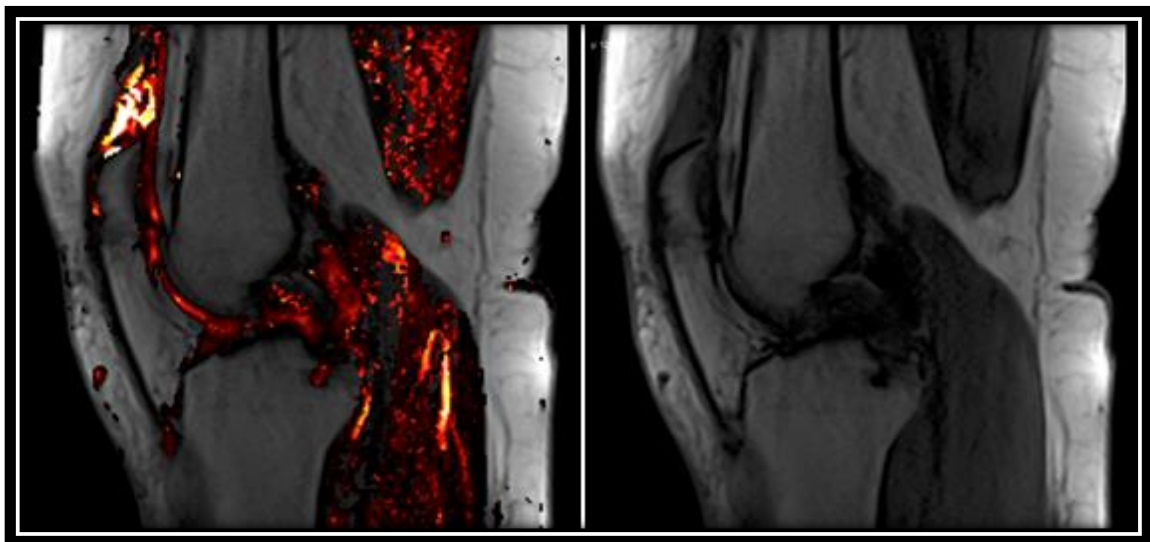




Tissue perfusion in knee osteoarthritis

Implications for exercise therapy



PhD thesis

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Implications for exercise therapy

Vævsperfusion ved knæartrose

Implikationer for terapeutisk træning

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Font page images: dynamic contrast-enhanced magnetic resonance imaging of a knee.

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PREFACE

This PhD thesis is based on the three original studies listed below. All studies were carried out at The Parker Institute, Department of Rheumatology, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen.

The manuscripts are enclosed in Appendices I-III

ORIGINAL STUDIES

Study I Associations between muscle perfusion and symptoms in knee osteoarthritis: a cross sectional study. Bandak E, Boesen M, Bliddal H, Riis RG, Gudbergesen H, Henriksen M. *Osteoarthritis Cartilage* 2015 Oct;23(10):1721-7.

Study II Exercise-induced pain changes associate with changes in muscle perfusion in knee osteoarthritis: exploratory outcome analyses of a randomised controlled trial. Bandak E, Boesen M, Bliddal H, Riis RG, Klokke L, Bartholdy C, Nybing JD, Henriksen M
In review in a journal with peer-review

Study III Adverse effects of exercise on synovitis in knee osteoarthritis: secondary outcome analyses of a randomised controlled trial. Bandak E, Boesen M, Bliddal H, Dagaard C, Hangaard S, Bartholdy C, Nybing JD, Kubassova O, Henriksen M
In review in a journal with peer-review

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SUMMARY

Knee osteoarthritis (OA) is a whole-joint disease with gradual degradation of the cartilage and local joint inflammation is present. Knee OA is the leading cause of pain and disability in the elderly population. Exercise therapy is a central component in the treatment of knee OA with beneficial effects on pain, function, and health-related quality of life. However, the effect on pain is moderate and the underlying complex mechanisms are not fully understood. Thus, there is a need for further improving the understanding of how exercise induces beneficial effects on pain in knee OA in order to optimise future treatment.

Magnetic resonance imaging (MRI) visualises all tissues in the knee joint. By adding a dynamic contrast-enhanced MRI sequence, it is possible to quantify perfusion of the knee joint-related tissues. Increased perfusion of synovium and Hoffa's fat pad has been associated with pain in knee OA.

The overall aim of this PhD project and the three studies that comprise it (Study I-III) was to investigate the effects of exercise therapy on perfusion in knee joint-related tissues using magnetic resonance imaging (MRI) and to investigate associations to knee pain in patients with knee OA.

In Study I, a cross sectional study, the associations between perfusion of the peri-articular knee muscles and pain were investigated in patients with knee OA. Muscle perfusion was quantified by dynamic contrast-enhanced MRI (DCE-MRI) and the Knee injury and Osteoarthritis Outcome Score questionnaire (KOOS) was used assessing knee pain. The results of Study I suggest that more widespread muscle perfusion is beneficial in knee OA as it associate with less pain. The association indicates that for each increment in the perfusion variable $N_{\text{voxel}}\%$ (Proportion of highly perfused voxels) the KOOS pain score is 0.41 higher, meaning less pain.

In Study II and III, the effects of 12 weeks of exercise therapy on perfusion in knee joint-related tissues (muscles (Study II), synovium, bone, Hoffa's fat pad, and Baker's cyst (Study III)) were investigated assessed by DCE-MRI in a randomised controlled trial (RCT).

Furthermore, it was investigated if perfusion changes in the knee joint-related tissues contribute in explaining the pain-reducing effect of exercise therapy in knee OA.

The results from Study II suggest that, exercise therapy increased muscle perfusion and reduced pain compared to a no-attention control group in knee OA. The statistical significant

group difference in favour of exercise therapy in pain change was -10.7 KOOS points (95%CI: -17.8 to -3.4). Changes in muscle perfusion were positively associated with changes in pain meaning higher muscle perfusion is associated with less pain. Thus, increased muscle perfusion may contribute to the pain-relieving effects of exercise therapy and be a potential objective marker on the effects of exercise in knee OA.

The results of Study III suggest that exercise therapy yields adverse effects on synovium (increased synovitis) compared to a no-attention control group in knee OA as there were significant group differences in DCE-MRI assessed synovitis in the anterior part of the knee with higher perfusion (adverse) values in the exercise group. No associations were found between pain changes and tissue perfusion investigated in Study III.

The overall conclusion of this PhD is that exercise therapy yields increased muscle perfusion that correlates with reduced pain, but is paralleled by adverse effect on synovitis in the anterior part of the knee when compared to a no-attention control group in patients with knee OA.

DANSK RESUMÉ (DANISH SUMMARY)

Knæartrose (slidgigt i knæet) er en sygdom, som omfatter hele leddet, hvor brusken gradvist nedbrydes og lokal betændelsestilstand er til stede. Knæartrose er den hyppigste årsag til smerte og nedsat fysisk funktion blandt ældre. Terapeutisk træning er en central del af behandlingen med gavnlige effekter på smerte, fysisk funktion og helbredsbedinget livskvalitet. Effekten på smerte er dog moderat og de underliggende mekanismer er ikke fuldt ud forstået. Dermed er der behov for at øge forståelsen for den smertenedsættende effekt af terapeutisk træning for derigennem at kunne optimere behandlingen på længere sigt.

Magnetisk resonans (MR) scanning kan visualisere alle væv i knæleddet. Ved at tilføje en dynamisk kontrastforstærket MR sekvens er det muligt at kvantificere blodgennemstrømning (perfusion) i vævene. Øget perfusion i synovium (ledslimhinden) og Hoffa's fedtleger (fedtpude, lokaliseret nedenfor knæskallen) har vist sig at være associeret med smerter ved knæartrose.

Det overordnede formål med denne ph.d. og de tre studier, som udgør den (Studie I-III) var at undersøge effekten af terapeutisk træning på perfusion af væv i og omkring knæleddet ved brug af MR-scanning samt at undersøge associationer til knæsmerter hos patienter med knæartrose.

I Studie I, som var et tværsnitsstudie, blev forholdene mellem perfusion i muskulaturen omkring knæet og smerte ved knæartrose undersøgt. Perfusion blev kvantificeret ved brug af kontrastforstærket MR-scanning og spørgeskemaet KOOS blev anvendt til at rapportere knæsmerter. Resultaterne fra Studie I tyder på, at mere udbredt perfusion i muskulaturen er gavnlig ved knæartrose, da det er forbundet med mindre smerte. Den fundne sammenhæng indikerer, at for hver stigning i variabelen Nvoxel% (andelen af væv med høj perfusion), så stiger KOOS scoren med 0.41 (jo højere KOOS score jo mindre smerte).

I Studie II og III, som var randomiserede kontrollerede studier, undersøgtes effekten af et 12 ugers terapeutisk trænings program på perfusion i væv med relation til knæledet (muskler (Studie II), synovium (ledslimhinden), knogle, Hoffa's fedtlegeme samt cyster (Baker's cyste) (Studie III)) ved brug af kontrastforstærket MR-scanning. Derudover blev det undersøgt om ændringer i perfusion i væv med relation til knæledet bidrager til at forklare den smertereducerende effekt af terapeutisk træning ved knæartrose.

Resultaterne fra Studie II tyder på, at terapeutisk træning øgede perfusion i muskulaturen og reducerede smerte ved knæartrose sammenlignet med en kontrolgruppe, som ikke modtog nogen behandling. Den statistisk signifikante gruppeforskel på ændringer i smerte til fordel for træning var -10.7 KOOS point (95% CI: -17.8 to -3.4). Ændringer i perfusion i muskulaturen var positivt forbundet med ændringer i smerte, hvilket betyder, at højere perfusion i muskulaturen er forbundet med mindre smerte. Dermed kan øget perfusion i muskulaturen muligvis bidrage til den smertereducerende effekt af terapeutisk træning og være et potentielt objektivt mål for effekten af terapeutisk træning ved knæartrose.

Resultaterne fra Studie III tyder på, at terapeutisk træning medfører uønsket effekt på synovium med øget synovit (betændelse i ledslimhinden) ved knæartrose sammenlignet med en kontrolgruppe, som ikke modtog nogen behandling med signifikante gruppeforskelle i synovit målt med kontrastforstærket MR i den forreste del af knæledet med højere perfusion (uønsket) i træningsgruppen. Der blev ikke fundet nogen sammenhæng mellem ændringer i perfusion og ændringer i smerte i vævene undersøgt i Studie III.

Den overordnede konklusion på denne ph.d. er, at terapeutisk træning giver øget perfusion i muskulaturen, som er forbundet med mindre smerte samt at terapeutisk træning har en uønsket effekt på synovit i den forreste del af knæet ved knæartrose sammenlignet med en kontrolgruppe, som ikke modtog nogen behandling.

