performed to gain insight into the course of cartilage adaptations following ACL injury and reconstruction and into the factors that might affect rate of change. Although the evaluation of methodological quality confirmed the need for more research, the first high-quality studies described an overall progressive decline in all cartilage plates from 2 weeks up to 11 years after surgery. While the lateral compartment initially undertook attempts to recover, maintained medial compartment degeneration was reported as soon as 3 weeks following injury next to progressive patellofemoral involvement. Although ACL-reconstruction was associated with pronounced morphological changes during the first year after injury, approximately 2 years of follow-up were required for MRI to clearly detect morphological change. Therefore, it was suggested for prevention strategies to focus on early (ultra-)structural cartilage decline identifying moderate-to-strong evidence for meniscal injury/meniscectomy, the presence of BML, length of follow-up and persistent altered biomechanics as factors affecting rate of cartilage change after ACL reconstruction. Additionally, the implications of the literature review were discussed regarding future research directions, prevention and treatment strategies including return to play decisions. With regard to the latter, return to play decisions were proposed to consider the long-term structural longevity of the knee next to surgical and rehabilitation variables. Whereas, on average, sports can be resumed at 6 months after surgery, this dissertation showed that – at this point in time – tibiofemoral cartilage in knees that were operated on with isolated ACL reconstruction displayed diminished *in vivo* resiliency after a 30-minute run next to decreased quality in terms of biochemical composition when compared to uninjured controls. In patients, the deleterious cartilage outcomes tended to aggravate in those returning to sports prior to 5 months after surgery and those operated on within 10 weeks from injury. Taken together, the results of the systematic review and the experimental study suggest that post-operative rehabilitation may benefit from a delayed return to sports activities, even in the case of low-risk activities such as running. Additionally, while rotational instability may be an unresolved maintaining factor in cartilage quality decline, the patients that performed high-risk pivoting sports before injury, may be advised to pursue activity modification similar to the contemporary recommendations in patients undergoing nonsurgical treatment options. Nonetheless it should be stressed that long-term follow-up studies are warranted to confirm the efficacy and safety of the proposed strategies aiming at cartilage health preservation.

In the first part of this dissertation, generalized joint load directed by surface areas was suggested to contribute to cartilage quality preservation whereas mature healthy knee cartilage showed being

able to functionally adapt to some extent to an *in vivo* loading regimen. Corroborated by the positive effects of weight-bearing neuromuscular and strength exercise in terms of PG turn-over and symptoms or function, exercise was suggested beneficial also in those individuals exhibiting early stages of OA disease. These individuals, however, were assigned at higher risk for accelerated OA progression. Considering the potential altered cartilage deformational responses to *in vivo* load in an OA joint, the second part of this dissertation additionally evaluated the effect of an *in vivo* 30-repetition squat exercise on the extent of tibiofemoral cartilage deformation and recovery in individuals diagnosed with early radiographic signs of OA (i.e., K/L grade of maximum 2, no history of knee surgical procedures). Similar to the patients that underwent ACL-reconstruction, equal deformation preceded a tendency towards delayed deformation in the patients when compared to the control subjects, requiring *at least* 15 minutes of recovery for all entire plates to restitute to baseline volumes after cessation of the squat exercise. It was hypothesized that neglect of adequate rest periods after weight-bearing exercise, especially in case of minimal symptomatic responses to the exercise, may compromise optimal chondrocyte metabolism furthering a negative downwards spiral towards cartilage deterioration and joint destruction.

In conclusion, while regular exercise may be an interesting asset in primary OA prevention, exercise implementation in those with cartilage deterioration up to early radiographic OA (K/L grade ≤2) should consider the altered deformational responses including delayed recovery after *in vivo* exercise that may predispose the cartilage to accelerated degeneration.
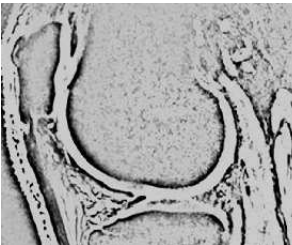
NEDERLANDSTALIGE SAMENVATTING