ABBREVIATIONS

BMI	Body Mass Index
BMLs	Bone marrow lesions
CE-MRI	Contrast-enhanced magnetic resonance imaging
CR	Conventional radiography
DCE-MRI	Dynamic contrast-enhanced magnetic resonance imaging
Gd	Gadolinium
IRE	Initial rate of enhancement
IRW	Initial rate of washout
KL	Kellgren and Lawrence grading scale
KOOS	Knee injury and Osteoarthritis Outcome Score questionnaire
ME	Maximal enhancement
MOAKS	MRI in Osteoarthritis Knee Score
MRI	Magnetic resonance imaging
Nvoxel%	Proportion of highly perfused voxels
OA	Osteoarthritis
PROMs	Patient reported outcome measures
ROI	Region of interest
SI-curves	Signal-intensity curves
VOI	Volume of interest

1. INTRODUCTION

Osteoarthritis (OA) is the most common degenerative joint disease resulting in gradual destruction of the cartilage and involvement of all tissues of the joint (1). OA causes pain and disability that lead to reduced health-related quality of life (2). As there is yet no cure, treatment of OA consist of pain management, improving disability, and reducing disease progression (3).

Exercise therapy is a part of the recommended core treatment of knee OA (4-6) based on extensive evidence on beneficial effects on pain, function, and improvement in health-related quality of life (7-10). The effect sizes of exercise therapy are consistently small to moderate irrespective of exercise modality and dosages (11). Furthermore, the effect is known to decline after cessation of exercise therapy (12). In order to improve the understanding of a possible mode of action, several factors have been suggested mediating the pain-reducing effects of exercise (13-17). However, still the underlying mechanisms remain unclear (18).

As muscle tissue is the primary tissue affected by exercise; any physiological changes caused by exercise are expected to be reflected in the muscles. Furthermore, exercise is suggested to have a systemic anti-inflammatory effect (19-21) and local anti-inflammatory response in both intra-articular and peri-synovial with increased concentrations of IL-10 have been reported in knee OA after one exercise session (22).

Knee OA is a complex disease involving both central and peripheral mechanisms (18) including local joint inflammation (23). Inflammation of the synovium (synovitis) is the hallmark of intra-articular inflammation in knee OA (24-27) and a common finding together with bone marrow lesions (BMLs) (28). Synovitis and BMLs are associated with knee OA pain (29-31). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a sensitive method to assess signal intensity based measurements of perfusion in synovium and BMLs reported to correlate with pain in knee OA (32-34). DCE-MRI is a sensitive method to assess muscle perfusion as well (35).

Investigating the effect of exercise on knee joint-related tissue perfusion and the relation to knee OA pain may contribute to the understanding of the pain-reducing mechanisms of exercise therapy in knee OA and give implications for exercise therapy.

2. AIM AND HYPOTHESES

The overall aim of this PhD project and the three studies that comprise it was to investigate the effects of exercise therapy on perfusion in knee joint-related tissues using magnetic resonance imaging (MRI) and to investigate associations to knee pain in patients with knee OA.

This was addressed using two different approaches: first, the relationships between perfusion of the peri-articular knee muscles assessed by dynamic contrast- enhanced magnetic resonance imaging (DCE-MRI) and pain (KOOS) in patients with knee OA were investigated (Study I).

Secondly, the effects of exercise therapy on perfusion in knee joint-related tissues (muscles, synovium, bone, Hoffa's fat pad, and Baker's cyst) assessed by DCE-MRI and associations between changes in the knee joint-related tissues and changes in pain in patients with knee OA compared to a no-intervention control group were explored (Study II and III).

Hypotheses

1. Higher levels of perfusion (higher contrast enhancement assessed by DCE-MRI) in the peri-articular knee muscles are associated with more pain in patients with knee OA (Study I).

As the first hypothesis was rejected (in fact the opposite was the case as more widespread perfusion was associated with less pain) the hypothesis for Study II was formulated as follows:

2. Exercise therapy increases muscle perfusion in parallel with reduced pain in patients with knee OA when compared with a no-attention control intervention (Study II).
3. Exercise therapy reduces synovitis and BMLs in parallel with reduced pain in patients with knee OA when compared with a no-attention control intervention (Study III).

3. KNEE OSTEOARTHRITIS

Epidemiology

Osteoarthritis (OA) is the most common degenerative joint disease (36) with knee OA being the leading cause of pain and disability in the elderly population leading to reduced quality of life (2). OA is the 12th leading cause of years lived with disability (37) with knee OA accounting for more than 80% of the disease's total burden (38).

The prevalence of OA has doubled since the mid-20th-century and is expected to continue due to an increasing ageing population and obesity (36, 39). 15 % of all individuals aged 56-64 years are estimated to have symptomatic or clinically knee OA increasing in prevalence to 17-33% in individuals aged 75 years or older (40, 41). Radiographically verified knee OA affects around 17- 26% of all individuals aged 56-64 years and 25 -50% of all individuals aged 75 years or older (40, 41).

Etiology and pathogenesis

The etiology of knee OA is not fully understood but is suggested to be multi-factorial with both non-genetic and genetic risk factors as age, gender, overweight, obesity, inactive lifestyle, joint injury, knee malalignment and occupation with high biomechanical knee load (1, 42-45). Low-grade systemic inflammation has been suggested as a risk factor as well (46, 47).

Knee OA is a whole-joint disease involving all the tissues of the synovial joint including articular cartilage, menisci, bone (subchondral bone and bone marrow), ligaments, synovium, capsule, adipose tissues, and muscles (1, 43, 48, 49). OA is characterised by a repair process with a combination of tissue attrition and new tissue production, manifesting typically as focal loss of articular cartilage, bone remodeling, and marginal osteophyte formation. Varying degrees of synovitis, increased synovial fluid volume capsular thickening, degenerative changes in the menisci and ligaments are also recognised features (1, 43, 48-50).

Diagnostic criteria and classification

A clinical diagnosis of knee OA is based on symptoms and clinical examination. Several clinical diagnostic criteria for knee OA have been proposed (51) with the criteria suggested by the American College of Rheumatology requiring the presence of knee pain being widely used (52) (Table 1).

In epidemiologic studies, the severity of knee OA on conventional radiography (CR) is often classified using The Kellgren and Lawrence (KL) grading scale from 0-4 (53). The threshold for a radiographic diagnosis of knee OA is at KL grade ≥ 2 with definite osteophyte and possible joint space narrowing (53). The medial tibiofemoral compartment is most frequently affected by OA (54). Symptomatic knee OA is when both radiographic and clinical diagnosed knee OA is present (40, 55).

Table 1. The American College of Rheumatology diagnostic criteria for knee OA (Adapted from Altman 1986 (52))

Clinical criteria	Clinical and radiographic criteria
Knee pain + at least 3 of the following:	Knee pain and osteophytes + at least 1 of the following:
Age > 50 years Stiffness < 30 minutes Crepitus Bony tenderness Bony enlargement No palpable warmth	Age > 50 years Stiffness < 30 minutes Crepitus

Clinical presentation and symptoms

Clinically, the osteoarthritic knee is often characterised by tenderness when palpating the joint line, swelling, stiffness and reduced range of motion. Crepitus, bony enlargements, deformity of the bones, instability of the joint, altered gait, muscle atrophy or weakness are other typical features often present in knee OA (1, 2, 56).

The main symptom of knee OA is pain which includes activity-induced pain relieved by rest. At later disease stages pain may also occur at rest. Impaired physical function, stiffness, joint instability, impaired muscle strength and pain-related psychologic distress if pain persists are all frequent symptoms in knee OA (1, 2, 55, 56).

Pain and pain mechanisms

The mechanisms causing knee OA pain are complex and not fully understood (57). Nociceptive and neuropathic mechanisms both at central and local levels are suggested to be involved (58, 59) including local inflammation of the joint structures (18, 23).

Pain in OA is heterogeneous and may present with different patterns as constant and intermittent pain, with or without a neuropathic component, and with or without sensitization (57, 60). Pain sensitization i.e. altered nociceptive processing within the peripheral and central nervous system has been suggested to play a central role in knee OA (59, 61, 62). The mechanisms by which sensitization may occur in people with knee OA are still unclear (61, 63).

There is a discordance between structure and symptoms in OA (64, 65) indicated by OA pain not being correlated with radiographic joint damage (66, 67). This may be because cartilage is not innervated (68). Despite largely innervation of osteophytes, bone, meniscal changes, and ligament tears they are not with OA pain (69). BMLs, synovium (synovitis), and Hoffa's fat pad are all largely innervated, correlated to pain and suggested playing important roles in the generation of pain in knee OA (64, 70-74).

OA pain perception is influenced by multiple factors including biological, psychological, social and environmental factors (56) (75-78). These factors together with the discordance between the degree of joint damage and pain (66, 67) underline the importance of not only focusing on the joint structures when trying to clarify the underlying mechanisms of OA pain (79). Instead, a bio-psychosocial approach is advised (56, 65).

4. INFLAMMATION AND RELATION TO PAIN IN KNEE OSTEOARTHRITIS

The evidence of the role of inflammation in the pathogenesis of osteoarthritis is evolving. Inflammation is now considered a key factor in the generation and progression of structural degeneration and symptoms (23, 46, 47).

Within the osteoarthritic joint local inflammatory processes take place triggered by tissue damage and stress responses in the cartilage, bone and synovium (23, 46-48, 80). Cartilage degradation products together with pro-inflammatory chondrocyte derived-products affect the synovium. This leads to the synovium producing inflammatory mediators that feedback on chondrocytes leading to further deregulation that again lead to increased cartilage degradation (23, 25, 46-48). The inflammatory process is suggested to increase the intra-articular pressure (81) which may activate nociceptors (82, 83).

Furthermore, joint inflammation is suggested to promote pain sensitisation in knee OA through sensitisation of the joint nociceptors leading to enhanced pain facilitation (84, 85). Thus anti-inflammatory treatments may prevent sensitisation and mediate pain reduction in knee OA (32, 86). However, long-term repeated anti-inflammatory treatment using intra-articular corticosteroids did not reduce pain but had adverse effect on cartilage in knee OA (87).

Systemic markers of inflammation

Systemic markers of inflammation are modestly elevated in OA (CRP, TNF- α , TNF-R1, TNF-R2, TNFR1 and sTNFR2) (88) and suggested to contribute to the pathophysiology of knee OA (89). Furthermore, higher levels of pro-inflammatory markers in serum were associated with pain and impaired physical function in patients with knee OA (90, 91).

Adipokines are systemic factors (e.g. leptin, adiponectin, resistin) released by adipose tissue associated with OA severity and inflammation (92-95). Overweight is shown to double the relative risk of hand OA (96), which further supports the assumption that adipose tissue generate substances with negative influence in the OA process. Obesity and metabolic syndrome are associated with increased systemic low-grade inflammation and are known to increase the risk of OA (89, 94, 97).

Local inflammation

Synovial inflammation (synovitis)

Synovitis is the hallmark of intra-articular inflammation and a common finding in all stages of knee OA (24-27). Further, synovitis is an independent cause of incident knee OA (98). Synovitis can be assessed by histology, arthroscopy and using ultrasonography and contrast-enhanced MRI (49, 99-101). Histological changes of the synovium include hypertrophy and hyperplasia with increased number of lining cells (24, 49).

The development of synovitis is suggested to be mediated by dysfunctional chondrocytes and cartilage (25, 48, 102). Synovitis is associated with pain (29, 31, 70, 73, 103, 104) and pain sensitisation in knee OA (61, 85). Worsening of synovitis is associated with increased risk of frequent and more severe pain (70, 72, 104), radiographic disease progression and cartilage deterioration (29, 105, 106), whereas reductions of synovitis is reported correlated with reduced pain (86). Therefore synovitis is of particular interest as a target for preventive and therapeutic interventions in knee OA (72, 86, 105). Recent results indicate that long-term treatment of synovitis using intra-articular corticosteroids did not reduce pain and might have adverse effect on cartilage (87, 107).

Inflammatory activity in the synovial fluid has been identified and associated with symptoms and joint damage (24, 108-110). The number of leukocyte counts in the synovial fluid is suggested to be a future marker of inflammation as the leukocyte number correlate with synovial volume (111).

The infrapatellar fat pad (Hoffa's fat pad)

Hoffa's fat pad is an intra-capsular extra-synovial structure located behind the patellar tendon covered by synovial membrane posteriorly consisting of adipose tissue (112). It is considered to contribute to the pathophysiological changes in knee OA via production of cytokines and adipokines mediating joint inflammation (113-115). Inflammation in Hoffa's fat pad is reported to be a source of pain (104, 116, 117) and associated with disability (117). The inflammatory cell composition in Hoffa's fat pad is suggested to be similar to that of the synovium in knee OA (116).

Cysts

Intra- and peri-articular cyst-like lesions are a well-known phenomenon in knee OA with Baker's (popliteal) cyst being the most frequent one (118-120). There is conflicting evidence regarding the association of Baker's cysts and pain in knee OA (118, 120-122). The presence of Baker's cysts has been reported to be associated with joint effusion (119, 123) and the presence of joint effusion is associated with pain in knee OA (69). The presence of Baker's cysts does not seem to predict radiographic OA severity (120) and it may be a secondary phenomenon rather than a primary trigger of knee OA pain (119).

Muscles

The role of muscle tissue in the symptomatology of knee OA is not clear (12, 124), but findings of inflammatory markers in peri-articular knee muscles in patients with knee OA suggest that inflammatory processes are not only confined to articular structures (125, 126). Muscle inflammation may well contribute to the development of pain and the functional decline in knee OA. This is supported by the association between pro-inflammatory markers in knee muscles (MCP-1 and STAT-3) and reduced biomechanical function of the knee during walking, lower habitual walking speed, and self-reported reduced physical function (126).

Improving muscle quality by reducing adipose tissue and thereby reducing the amount of adipokines secreted may potentially play a metabolic and anti-inflammatory role in OA pathogenesis (127). Adipose tissue is considered an endocrine organ that secretes inflammatory cytokines such as IL-1 β and TNF- α and adipokines such as adiponectin, resistin and to a lesser degree leptin that have been implicated in the pathogenesis of OA (93, 128). Indirect support of the role of muscle in the regulation of inflammatory pathways has been reported at a genetic level (129).

Local changes in subchondral bone

Bone marrow lesions

Bone marrow lesions (BMLs) are a common finding in knee OA (28) and located in the subchondral bone. On radiographs BMLs are non-cystic and histologically characterized by fat necrosis, localized marrow fibrosis and micro-fractures of the trabecular bone (130). BMLs quantified by MRI are non-cystic subchondral areas of ill-defined hyperintensity on T2-weighted or proton density-weighted, fat suppressed fast spin echo images (28, 130).

The pathophysiology of BMLs is poorly understood and suggested to reflect mechanical injury of the bone (131). BMLs associate with active bone remodeling and repair (132, 133) and tend to associate with regions of cartilage damage in knee OA (134, 135).

BMLs are associated with pain (30, 73, 75, 136) but do not seem to contribute to sensitisation in knee OA (61). Worsening of BMLs is associated with increased risk of frequent and more severe pain (70, 72, 86, 104, 137), radiographic disease progression and cartilage deterioration (29, 98, 105, 138), whereas reductions of BML volume correlate with reduced pain (72, 86, 136). Therefore BMLs are of particular interest as a target for preventive and therapeutic interventions in knee OA (72, 139). However, no relation was found between the presence or absence of BMLs and treatment outcomes (pain and global perceived effect) after an 18-week exercise intervention (140).

5. MANAGEMENT AND TREATMENT OF KNEE OSTEOARTHRITIS

As there is yet no treatment available to alter structural progression in knee OA the management focus is on reducing symptoms and improving physical function. The optimal management of OA requires a combination of non-pharmacological and pharmacological modalities (56, 141, 142) and if not sufficient, surgery may be an option.

Non- surgical management

Several evidence-based consensus recommendations on non-surgical core treatments appropriate for all individuals with knee OA include self-management and education, weight management, biomechanical interventions, exercise, and intra-articular corticosteroids (3-6).

Non-pharmacological management

Self-management and patient education

Health education and self-management are widely recommended as a part of the core treatment in knee OA (3-5, 141-143) with only limited effect on pain (141).

As chronic pain is maintained and influenced by maladaptive emotional, cognitive and behavioral factors (144, 145) interventions addressing these factors are recommended (146).

Weight management and weight loss

Weight management is well-established in the primary management (prevention before clinical symptoms or structural disease development) and secondary preventive strategy for OA (to slow down progression) (142). Therapeutic weight reduction in knee OA was in a meta-analysis found to improve pain and physical function (147).

Biomechanical interventions and assistive devices

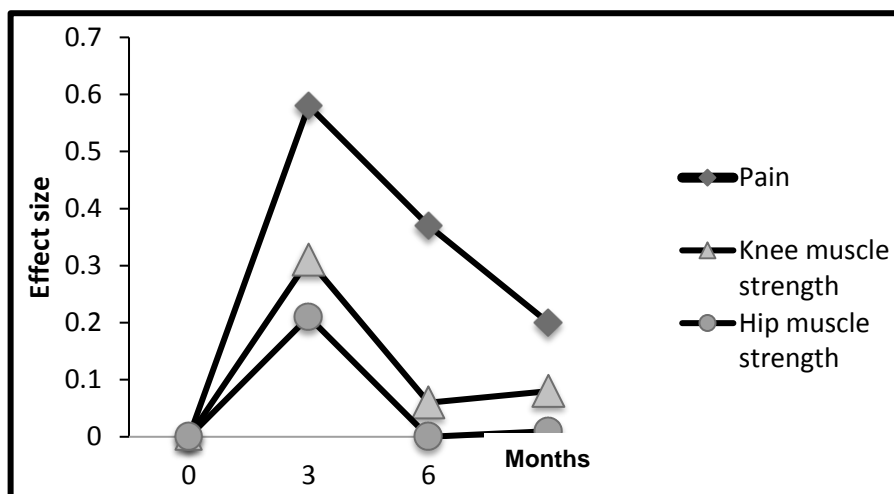
Biomechanical interventions as the use of braces and orthoses as directed by an appropriate specialist (148) are recommended in several guidelines (3-6). A recent study suggests that knee brace intervention is effective in reducing BMLs in patellofemoral knee OA (149).

Exercise therapy

There is extensive evidence on exercise therapy-induced improvement of pain, physical function (7, 8, 150), and quality of life (10) in knee OA with small to moderate effect sizes comparable with estimates reported for non-steroidal anti-inflammatory drugs (7). Unfortunately, the beneficial effects of exercise therapy decline after end of treatment (Figure 1) (7, 12).

The effect sizes for pain and physical function are independent of whether exercise therapy is individual or delivered as group-based or home-programs. However, individually delivered programs might result in greater reductions in pain and improvements in physical function, compared to group-based or home-programs. Furthermore, the effect rates seem not to be affected by different types of exercise therapy (e.g. quadriceps strengthening only, aerobic exercise, walking or a combination) (7). However, stratified analyses have shown that exercise programs focusing on quadriceps strength only, despite differences in radiographic severity and baseline pain, were more beneficial in reducing pain and reducing disability than programs aiming at improving general lower limb strength (11).

Figure 1. Effects of exercise declines after cessation. (Adapted from Bennell et al 2013 (12))



Increased number of face-to-face contacts with the healthcare professional supervising the exercise program improved the immediate effect size of exercise on pain and physical function (7). This is supported by Juhl et al. (11) who found a positive dose-response effect on pain improvement and larger number of supervised sessions of aerobic exercise, however this was not the case for disability (11).

Pharmacological management

Analgesics

Despite taking all their prescribed medicine most people with OA have persistent pain (1). Oral acetaminophen (paracetamol) (141, 142, 151) and non-steroidal anti-inflammatory drugs (NSAIDs) (152, 153) are first-line therapies with small to moderate effect on pain. However, the treatments are associated with considerable side effects as gastrointestinal and cardiovascular complications (154-157) which calls for reconsideration of the present recommendations of using paracetamol and NSAIDs in the pain management of knee OA (155, 158).

Celecoxib (NSAID oral COX-2 inhibitor) might be slightly better than placebo and topical NSAIDs in reducing pain and improving physical function (154). Topical NSAIDs are widely recommended for treating symptomatic knee OA as alternative or adjunctive therapy (142, 151). Conflicting evidence on the effect of oral or transdermal opioids are reported (158). Adverse events are frequent and include dizziness, nausea, constipation and falls, which limit the clinical use of opioids (158, 159).

Intraarticular corticosteroids

Intraarticular corticosteroids are widely recommended in the management of knee OA (4, 5, 160). However a recent meta-analysis (161) questions the routine use in clinical practice as it remains unclear whether there are clinically important benefits one to six weeks after corticosteroid injection (161). Furthermore, long-term use of intra-articular corticosteroids did not reduce pain and increased cartilage degradation compared with controls in knee OA (87, 107)

Surgical treatment

Joint replacement surgery is universally recommended in existing treatment guidelines if a combination of non-pharmacological and pharmacological interventions fail to improve pain, physical function and health-related quality of life in knee OA (142, 143, 162, 163).

Joint replacement surgery is generally accepted as a reliable and appropriate procedure to restore function and improve health-related quality of life in patients with knee OA. The prognosis of persisting pain occurring after total knee replacement is affected by independent risk factors as mental health disorder, catastrophizing, pain at multiple sites and preoperative knee pain (164, 165). The age at the time of surgery is of major importance when it comes to the risk of needing a revision. Age above 62 years at the time of surgery implies a low risk of revision (166).

6. IMAGING IN KNEE OSTEOARTHRITIS

Conventional radiographs

Conventional semiflexed standing radiography (CR) is the standard method in clinical practice for an imaging-based diagnosis of knee OA and to assess structural progression in knee OA (Figure 2) (167-169). The typical changes detected by CR are osteophytes, joint space narrowing or decreased joint space width, subchondral sclerosis, and subchondral cysts (170). Semi-quantitative scoring systems are used in the assessment of the severity of radiographic knee OA (170) with the Kellgren and Lawrence grading system being one of the most widely used in defining radiographic OA (53).

There is a poor association between CR and clinical features (67, 171). This might be due to the fact that CRs are insensitive to cartilage loss, and joint space narrowing is a composite measure of meniscal extrusion and cartilage loss and CRs cannot capture key elements of knee OA such as soft tissue pathology and inflammation (171, 172).