Osteoartrose (OA) ter hoogte van het kniegewricht wordt omschreven als één van de belangrijkste en meest voorkomende invaliderende aandoeningen in onze Westerse samenleving. Deze ziekte-entiteit betreft het gehele gewrichtsorgaan waarbij alle synoviale structuren kunnen aangetast worden of een oorzakelijke rol kunnen spelen maar waarbij hoofdzakelijk kraakbeenverlies als een marker voor ziekteprogressie wordt aangeduid. Aangezien onderzoeks- en behandelstrategieën zich traditioneel voornamelijk richtten op vergevorderde stadia en palliatie, werd een verandering in het denkpatroon voorgesteld. Deze verandering hield in dat de aandacht zou moeten worden gericht op die individuen met een verhoogd risico op het ontwikkelen van OA en die individuen die reeds tekenen van vroegtijdige ziekte vertonen. Immers, bij deze groepen zou preventie (of behandeling) van progressieve kraakbeenbeschadiging mogelijks nog een optie kunnen zijn. Bovendien werd, omwille van de zwakke correlatie tussen symptomatologie en gewrichtskwaliteit zeker in de eerste stadia van OA, voorgesteld om bij het opstellen van onderzoeksdesigns het onderscheid te maken tussen effecten van behandeling op het gewrichtsniveau enerzijds en het ziekteniveau (d.i., de perceptie van de patiënt omtrent symptomen en functieverlies) anderzijds. Hoewel hieromtrent de huidige literatuur niet rechtlijnig blijkt te zijn, wordt gesuggereerd dat bij individuen met intra-articulaire letsels of met een verhoogd risico op een versnelde progressie van OA, licht tot matige (sport-) activiteit – alleszins in de initiële fase – een bevorderlijk effect zou kunnen hebben op de structurele integriteit van het gewrichtskraakbeen. Niettegenstaande aangepaste oefenprogramma's kunnen zorgen voor symptoomverlichting en verbetering van functie, blijkt verdere exploratie naar de effecten van oefening op de structurele integriteit van het kraakbeenweefsel bij patiënten met OA noodzakelijk. Immers, kraakbeen van door OA aangetaste gewrichten zou een veranderd deformationeel gedrag vertonen wanneer (*in vivo)* belast.

Het doel van dit doctoraal proefschrift was tweevoudig. In een eerste deel werd, met het oog op preventie van OA, gepoogd bij te dragen aan het begrip over de manier waarop *in vivo* oefening of sportbeoefening kan worden aangewend in de bescherming tegen kraakbeendegeneratie. Een tweede deel onderzocht hoe oefening of sportbeoefening kan worden geïmplementeerd bij individuen met een verhoogd risico op het ontwikkelen van radiografische tekenen van OA of versnelde progressie van OA (d.i., gediagnosticeerd met radiografische tekenen van vroegtijdige OA, K/L graad ≤2).

Het eerste deel van dit proefschrift onderzocht het *in vivo* deformationeel gedrag van het kraakbeen in het bovenste spronggewricht van de enkel dat zelden wordt aangetast door de idiopathische vorm van OA. Immers, met het oog op belastings-afhankelijke matrix compositie en kraakbeenduurzaamheid zou het inzicht in de manier waarop dit gewricht *in vivo* belasting transfereert kunnen helpen in de identificatie van eigenschappen waarover oefening zou moeten beschikken als kraakbeenbescherming als doel wordt vooropgesteld. Er werd aangetoond dat het tibiotalaire kraakbeen een aanzienlijke vervorming ondergaat na *in vivo* belasting vergeleken met de beschikbare studies in de literatuur over het kniegewricht. Na het uitvoeren van een squat oefening

met 30 herhalingen werd vastgesteld dat voor het talaire kraakbeen, na aanzienlijke deformatie, een periode van 30 minuten vereist was om kraakbeenvolumes te laten herstellen na de oefening. De mate van deformatie bleek tevens gestuurd door de mate waarin de gewrichtsoppervlakken betrokken waren in de gewrichtsarticulatie. Er werd daarom gesuggereerd dat veralgemeende belasting van de gewrichtsoppervlakken een eigenschap zou kunnen zijn waarover oefeningen zouden moeten beschikken in het geval dat behoud van kraakbeenkwaliteit wordt nagestreefd. Opdat oefening-gerelateerde kraakbeenbescherming zou kunnen worden vertaald naar het kniegewricht, onderzocht het eerste deel van dit proefschrift bijkomend in een longitudinale studie of volwassen kraakbeen van de knie in staat is zich functioneel aan te passen aan een gradueel opgebouwd loopprogramma voor beginnende lopers, het Start To Run programma. Er werd aangetoond dat bij relatief jonge vrouwen zonder loopervaring, volwassen femoraal kraakbeen in de knie zich in zekere mate aanpast aan veranderingen in fysieke activiteit. Ondanks de nood aan verder onderzoek in grotere en verscheidene onderzoekspopulaties gedurende langere opvolgperiodes, werd gesuggereerd dat dit programma voor beginnende lopers potentieel biedt bij de primaire preventie van kraakbeendegeneratie bij relatief jonge en onervaren volwassenen (d.i., <40 jaar oud). Tevens zouden deze resultaten het aanmoedigen van regelmatige sportbeoefening in onze Westerse samenleving kunnen ondersteunen.