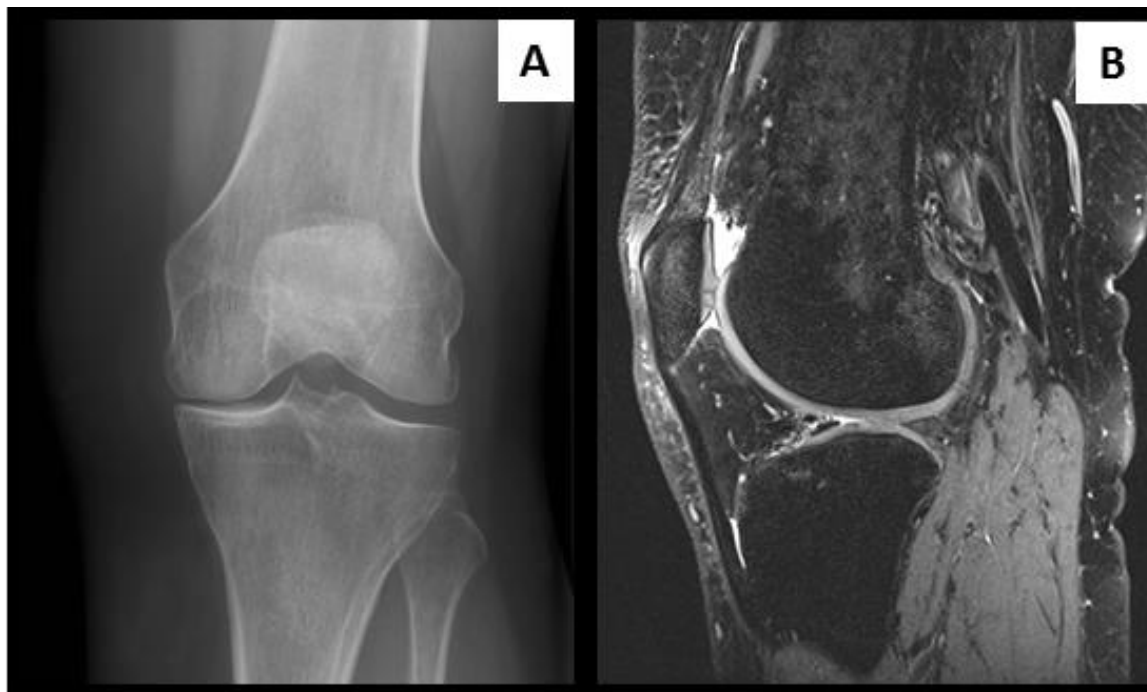
MRI

The use of MRI has contributed strongly to the understanding of knee OA as a whole-joint disease (167). MRI provides a unique 3-D visualization of all the anatomical structures of the knee including cartilage, menisci, ligaments, synovium, effusions, muscles, Hoffa's fat pad and bone marrow (Figure 2) (167, 172). MRI-based whole-joint assessment has shown that the structural changes in knee OA also are common in knees not showing definite radiographic changes (173). Associations between clinical features (including pain) and MRI assessed structures (synovitis, BML, Hoffa's fat pad) underline the importance of MRI in the understanding of knee OA pathophysiology and management (69, 167, 174).

Semiquantitative MRI scoring systems for non-contrast-enhanced MRI evaluating all knee joint related structures in knee OA are well-established (175-179). In the evaluation of synovitis and effusions, only contrast-enhanced MRI (CE-MRI) is able to differentiate the contrast-enhancing synovium from effusion with synovitis correlating with histological synovitis (180). The "CE-synovitis score" has been proposed in the assessment of synovitis on CE-MRI in knee OA (73).

Figure 2.

Conventional semiflexed standing radiograph (KL grade 3) (A) and MRI (proton density (Pd)-weighted) (B) of the left knee.



Dynamic contrast-enhanced MRI (DCE-MRI) is based on imaging before, during and after intravenous bolus infusion of a Gadolinium (Gd) contrast agent. The distribution of the contrast agent depends on the tissue perfusion and with DCE-MRI it can be quantified through temporal variations of the MRI signal intensity (32, 181, 182). Thus DCE-MRI variables can be used as surrogate markers of perfusion. Perfusion parameters can be extracted using signal-intensity curves (SI-curves) reflecting the speed, degree and distribution of the contrast over time in every single voxel within the image (183, 184). The SI-curves can be analysed quantitatively using either a pharmacokinetic (185) or a heuristic approach (186), which is the approach used in this PhD (more details later).

In knee OA, the rate and degree of DCE-MRI assessed contrast enhancement of the synovium correlate with histological synovitis in knee OA (100, 101). DCE-MRI assessment of the synovium has shown to be an appropriate measure of synovitis as they are more responsive and correlate better with symptoms in knee OA than conventional static MRI methods (32, 33, 187). DCE-MRI assessed perfusion of BMLs (34, 188, 189), Hoffa's fat pad (117), and muscles (Study I and II) in knee OA have been reported.

7. METHODOLOGY

The methods used in the studies comprising this PhD project (Study I-III) will be described in the following sections. Further details can be found in the original manuscripts appended to the thesis (Appendices I-III).

Study designs

In Study I, cross sectional data at one-year follow up from a weight-loss maintenance study were used (the LIGHT study; ClinicalTrials.gov: NCT00938808), (n=94). In Study II and III exploratory and secondary outcome analyses, respectively, were performed based on data from a randomised controlled parallel-group-trial investigating the effects of a 12-week therapeutic exercise programme on the pain sensitivity in patients with knee OA with per-protocol analyses, (n=33) (15).

Study populations

Inclusion criteria

A clinical diagnosis of knee OA verified by radiographs were an inclusion criterion in all studies. In Study I, eligibility criteria included age > 50 years and a body mass index (BMI) ≥ 30 kg/m². In Study II and III the eligibility criteria were age ≥ 40 years and a BMI index between 20 and 35 kg/m².

Exclusion criteria

Former or planned lower extremity joint replacements were exclusion criteria in all studies. In Study I, lack of motivation to lose weight, being in pharmacologic treatment for obesity, planned bariatric surgery, active joint disease besides OA including significant hip OA, and use of opioids were exclusion criteria. In Study II-III participation in exercise therapy within the previous 3 months, having inflammatory and autoimmune diseases were exclusion criteria.

Ethical considerations

All three studies were approved by the Regional Health Research Ethics Committee of The Capital Region of Denmark (Study I; (H-B-2007-088) and Study II and III; (H-2-2011-159)) and were conducted in accordance with the Declaration of Helsinki. Prior to inclusion in all three studies, all participants gave their oral and written informed consent to participate. Studies were registered prior to commencement at www.ClinicalTrials.gov: NCT00938808 (Study I) and NCT01545258 (Study II and III).

The use of intravenous Gd-containing MRI contrast-agents is in the large majority of cases safe (190). In all three studies, DCE-MRI was not performed in persons with known or suspected allergy against Gd-containing MRI contrast-agents in order to prevent anaphylaxis. As Gd-containing contrast-agents are primarily excreted renally, the administration of such contrast-agents may deteriorate an already impaired renal function. By consequence, DCE-MRI was contraindicated in persons with decreased renal function with an estimated glomerular filtration rate (eGFR <60 ml/min/1.73m²) as recommended by the Danish Health and Medicines Authority.

Assessment of perfusion and inflammation using MRI

Static conventional MRI was performed prior to a DCE-MRI sequence with intravenous infusion of Gd followed by a post contrast sequence in all the three studies comprising this PhD (Study I-III). The full MRI protocols are described in details in Ballegaard et al. (117) (Study I) and in Supplementary file (Study II and III).

Assessment of perfusion using DCE-MRI

Dynamika®

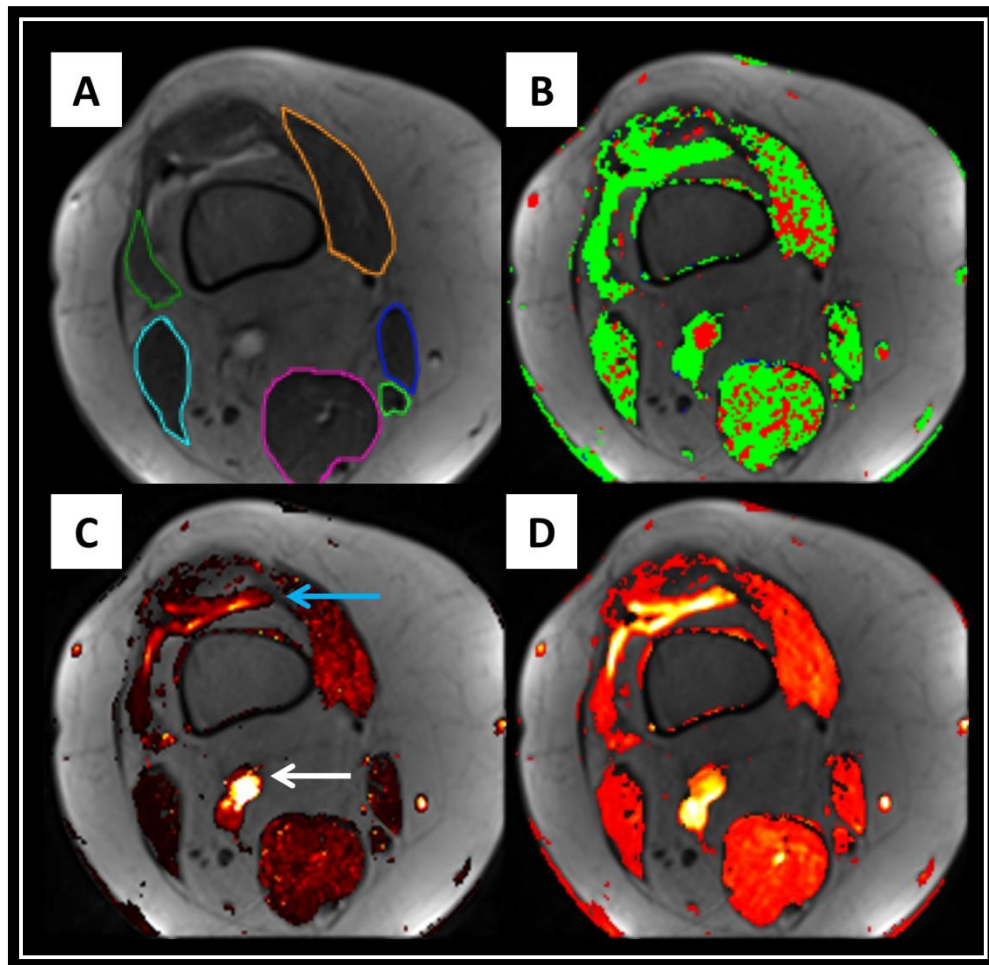
In all three studies, the analyses of DCE-MRI quantified perfusion were performed using the software Dynamika® (Image, Image Analysis Group, London, UK). The first steps of the analyses in Dynamika® are uploading of the DCE-MRI images followed by motion correction between temporal slices and determination of a baseline level of signal intensity (the two first time frames chosen in Study I-III) (191). Subsequently, regions of interest (ROIs) were manually drawn on all slices containing the structures listed below avoiding major vascular branches. For anatomical guidance and confirmation of the structures, additional MRI sequences were viewed in parallel (Figure 5, C).

Precise ROIs refer to drawings of the anatomical boundaries of the structure. Rough ROIs are drawings of the enhancing part of the structure and may contain non-enhancing parts as well. Only enhanced voxels contribute to the perfusion variables. Using precise ROIs enables calculation of the volume.

- Precise ROIs were manually drawn around the peri-articular muscle groups in Study I and Study II (Figure 3, A and 4 A, respectively).
- Rough ROIs were manually drawn around enhancing synovium (synovitis) divided into three regions in Study III (Figure 5):
 - Anterior synovium, Posterior synovium, and Hoffa synovium
- Precise ROIs were manually drawn around Hoffa's fat pad and Hoffa-related synovium (Hoffa anatomical) in Study III (Figure 5, A-B, Hoffa anatomical (orange)):
- Enhancing bone (BMLs) and Baker's cysts if present (Study III) (Figure 5, A-B).

The ROIs of each structure were summed and averaged into one single volume of interest (VOI) for each structure. The calculations in Dynamika® on a voxel-by-voxel basis within the ROIs and averaged across the VOI (33, 181). From the VOIs, mean values of DCE-MRI perfusion variables were extracted (Table 2).

Figure 3. Axial DCE-MRI (GRE T1 w VIBE sequence) of the knee (Adapted from Study I).



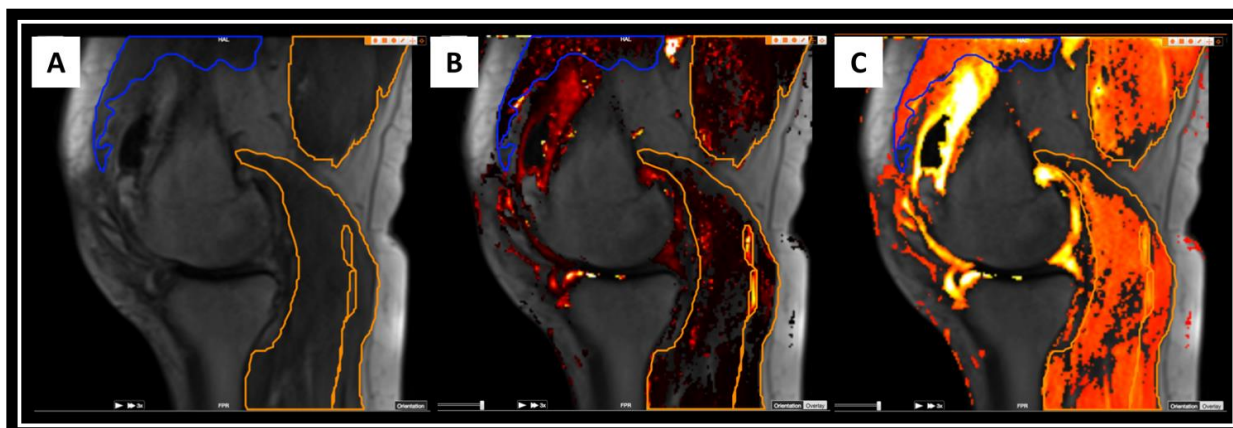
A: regions of interest (ROIs) were manually drawn on all the images containing peri-articular knee muscles exemplified with a single image.

B: contrast enhancement patterns: washout (red), persistent (blue), plateau (green), or no enhancement (no color).

C: maps of Initial Rate of Enhancement (IRE). White arrows: the popliteal artery. Blue arrows: contrast enhancement in the synovium in the retro-patellar space

D: maps of Maximum Enhancement (ME). Values of IRE and ME are colour-coded and superimposed on the grey scale dynamic images. The highest values are shown in bright yellow to white and lower values in a spectrum of red colours.

Figure 4. Sagittal DCE-MRI (GRE T1 VIBE) of the knee (Adapted from Study II).



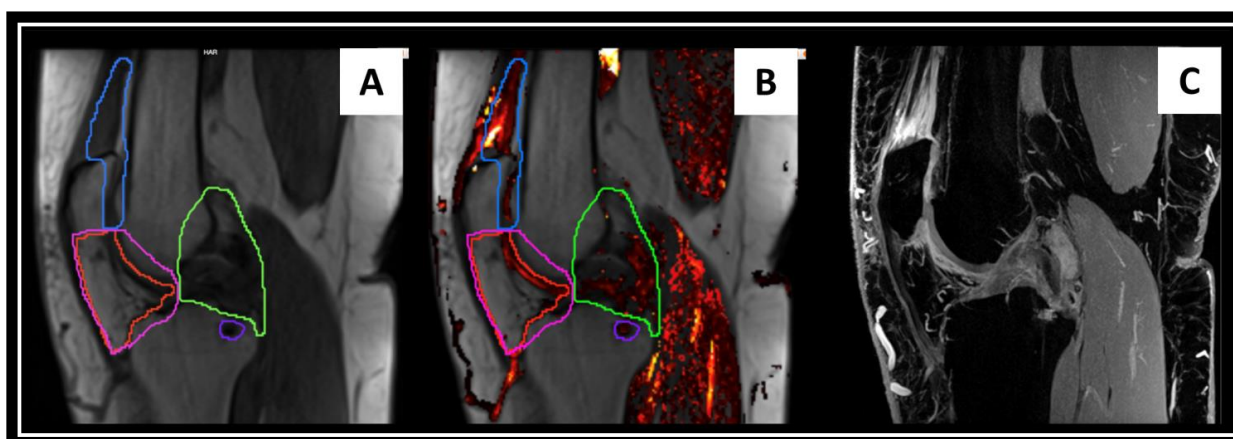
A: regions of interest (ROIs) were manually drawn on all the images containing peri-articular knee muscles exemplified with a single image: blue: extensor muscle; yellow: flexor muscles.

B: Initial Rate of Enhancement (IRE) map as shown in Dynamika®

C: Maximal Enhancement (ME) map as shown in Dynamika®.

Values of IRE and ME are colour-coded and superimposed on the grey scale dynamic image. The highest values are shown in bright yellow to white and lower values in a spectrum of red colours.

Figure 5. Regions of interest in Study III on sagittal DCE-MRI (GRE T1 VIBE) of the knee (Adapted from Study III).



A-C: Anterior synovium (blue); Posterior synovium (green); Hoffa synovium (pink); Hoffa anatomical (orange); Bone marrow lesion (purple).

A: without contrast map

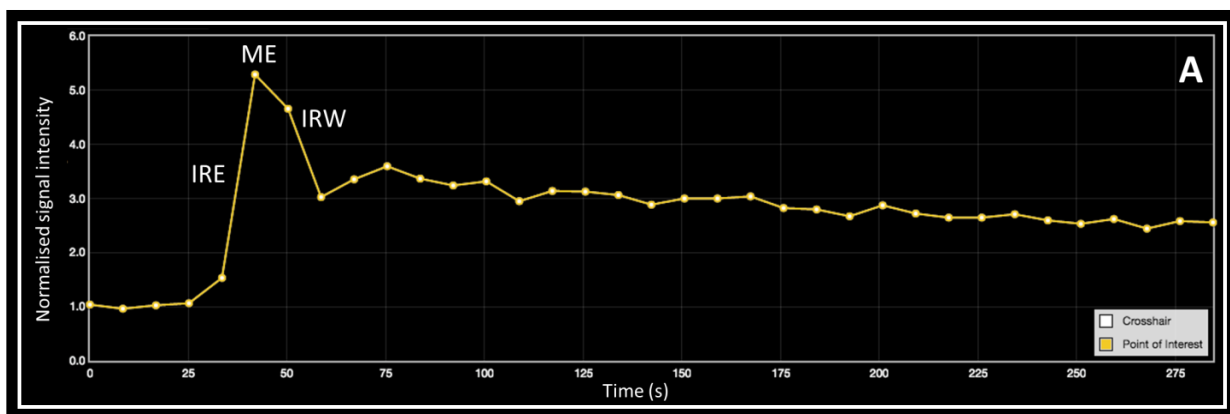
B: Initial Rate of Enhancement map as shown in Dynamika®.

C: The corresponding reconstructed 3D VIBE post contrast image with 5 mm slice thickness corresponding to the DCE-MRI image.

Heuristic DCE-MRI analyses

The DCE-MRI were analysed using a heuristic approach which is based on signal intensity changes over time in each voxel (33, 100, 181) calculated relatively to a defined baseline signal intensity level (set to the two first time frames in Study I-III). The signal intensity changes over time can be plotted as SI-curves (Figure 6) (33, 181).

Figure 6. Signal-intensity-curve (SI-curve)

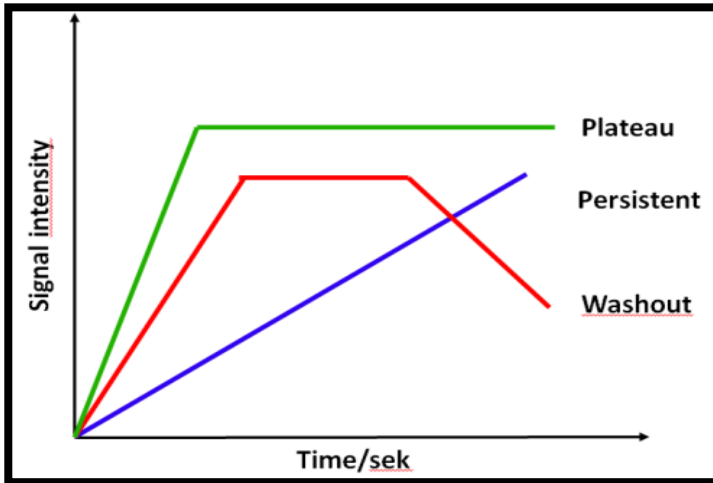


SI-curve from a point of interest in the popliteal artery with the characteristics of tissue with high perfusion: rapid increase in signal intensity, a plateau is reached with a subsequent rapid decrease (washout) (x-axis: time (s); y-axis: normalised signal intensity (baseline= 1.0)). IRE: initial rate of enhancement; ME: maximal enhancement; IRW: initial rate of washout.

The upslope on the SI-curve is referred to as the initial rate of enhancement measured in %/s (IRE) (Table 2). Tissues with high perfusion, such as arteries, have a steep upslope on the SI-curve (high IRE) due to a rapid uptake of Gd (Figure 6). Tissues with high perfusion will reach a peak, maximal enhancement (ME) or short plateau and a subsequent rapid decrease often referred to as washout phase (IRW) (Figure 6). Tissues with lower perfusion show a slower increase in the uptake of Gd and do not reach washout phase within the 5 minutes scan time of the DCE-MRI sequence.

Based on linear approximations of the SI-curves Dynamika® automatically assigns every voxel to one of four perfusion patterns: no enhancement (voxels without contrast uptake; no color), persistent (voxels that do not reach a plateau phase; blue), plateau (voxels that reach plateau but not a washout phase; green) and “Washout” (voxels that reach a washout phase; red) (33, 181, 192) (Figure 3, B and Figure 7).

Figure 7. Perfusion patterns based on linear approximations of the signal-intensity curves.



Various heuristic DCE-MRI perfusion variables can be extracted from the SI-curves with the variables included in Study I-III defined in Table 2.