Om tegemoet te komen aan de voorgestelde paradigma verschuiving, werd in het tweede deel van dit doctoraal proefschrift het effect van de implementatie van oefening of sportbeoefening op kraakbeen eigenschappen toegepast bij een groep patiënten die een verhoogd risico vertonen op OA ontwikkeling (d.i., voorste kruisbandletsel of -reconstructie) en bij een groep patiënten gediagnosticeerd met tekenen van vroegtijdige OA (d.i., K/L graad tot maximum 2). In eerste instantie werd een systematisch literatuur onderzoek uitgevoerd om meer inzicht te verkrijgen in de evolutie van kraakbeenadaptaties na een voorste kruisbandletsel en reconstructie en in de factoren die mogelijks snelheid van kraakbeenveranderingen zouden kunnen beïnvloeden. Hoewel de analyse van de methodologische kwaliteit van de geïncludeerde studies de nood naar verder onderzoek bevestigde, beschreven de eerste studies met goede kwaliteit een veralgemeend en progressief kraakbeenverval over een tijdspanne gaande van 2 weken tot 11 jaar na operatie. Desondanks een initiële poging tot kraakbeenherstel in het laterale compartiment van de knie, werd blijvende en progressieve degeneratie vastgesteld in het mediale compartiment ten vroegste gerapporteerd 3 weken na oplopen van het voorste kruisbandletsel, tezamen met een progressieve betrokkenheid van het patellofemorale gewricht. Waar een voorste kruisbandreconstructie werd geassocieerd met meer uitgesproken morfologische veranderingen gedurende het eerste jaar follow-up, bleek een periode van nagenoeg 2 jaar nodig te zijn vooraleer MRI duidelijk morfologische waarneembare kraakbeenveranderingen kon aantonen. Bijgevolg werd gesuggereerd dat preventiestrategieën de aandacht dienden te vestigen op vroegtijdige (ultra-) structurele verandering waarbij matig tot sterk bewijs werd geleverd voor de rol van meniscus letsels/meniscectomie, aanwezigheid van beenmergoedeem, duur van follow-up en persisterende

veranderde biomechanica als mogelijke factoren die de snelheid of mate van kraakbeenverandering na een voorste kruisbandreconstructie zouden kunnen beïnvloeden. Naar aanleiding van de resultaten van het literatuuronderzoek werden tevens de implicaties op het vlak van verder onderzoek, preventie en behandelstrategieën besproken m.i.v. de toelating tot sporthervatting. Met betrekking tot de toelating tot sporthervatting, werd voorgesteld dat deze beslissingen, naast chirurgische of revalidatie-gebonden variabelen, tevens de structurele integriteit van het kniegewricht op lange termijn in beschouwing zouden moeten nemen. Aangezien sporthervatting is toegelaten omstreeks gemiddeld 6 maanden na de operatie, toonde dit proefschrift aan dat, op dit tijdstip, het tibiofemorale kraakbeen in de knie behandeld met een voorste kruisbandreconstructie (d.i., geïsoleerde reconstructie met hamstrings autogreffe), na vergelijking met gewrichten bij blessurevrije controlepersonen, een verminderd herstellingvermogen vertoonde na een 30-minuten durende loopactiviteit gepaard gaande met een verminderde kwaliteit inzake biochemische compositie. Bij de geopereerde patiënten werd een tendens vastgesteld naar meer schadelijke kraakbeen uitkomsten in het geval van een terugkeer naar de sport voor 5 maanden na de operatie en bij die patiënten die werden geopereerd binnen 10 weken na het oplopen van het letsel. Als de resultaten van het literatuur onderzoek en de experimentele studie worden samengevat, kan worden gesuggereerd dat de postoperatieve revalidatie baat zou kunnen hebben bij een uitstel van sporthervatting, zelfs in het geval van sporttakken die een laag-risico betekenen voor recidiverende blessures, zoals lopen. Aangezien persisterende rotationele instabiliteit een onderhoudende factor zou kunnen zijn in progressief kraakbeenverval, werd tevens voorgesteld dat patiënten die voor het voorste kruisbandletsel participeerden in pivoterende sporten, sporten met een "hoog-risico" voor recidivering, zouden kunnen worden geadviseerd om te opteren voor aanpassing van activiteit na de operatie naar analogie met de huidige aanbevelingen die worden gehanteerd bij patiënten met conservatief behandelde voorste kruisbandletsels. Desalniettemin moet worden benadrukt dat het mogelijke effect en veiligheid van kraakbeen beschermende strategieën dient te worden ondersteund in klinische follow-up studies op lange termijn.