Table 2. Definitions of DCE-MRI perfusion variables used in Study I-III

Variable name	Abbreviation	Definition	Study
Initial Rate of Enhancement	IRE	The upslope on the Time intensity curve measured as the mean relative increase in signal intensity per second from enhancement onset until ME is reached (%/s). A surrogate of the degree of perfusion.	Used as input to other variables (below)
Maximal Enhancement	ME	The highest mean signal intensity value relative to the baseline intensity. A surrogate of the degree of perfusion.	
Highly Perfused Voxels	Nvoxel	The number of voxels with “plateau” or “washout” enhancement patterns, i.e. the highest perfused voxels, the most perfused tissue.	Study III
Initial Rate of Enhancement Composite Score	IRExNvoxel	The mean relative increase in signal intensity per second (%/s) (IRE) multiplied by the number of highly perfused voxels (Nvoxel). The variable becomes a composite parameter reflecting both the volume (voxels) and the degree of perfusion.	Study I Study II Study III
Maximal Enhancement Composite Score	MExNvoxel	The highest mean signal intensity value relative to the baseline intensity (ME) multiplied by the number Highly Perfused Voxels. The variable becomes a composite parameter reflecting both the volume (voxels) and the degree of perfusion.	Study I Study II Study III
Proportion of Highly Perfused Voxels (%)	Nvoxel%	The proportion of highly perfused voxels reaching either a “plateau” or “washout” enhancement pattern (Nvoxel) in percentage of the total number of voxels within the VOI.	Study I Study II
Initial Rate of Enhancement Index	IRExNvoxel%	The mean relative increase in signal intensity per second (%/s) (IRE) multiplied by the proportion of highly perfused voxels (Nvoxel%).	Study II
Maximal Enhancement index	MExNvoxel%	The highest mean signal intensity value relative to the baseline intensity (ME) multiplied by the Proportion of Highly Perfused Voxels.	Study II

Conventional static MRI assessment of synovitis and BML

The MRI in Osteoarthritis Knee Score (MOAKS)

In Study III, static, non-contrast-enhanced (CE) MRI sagittal and reconstructed axial pre-Gd 3D PDw sequences were used to assess BMLs, synovitis and effusion as recommended in the MRI in Osteoarthritis Knee Score, MOAKS (177). BMLs were scored in 15 sub-regions (2 patellar, 6 femoral, 7 tibial) based on standardized regions. “Effusion-Synovitis” (a score for assessment of degree of hyperintensity within the articular cavity) and “Hoffa-Synovitis” (a score for assessment of degree of hyperintensity in Hoffa’s fat pad) were each scored from 0-3 and collapsed into one single “MOAKS-Synovitis” score (0-6).

The whole-knee synovitis score by Guermazi et al.

In study III, static CE-MRI using the post-Gd sagittal and reconstructed axial T1w TSE images (Vibe) were used to assess synovitis in 11 standardized regions (0-2) based on the thickness of the synovium according to the whole-knee synovitis score, “CE-Synovitis” as proposed by Guermazi and colleagues (73). All the sequences were viewed in Impax (version 6.6.1.7523 2017).

Assessment of pain

In all three studies the Knee injury and Osteoarthritis Outcome Score questionnaire (KOOS) was applied (193, 194). KOOS is a self-administered patient-reported outcome measurement instrument, assessing five domains of importance to patients with knee OA (Pain, Symptoms, Function in daily living, Function in sport and recreation, and Knee related quality of life) relating to target knee during the previous week. Each domain includes individual items answered on a 0-4 Likert scale. A normalized 0-100 score indicate how many percentages of the maximum 100 that has been reported (0 indicating the worst, 100 indicating the best) is calculated for all domains (193-195). A validated Danish touch screen version of the KOOS was applied (196).

Exercise therapy intervention

The intervention in Study II and III consisted of facility-based, functional and individualised exercise therapy supervised by a trained physiotherapist 3 times weekly for 12 weeks. The exercise program lasted approximately 1 hour and consisted of a warmup phase (10-minutes of ergometer bicycling at moderate intensity) followed by a circuit training program focusing on strength and coordination exercises of the trunk, hips, and knees. The exercises were performed with free weights, elastic rubber bands, or body weight as resistance. Progression of resistance or coordination difficulty was made on an individual basis according to a pre-specified progression protocol (for more details see Appendix, Study II).

A personal training diary was used at each visit recording the level of each exercise, including external load, number of repetitions, or duration together with current knee pain before an exercise session on a 0–10 numeric rating scale. If symptomatic exacerbation was reported upon attending an exercise session (the current knee pain exceeded a score of 5) a “rescue” training program was applied for that session excluding any weight bearing activities to avoid unwarranted symptom provocation during a period of symptomatic flare-up. Protocol adherence was defined as attendance at a minimum of 24 sessions of 36 possible sessions.

The control group received no attention during the 12 weeks and was requested not to engage in exercise therapy during the study period. The control group were invited to participate in another study on exercise (<http://clinicaltrials.gov/ct2/show/NCT01945749>) after study completion.

The methods and measurements used in the studies of the PhD

Table 3. Overview of the methods and measurements used in Study I-III

	Study I	Study II	Study III
Design			
Cros sectional	X		
RCT		X	X
MRI			
Non CE-MRI			
MOAKS-Synovitis			X
MOAKS-BML			X
CE-MRI			
CE-Synovitis			X
DCE-MRI perfusion			
Muscle	X	X	
Synovium			X
Baker's cyst			X
Hoffa			X
BMLs			X
PROMs			
KOOS	X	X	X
Reproducibility tested			
DCE-MRI	X	X	X

8. DISCUSSION OF RESULTS

Muscle perfusion, pain and exercise therapy in knee osteoarthritis

Prior to conducting the studies comprising this PhD., observations were made in our research group during analyses of DCE-MRI quantified perfusion of intra-articular structures in patients with knee OA (117, 197). It was observed that contrast enhancement was not confined to intra-articular structures, but different patterns of contrast enhancement were observed in the peri-articular knee muscle tissue as well. No evidence was available to explain if the degree of enhancement in these muscles was pathological as observed in intra-articular structures (100, 101, 113-115, 198) and contributed to the symptoms in knee OA, or if the contrast uptake seen was due to the basic perfusion of the skeletal muscle tissue (199-201).

Findings of inflammatory markers in vastus lateralis in knee OA suggest that inflammatory processes are not only confined to intra-articular structures (125, 126) and inflammation may contribute to the development of pain (63, 84). However, no correlations were found between pain and muscle inflammation (in biopsies) (126). Understanding the role of muscle perfusion in knee OA symptomatology may contribute to the understanding of a possible mode of action of exercise therapy in knee OA.

Therefore, in Study I, a cross sectional study on 94 patients with knee OA, it was hypothesised that higher contrast-enhancement (higher perfusion) in the peri-articular muscles, would associate with worse symptoms. The hypothesis was rejected as the results showed that more widespread muscle perfusion in the peri-articular knee muscles was associated with lower pain intensities in patients with knee OA. The association indicates that for each increment in the perfusion variable $N_{\text{voxel}}\%$ (Proportion of highly perfused voxels) the KOOS pain score is 0.41 higher, meaning less pain (Table 4). The relationship was opposite to that described between pain and perfusion of the intra-articular tissues (synovium and Hoffa's fat pad) in knee OA with increased intra-articular perfusion being associated with more pain (103, 117, 197).

The results from Study I suggest that more widespread muscle perfusion is not pathological and should not be interpreted as inflammation and may be considered beneficial in knee OA (Study I). This could possibly be a parallel to the changes in muscle capillarisation following exercise as observed in other studies (202-204) and variations in muscle perfusion may reflect variations in the muscle status in the population studied.

Table 4. Multiple regression analyses (Adapted from Study I).

Dependent variable	Independent variables	Model	Reduced model 1	Reduced model 2	Adjusted for BMI, age, sex
KOOS pain	IRExNvoxel	-0.016 (0.019) p=0.4081	-0.011 (0.016) p=0.4691	-	-
	MExNvoxel	0.000052 (0.00013) p=0.6805	-	-	-
	Nvoxel%	0.41 (0.18) p=0.0258	0.45 (0.15) p=0.0040	0.41 (0.14) p=0.0048	0.39 (0.15) p=0.0119

KOOS pain as dependent variable and DCE-MRI perfusion variables as independent variables. Slope (SE), p-value.

The results of Study I are not compatible with the findings of muscle inflammation in the vastus lateralis in individuals with knee OA (125, 126). With the presence of muscle inflammation (quantified by biopsies), the level of perfusion would be expected to be higher compared to non-inflamed tissue as increased perfusion has been associated with inflammation (33, 100, 192, 198) and associate with higher pain (117, 197). However as mentioned earlier, no correlation was seen between muscle inflammation in the biopsies and pain (126). It could be speculated that muscle inflammation detected in biopsies reflect a focal area of inflammation present in knee OA (125, 126) (opposite from widespread), and might explain why no association between muscle inflammation and pain was found in vastus lateralis (126). The volume of muscle perfusion quantified was larger than the volumes of biopsies where muscle inflammation was detected (126), further supporting that two different phenomena are measured.

During exercise, muscle tissue is the tissue primarily affected. Thus, any physiological changes caused by exercise are expected to be reflected in the muscles, although not necessarily measurable with current techniques. The association between more widespread muscle perfusion and lower pain intensities is indirectly supported by muscle weakness being typical in knee OA (12) and the fact that exercise has beneficial effects on pain in knee OA (7) that may be mediated by more widespread muscle perfusion that may be attributable to physiological changes in the muscles, e.g. changes in muscle capillarisation (202-204).

As mentioned earlier, the underlying analgesic mechanisms of exercise therapy in knee OA are not fully understood (13, 18). Several aspects have been suggested to mediate the

pain-reducing effects of exercise including increased muscle strength, knee range of motion, improvements in proprioception (13, 14), reduced pain sensitivity (15), and improved psychological well-being (16, 17). The results of Study I indicate that changes in muscle perfusion could contribute in explaining the underlying analgesic mechanisms of exercise interventions in knee OA. This is novel and introduces an objective DCE-MRI measurement that may be a potential measure of the effects of exercise. However, as Study I was cross sectional, causality cannot be determined.

In order to draw conclusions on causality a secondary outcome analysis of a randomised controlled trial (15) on the effects of 12 weeks of exercise therapy was conducted (Study II). It was investigated if change in muscle perfusion is a contributing factor explaining the beneficial outcomes of exercise therapy on pain. Based on the results of Study I, it was hypothesised that an exercise therapy intervention would increase muscle perfusion in parallel with improved pain when compared with a no-attention control intervention in knee OA.

The results from Study II supported the hypothesis as increased muscle perfusion was associated with reduced pain (higher KOOS pain score) across the population (Table 5).

Table 5. Spearman correlation coefficients of changes between KOOS Pain and DCE-MRI variables (Flexor VOI) (Adapted from Study II).
(For correlations of Total and Extensor VOIs see Supplementary file, Study II).

Change	Flexor VOI				
	Nvoxel%	IRExNvoxel	IRExNvoxel%	MExNvoxel	MExNvoxel%
KOOS Pain	0.39 P=0.02	0.35 P=0.04	0.29 P=0.10	0.42 P=0.01	0.37 P=0.03

The cross sectional results from Study I were confirmed in Study II indicating that more widespread and increased muscle perfusion is beneficial in knee OA in contrast to the suggested perfusion of intra-articular structures (e.g. synovium and Hoffa’s fat pad) being pathological (31, 103, 117, 197). Increase in muscle perfusion may be a parallel to changes in muscle capillarisation following exercise (202-204) and variations in muscle perfusion may reflect variations in the muscle status in the population studied.

Furthermore, the results of Study II are indirectly supporting the part of the hypothesis tested that exercise therapy would increase muscle perfusion as the participants in the

exercise therapy group exhibited a constant level of muscle perfusion in contrast to decreased perfusion in the control group over the 12-week intervention period (Table 6).

The results of Study II do not provide insight into the underlying mechanism of the exercise-induced differences in muscle perfusion. From the available evidence, exercise has shown to increase capillarisation or cause angiogenesis as a physiological response (202, 204, 205). Based on the results of Study II, it could be speculated that this specific therapeutic exercise intervention prevented such loss of capillarisation in the muscle tissue in knee OA. The exercise intervention used (15) was designed to improve physical functioning in every-day tasks, and albeit there are elements of peri-articular muscle strengthening, this was not a specific focus and no differences were seen in the changes in muscle strength (Table 6).

Exercise therapy interventions more specifically aiming at physiological muscle adaptations (e.g. resistance or cardiovascular training) are also beneficial in terms of pain (12, 206) and may prove even more effective in inducing changes in muscle perfusion. Future research is necessary to investigate the effect of such exercise interventions and to explore the relation to pain changes. This knowledge can be used to optimise exercise therapy in the management of knee OA.

The population studied in Study I is the same cohort studied in Ballegaard et al. (117) and in Riis et al. (197) which underline the findings of different pain-perfusion relationships in muscles and intra-articular structures, respectively. When it comes to generalisability, the findings in Study I and Study II support each other across different study designs and altogether strengthen the confidence in the results of Study II. Furthermore, different knee OA populations were studied. In Study I (a part of a weight-loss trial) the average body mass index (BMI) was 32.3, whereas BMIs in study II were 27.7 and 29.0 for the exercise and control groups, respectively. With higher BMI a larger amount of white adipose tissue would be expected. White adipose tissue is the most common known source of adipokines (89) being central in the association between obesity, inflammation and OA (89, 207). Nevertheless, the pain-muscle perfusion relationship did not seem to be affected by the differences in BMI in the populations studied.

Altogether, the results from Study I and II indicate that more widespread and increased muscle perfusion is associated with reduced pain in knee OA. Furthermore, increased muscle perfusion may contribute to the pain-relieving effects of exercise therapy and may be a potential objective marker of the effects of exercise therapy in knee OA.

Table 6. Comparison of changes (ANCOVA) adjusted for baseline values

(Adapted from Study II).

	Exercise group (n=16)	Control group (n=17)	Comparison	
	Mean (SE)	Mean (SE)	Mean Difference (95% CI)	P value
KOOS				
Pain	8.4 (2.5)	-2.1 (2.4)	-10.6 (-17.8;-3.4)	0.0054
DCE-MRI perfusion variables				
Total Muscle VOI				
Nvoxel%	2.0 (2.3)	-4.6 (2.3)	-6.534 (-13.2;0.09)	0.0531
IRExNvoxel	-50.4 (26.9)	-155.61(26.09)	-105.2 (-182.1;-28.3)	0.0090
IRExNvoxel%	-0.03 (0.02)	-0.13 (0.02)	-0.10 (-0.17;-0.04)	0.0040
MExNvoxel	327.5(3,804.1)	-9,014.3 (3,688.7)	-9,341.8 (-20,249;1,565.7)	0.0905
MExNvoxel%	1.7 (3.0)	-7.8 (2.973)	-9.5 (-18.1;-0.9)	0.0315
Extensor VOI				
Nvoxel%	0.2 (3.1)	-6.9 (3.0)	-7.1 (-16.00;1.9)	0.1162
IRExNvoxel	-18.4(11.6)	-59.3 (11.3)	-40.8 (-74.0;-7.7)	0.0174
IRExNvoxel%	-0.03 (0.04)	-0.21 (0.04)	-0.18 (-0.29;-0.06)	0.0033
MExNvoxel	-1,040.2 (1,328.3)	-3,582.4 (1,288.6)	-2,542.2 (-6321.6;1237.3)	0.1797
MExNvoxel%	-0.6 (4.5)	-11.9 (4.4)	-11.3 (-24.2;1.6)	0.0837
Flexor VOI				
Nvoxel%	2.4 (2.244)	-4.4 (2.2)	-6.7 (-13.1;-0.3)	0.0398
IRExNvoxel	-31.8 (17.9)	-96.6 (17.4)	-64.8 (-116.0;-13.6)	0.0149
IRExNvoxel%	-0.03 (0.02)	-0.11(0.02)	-0.08 (-0.14;-0.02)	0.0076
MExNvoxel	1,830.9 (3,061.8)	-5,867.8 (2,967.6)	-7,698.7 (-16,541;1,143.9)	0.0855
MExNvoxel%	2.3 (2.8)	-7.1 (2.8)	-9.4 (-17.5;-1.4)	0.0237
Muscle strength Knee extension, Nm/kg				
0°/s	-1.1 (4.1)	4.9 (3.9)	5.9 (-6.3;18.2)	0.3234
60°/s	-4.7 (2.9)	0.7 (2.8)	5.4 (-3.1;13.9)	0.2035
120°/s	-4.6 (3.0)	-3.1 (2.9)	1.5 (-7.5;10.6)	0.7311
180°/s	-2.4 (2.8)	-1.6 (2.7)	0.8 (-7.6;9.2)	0.8432
Muscle strength knee flexion, Nm/kg				
0°/s	4.7 (3.1)	2.6 (2.9)	-2.1 (-11.2;7.1)	0.6394
60°/s	1.6 (2.4)	2.2 (2.3)	0.6 (-6.4;7.7)	0.8533
120°/s	0.3 (1.8)	3.7 (1.8)	3.3 (-2.1;8.7)	0.2142
180°/s	-0.9 (2.0)	2.54 (2.0)	3.4 (-2.6;9.5)	0.2558
6-min walk distance, m	40.1 (13.3)	1.1 (12.5)	-39.0 (-76.9;-1.1)	0.0441

Intra-articular effects of exercise in knee osteoarthritis

Synovitis and BML

As described earlier, synovitis is a marker of inflammation (24-27) and as BMLs associated with pain in knee OA (30, 73, 75, 103, 197). Worsening of synovitis and BMLs is associated with increased risk of frequent and more severe pain (70, 72, 86, 137, 208), whereas reductions of synovitis and BMLs correlate with reduced pain (72, 86, 136). Therefore, synovitis and BMLs are of particular interest as a target for preventive and therapeutic interventions in knee OA (72, 139).

Exercise is across populations suggested to have a systemic anti-inflammatory effect (19-21, 209). In a knee OA population a single exercise session induced a local anti-inflammatory response in both intra-articular and peri-synovial concentrations of IL-10 (22). Further, a study reported systemic anti-inflammatory effect after a 12-week exercise intervention in knee OA as reductions were seen inflammatory markers (plasma taken from peripheral blood sTNFR1 and sTNFR2) (210). However, 18 months of exercise had no effect on systemic measures of inflammation (serum IL-6) in knee OA (211).

In order to investigate if a therapeutic exercise intervention would reduce local inflammation (synovitis) and reduce BML perfusion in knee OA, which could contribute in explaining the exercise-induced pain reduction Study III was conducted. It was hypothesised that exercise therapy would reduce synovitis and BMLs (assessed by conventional and DCE-MRI) in parallel with changes in pain in knee OA when compared to a no-attention control intervention.

The results from Study III demonstrated that 12 weeks of exercise therapy did not reduce synovitis and BML among exercise adheres compared to a no-attention control group in knee OA (Table 7). In fact there were adverse effects on synovitis (higher perfusion) in the anterior part of the knee in the exercise group when compared to the control group (Table 7). This was indicated by statistically significant group differences in all DCE-MRI perfusion variables of the anterior synovium. Similar trends were observed in both DCE-MRI and static MRI variables (positive values in the exercise therapy group and negative values in the control group), although not all statistically significant, across all the assessed regions of synovium and BMLs (Table 7).

Table 7. Comparison of changes from baseline to follow-up adjusted for baseline value, age, weight and gender (Adopted from Study III).

	Exercise group (n=16)	Control group (n=17)	Comparison		
	Mean difference (SE)	Mean difference (SE)	Group Mean Difference	95% CI	P-value
KOOS					
Pain	10.6 (3.1)	-1.1 (2.9)	-11.7	-20.1;-3.4	0.0075
Non CE-MRI					
MOAKS-Synovitis	0.26 (0.14)	-0.18 (0.14)	-0.44	-0.88;0.0033	0.052
MOAKS-BML	0.21 (0.41)	0.22 (0.40)	0.012	-1.26;1.28	0.984
CE-MRI					
CE-synovitis	0.77 (0.45)	-0.31 (0.43)	-1.08	-2.47;0.31	0.122
DCE-MRI					
Total synovium					
Nvoxel	1,388.6 (1,541.5)	-2,085.7 (1,489.2)	-3,474.3	-8,165.0; 1,216.3	0.140
IRExNvoxel	23.3 (28.6)	-61.2 (27.6)	-84.5	-171.5;2.6	0.057
MExNvoxel	1,856.7 (2,576.9)	-4,619.0 (2,489.6)	-6,475.7	-14,318.0;;366.7	0.102
Anterior synovium					
Nvoxel	1,039.2 (735.1)	-1,567.1 (710.1)	-2,606.2	-4,844.4;-368.0	0.024
IRExNvoxel	5.2 (13.3)	-36.4 (12.8)	-41.6	-82.0;-1.2	0.044
MExNvoxel	1,420.8 (1,207.6)	-3,238.7 (1,166.5)	-4,659.4	-8,338.9;-980.0	0.015
Posterior synovium					
Nvoxel	112.0 (557.9)	-415.7 (538.9)	-527.7	-2,225.4;1,170.0	0.529
IRExNvoxel	8.8 (13.4)	-14.7 (12.9)	-23.5	-64.3;17.4	0.249
MExNvoxel	16.0 (945.6)	-852.7 (913.5)	-868.6	-3,745.9;2,008.7	0.541
Hoffa synovium					
Nvoxel	199.7 (239.11)	-129.6 (231.0)	-329.3	-1,057.0;398.4	0.361
IRExNvoxel	9.2 (6.1)	-8.7 (5.9)	-17.9	-36.4;0.7	0.058
MExNvoxel	323.8 (392.9)	-474.4 (379.6)	-798.2	-1,993.8;397.3	0.182
BML*					
Nvoxel	175.5 (181.2)	-30.0 (146.4)	-205.5	-737.5;326.5	0.432
IRExNvoxel	-0.04 (4.4)	-5.7 (3.6)	-5.7	-18.7;7.3	0.374
MExNvoxel	255.7 (271.4)	-127.4 (218.8)	-383.1	-1,183.4;417.2	0.332
*: Exercise therapy group: n=12, control group : n=17					

Study III confirmed the beneficial effects of exercise therapy on pain in knee OA (7) (Table 7, KOOS). The improvements in pain were paralleled by adverse effects on synovitis in the anterior part of the knee. However, no associations were found between the reductions in pain changes in synovitis or BML perfusion.

Synovitis

Study III is the first randomised controlled trial to evaluate the effect of exercise on synovitis and BML in knee OA, and hence there are no studies for directly comparison. However, there are studies indirectly corroborating the findings as exercising an inflamed joint can provoke inflammation with the motion of the synovial fluid inducing shear forces in the synovial membrane (212). Adverse effects of exercise in knee OA was seen as number of knees with effusions after a 3 months exercise program increased (213) and increased expenditure for NSAIDs during a 2 year home exercise program in comparison with controls (214) have been reported. Furthermore, in animals this adverse effect of exercise has been demonstrated where daily high intensity exercise (running) for 4 weeks was associated with cartilage damage of the knee and development of synovitis in otherwise healthy rabbit knees compared a control group. Higher levels of IL-15 expression in the synovial fluid and tissues were seen in the high intensity exercise group compared with the control group (215).