In het eerste deel van dit doctoraal proefschrift werd gesuggereerd dat veralgemeende belasting van de gewrichtsoppervlakken zou kunnen bijdragen aan het behoud van de kraakbeenkwaliteit en werd aangetoond dat volwassen kraakbeen in de knie in zekere mate in staat is om zich functioneel aan te passen aan een *in vivo* belastingregime. Ondersteund door de positieve effecten van belaste neuromusculaire en spierkrachtoefeningen op PG synthese en symptomatologie of functie, werd gesuggereerd dat oefening positieve bevorderlijke effecten zou kunnen hebben bij individuen in de vroegtijdige fase van OA. Echter, er werd tevens beschreven dat deze individuen een verhoogd risico vertonen op versnelde OA progressie. Het mogelijks veranderde deformationeel gedrag van het kraakbeen in beschouwing genomen, evalueerde het tweede deel van dit proefschrift tevens het effect van een *in vivo* squat oefening met 30 herhalingen op de mate van deformatie en duur tot herstel van het tibiofemorale kraakbeen bij individuen met radiografische tekenen van vroegtijdige OA (d.i., K/L graad maximum 2, geen voorgeschiedenis van chirurgische procedures ter hoogte van

het kniegewricht). Naar analogie met de patiënten geëvalueerd na een voorste kruisbandreconstructie, werd er een gelijkaardige graad aan deformatie vastgesteld bij de patiënten vergeleken met controlepersonen, opgevolgd door een tendens naar een vertraagd herstel van kraakbeenvolumes na de oefening bij de patiënten. Immers, na het beëindigen van de squat oefening was er bij de patiënten *ten minste* 15 minuten hersteltijd nodig opdat al de kraakbeenplaten in hun totaliteit rustvolumes hadden bereikt. De hypothese werd gesteld dat het negeren van voldoende rustperiodes na en tussen belaste oefeningen, zeker in het geval van minimale symptomatische reacties op de oefening, de condities voor een optimaal kraakbeenmetabolisme in het gedrang zouden kunnen brengen. Bijgevolg zou op deze manier de negatieve neerwaartse spiraal naar kraakbeenschade en gewrichtsdestructie in de hand kunnen worden gewerkt.

Als algemeen besluit kan worden gesteld dat daar waar regelmatige sportbeoefening een voordeel zou kunnen betekenen in de primaire preventie van OA, men bij de implementatie van oefening of sportbeoefening bij diegenen met kraakbeenbeschadiging tot vroegtijdige OA rekening zou moeten houden met het veranderde deformationele gedrag inclusief vertraagde herstelprocessen van het kraakbeen na *in vivo* belasting. Dit veranderde deformationele gedrag zou immers een voorbeschikkende factor kunnen zijn voor versnelde kraakbeendegeneratie.
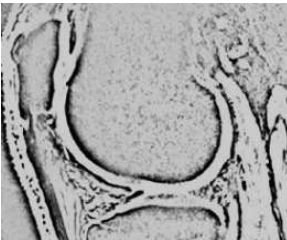
ACKNOWLEDGMENTS

"Two little mice fell in a bucket of cream. The first mouse quickly gave up and drowned. The second mouse, wouldn't quit. He struggled so hard that eventually he churned that cream into butter and crawled out."

("Catch Me If You Can", 2002)

Deze kleine muis heeft de voorbije vier jaar intensief gesparteld en is al wel eens "kopje onder" gegaan, maar – bovenal – ze is fier op het eindresultaat! Dit is dan ook het uitgelezen moment om iedereen te bedanken die op een directe en indirecte manier geholpen hebben, advies of een schouderklopje gegeven hebben, waardoor uiteindelijk de kleine muis bijna niet meer van ophouden wist!

In de eerste plaats zou ik mijn promotor, Prof Dr Erik Witvrouw, willen bedanken. Erik, dank je om potentieel in mij te zien en om me de kans te geven een doctoraat te maken. Uiteindelijk ben ik een weg ingegaan die misschien initieel niet "voorzien" was (Hoe zat dat ook alweer met die risicofactoren?) en die daardoor ook niet altijd de makkelijkste was, maar ik heb er alleszins geen spijt van. Als ik terugkijk moet ik opmerken dat jouw ultra-snelle-paper-reading vaardigheden (zit je nu in Gent of in Qatar), efficiënt knoop-doorhak vermogen, occasionele frustratie-ventilatie functie (van mijn kant uitwaaiend dan) en Eriks-gewijze peptalk zeker hebben bijgedragen tot (de omvang van) het eindresultaat.

Ook al staat er een promotor achter jou, geen onderzoeksgeld = geen doctoraat. Dit project zou niet mogelijk geweest zijn zonder de ondersteuning van het Fonds voor Wetenschappelijk Onderzoek Vlaanderen en het Bijzonder Onderzoek Fonds UGent.

Naast mijn promotor, zou ik ook de leden van de begeleidingscommissie, Prof Dr Philip Roosen, Prof Dr Koenraad Verstraete en Prof Dr Fredrik Almqvist, willen bedanken voor hun opmerkingen en input bij de opstart en verdere opzet van de studies in dit doctoraat.

To the members of the Jury, Prof Dr May Arna Risberg, Prof Dr Johan Bellemans, Prof Dr Dieter Van Assche, Prof Dr Nele Mahieu, Dr Annelies Maenhout and, Prof Dr Jan Victor, the chairman, thank you for preserving some time in your busy schedules for reading and commenting on my dissertation, and traveling to Ghent. Your comments and questions shaped this dissertation too and kept me on my toes.

Dat dit project de nodige technische en logistieke uitdagingen kende is geen overstatement. Maar… "binnen de moeilijkheid ligt de mogelijkheid", en, naast de grotere dingen, zat de mogelijkheid soms ook in de kleinere dingen. Een welgemeende dankjewel aan Dr Greta Vandemaele voor het optimaliseren van de MRI scansequenties; Mevr. Claire Schepens, hoofdverpleegkundige van de MRI afdeling, voor het telkens mee inplannen van mijn experimenten in de drukke kliniek agenda; Dr Tom Van Hoof en de geduldige Materialize help desk voor de Mimics introductie en de dienst

orthopedie voor de licentie details; Dr Nick Baelde, Radiologie AZ Jan Palfijn, voor de onverwachte nieuwe mogelijkheid voor de dGEMRIC studie - het eerste "MR plan" maar achteraf bekeken ook het meest uitdagende; de knie chirurgen van de dienst orthopedie UZGent voor het ter beschikking stellen van hun patiëntenbestand; de dienst sportkinesitherapie UZGent met in het bijzonder Bert Sticker, voor de hulp bij het opvissen van potentiële kandidaat-deelnemers; en natuurlijk al de patiënten of "proefpersonen" die hun avonden hebben opgeofferd voor 'een ritje in de MRI'.

Ik val misschien in herhaling, maar het is gewoon waar! De positieve werksfeer die onder de REVAKI-collega's heerst zorgt er zeker ook voor dat je er blijft voor gaan! Dank je wel aan de (ex-) assistenten zowel voor het advies (waaronder planning coaching en anti-lanterfant acties) en small talk alsook voor de afleiding met etentjes, cafeetjes/terrasjes, housewarmings, colamomentjes met ceremoniemeester(es) inbegrepen, loopmomentjes (Veerle, jammer dat we de draad niet meer hebben kunnen oppikken), congres momentjes ("What happens in XXX, stays in XXX") ... Kortom, tegen alle duurzaamheids-alias-milieu-pacten in stond jullie deur open. Een bijzondere dankjewel ook aan mijn bureaugenootjes vroeger en nu voor de "intellectuele en diepgaande gesprekken" over "Neveneffecten stofzuigerverkopers" tot het "Mixed Model Intercept", voor de mopjes van de dag, voor de klankborden, en gewoon voor het feit dat er "nen hoek af mocht zijn"(tot hier de details...) .

Dankjewel aan alle familie en vrienden van in de Kempen of van op andere verre bestemmingen die (onrechtstreeks) gesupporterd hebben aan de zijlijn gewoon door af en toe eens te vragen hoe het met het doctoraat ging.

Last but not least, mijn trouwste supporters, moeke, voke, Jo, Rein, Niels, Wouter en Kaat & Len, jullie zijn altijd mijn "toevluchtsoord" geweest (met gepersonaliseerde B&B en/of restaurant) zowel in Gent als in de Kempen. Jullie zorgden naast persoonlijk wetenschappelijk advies (voke, kraakbeenrevalidatie is geen oncologie maar ik heb je "tips and tricks" gaandeweg altijd geapprecieerd) alsook voor een portie relativering (die twee kleine prutsen weten daar wel raad mee!). Ik ben de laatste maanden niet veel meer op bezoek kunnen komen in de Kempen, maar tante Ans maakt dat zeker goed!!!

Dank U Wel!

Ans Van Ginckel,

Gent, 27 juni 2013

– The End –