Similar to the results of Study III, a study of bracing for patellofemoral OA showed pain-reductions in the intervention group and change in synovitis assessed by DCE-MRI was in favour of the control group (no intervention) (216). Furthermore, similar to Study III, changes in synovitis were not associated with changes in pain (216). The lacking association indicate that pain-reductions are not due to changes in synovitis which is supported by results from a longitudinal study (105).

As previously described, OA pain perception is influenced by several factors (56, 75-78). The International Association for the Study of Pain (IASP) defines pain as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (217). In accordance, pain perception in knee OA is shown to be affected by depression and quality of life (76). As exercise therapy improves psychological well-being (10, 16, 17) it is plausible that pain-reductions may be mediated through improvements in psychological well-being and not only changes in joint structures (79).

BMLs

Contradictory results for the effect of physical activity and exercise therapy on BMLs have been reported (218-220) (Study III) and will be discussed in the following. Reasons for the different

results may be explained by a dose-response relationship and due to the context in which the physical activity or exercise is performed (knee alignment, presence of structural lesions etc.) (218).

The suggested dose-response relationship between physical activity and BML development and progression is supported by a study finding that physical activity consisting of $\geq 10,000$ steps/day are deleteriously associated with BML progression compared with physical activity with $\leq 10,000$ steps/day (219). Furthermore, temporarily unloading of the knee (by knee joint distraction) may induce beneficial effects on OA-related structural changes at long term follow up in terms of increased cartilage thickness and radiographic joint space width and a decrease of the denuded area of subchondral bone, however this study did not on BML (220).

After a 12-week therapeutic exercise intervention no BML group differences were detected in the changes in DCE-MRI perfusion variables and static MRI variables (Study III). This may be due to a limited sample size, meaning that a power issue may have been the reason for not detecting any difference in group changes. Therefore, caution should be made in the interpretation of the results. However, as the intervention in Study III has limited weight-bearing activities (it consisted of low load activities and unloading) and a rescue program with even less weight-bearing activities was offered on days with VAS pain > 5 , the impact of the intervention may not be deleteriously associated with BML progression which is in support of the findings on the dose-response relationship and development of BML (219) and beneficial effects of unloading on knee OA-related changes in a knee OA population (220).

Cartilage

In healthy populations vigorous physical activity improves the cartilage of the knee (221, 222) whereas in knee OA, vigorous physical activity levels have been associated with knee joint replacement surgery (223). In parallel, vigorous physical activity did not affect the cartilage of healthy knees (224) but in knees with BMLs; adverse effects on cartilage were seen. Similar to this, physical activity in individuals with BMLs and meniscal pathology was associated with cartilage damage and increased meniscal pathology score (219).

In a population with mild knee OA, 12 months of high-impact exercise did not improve tibial and femoral cartilage (quantified by delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC)) compared to a non-exercising control group (225). However, in a population at high risk of developing knee OA, 4 months of moderate supervised

exercise improved femoral cartilage (dGEMRIC) compared with a non-exercising control group. The improvement in cartilage correlated with self-reported clinical status (KOOS) (226). The different results of these studies are suggested to be due to differences in the age of participants with the population with mild knee OA being more than 10 years older than those at high risk of developing knee OA (225). Further, the pathophysiological condition of knee cartilage, mild versus no knee OA may contribute to the different results. Moreover, the exercise loading modalities may explain different cartilage responses (moderate versus high-impact) (225). Future studies are warranted to clarify whether high-impact exercise is optimal to populations with more severe knee OA (221, 225).

The evidence suggests that exercise improves the cartilage of the knee prior to cartilage damage and may be damaging to the cartilage if initiated after cartilage degeneration has begun (227).

Altogether, the available evidence on the effect of exercise therapy on intra-articular tissue as synovitis, BMLs and cartilage in knee OA is ambiguous. This may be due to the health status of the joint at the time of exercise (1, 219, 225), different exercise modalities and dosages and heterogeneous populations.

Study III extends the current knowledge on the intra-articular effects of exercise therapy on synovitis and BML suggesting potential undesirable pro-inflammatory effects of exercise in knee OA.

9. SCIENTIFIC CONSIDERATIONS

Strengths and limitations

Cross-sectional versus randomized controlled trials and sample size

The cross-sectional design in Study I is a limitation that precludes an evaluation of the causality between changes in symptoms and DCE-MRI perfusion variables. However, the relatively large sample size (n=94) is a strength of Study I.

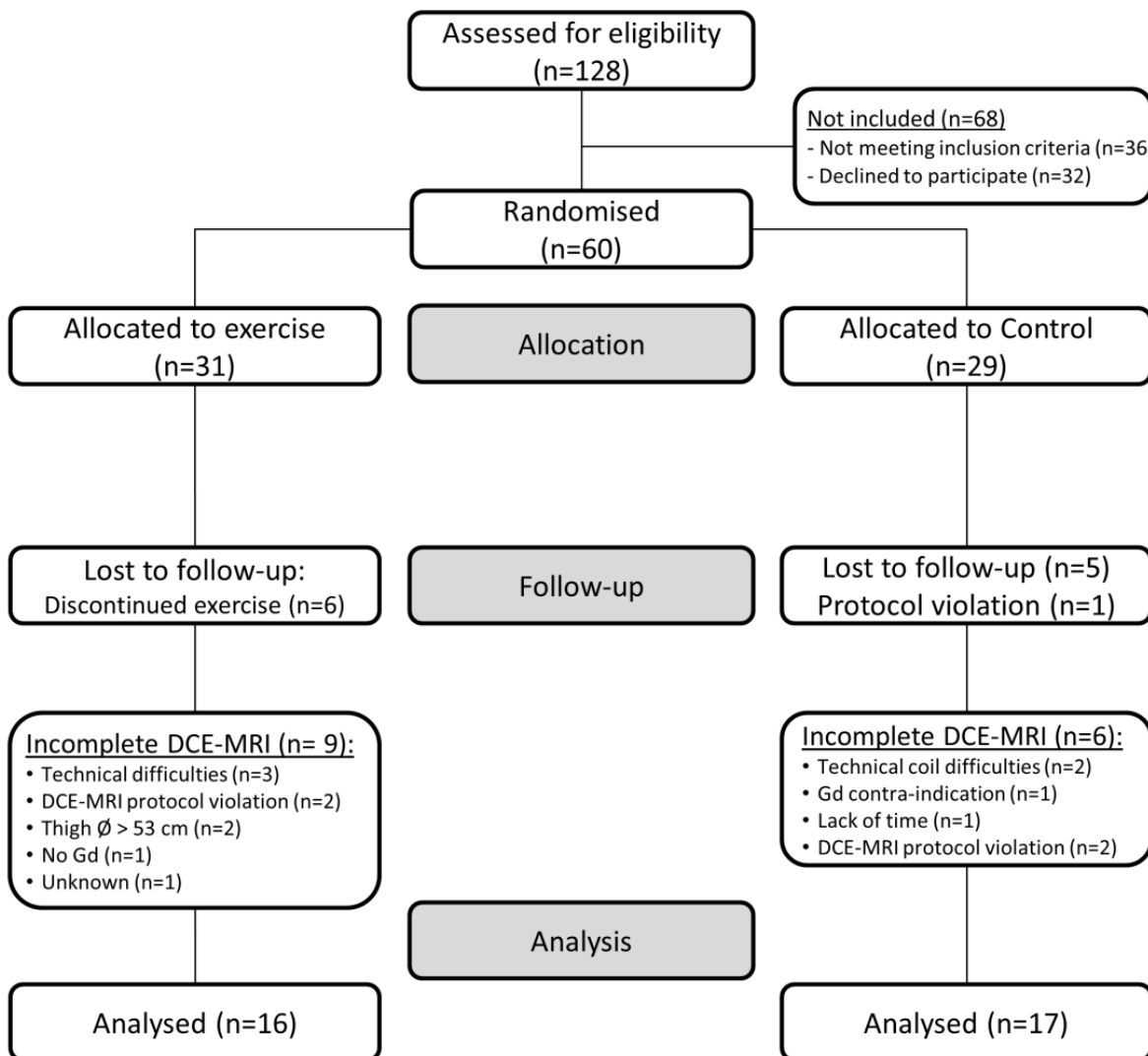
The use of a rigorous randomised study design in Study II and III is a major strength. On the other hand, the limited sample size due to incomplete DCE-MRI data and actual drop-outs is a limitation (Figure 8). Imbalances were seen at baseline and for sensitivity purposes the analyses were repeated with inclusion of age, weight, and gender as covariates, which did not affect the results significantly.

The fact that the study was designed and powered for another outcome are limitations that calls for replication in larger studies specifically designed for DCE-MRI. Nevertheless, differences associated with the effects of exercise therapy were detected. A proper mediation analysis could have strengthened our findings but the limited sample size weakens the statistical power for such mediation analyses. The results do not have immediate implications (meaning) in a clinical setting, but the results are important steps forward in the advancement of identifying the optimal exercise program for patients with knee OA.

Multiple testing

Multiple statistical tests increase the likelihood of a false positive finding. However, the results of Study I seem robust even after adjustment for covariates. With a Bonferroni adjustment for multiple tests (6 regressions) the threshold of statistical significance is 0.0083. The statistically significant regression results in KOOS pain respect this threshold for significance (slope (SE) 0.41 (0.14), P=0.0048), except the covariate model adjusted for BMI, age, and sex (slope (SE) 0.39 (0.15), P=0.0119)) (Table 4).

Figure 8. Flowchart (Adapted from Study II and III).



Per-protocol versus intention to treat analyses

The pre-specified focus on the per-protocol population in Study II and III allowed for a description of the mechanisms of exercise therapy reliably. The reason for using a per-protocol analysis in the main trial was in order to examine the underlying mechanisms of a 12-week therapeutic exercise program on the pain sensitivity in patients with knee OA that adhere to the exercise protocol. In that sense only included participants adhering to the protocol (i.e. actually received exercise in an adequate dosage vs. did not receive exercise) could contribute answering the research question and constituted the per-protocol population.

The exercise intervention was not designed specifically to improve muscle perfusion, but to improve pain and disability in knee OA. As stated earlier, the underlying mechanisms on the effects of exercise (particularly pain) are unclear and thereby a per-protocol analysis could contribute increasing the knowledge among exercise adherers. Intention-to-treat analyses would not provide insights into the underlying mechanisms.

DCE-MRI reproducibility, feasibility and reference material

The use of a 3T MRI system, pre-specified methods, using standardised and validated image analyses with adequate reproducibility together with pre-specified analyses plans are major strengths of the studies.

As a send/receive knee coil was used the structures analysed were defined by the coverage of the knee coil. Hence the total volumes of the peri-articular knee muscles were not available for analyses and the DCE-MRI perfusion variable Nvoxels was normalised in percentage of the analysed volume, Nvoxels%, the percentage of highly perfused voxels. In Study III, the area of the knee analysed at baseline and follow up was standardised with the shortest distance from patella to the proximal range of the image defining the upper area of interest in both scans.

DCE-MRI is not feasible as a standard clinical assessment, but is considered a relevant a method discovering the underlying mechanisms of exercise therapy in knee OA.

As no reference material on a healthy population is not available the interpretation of the DCE-MRI is limited.

Objective methods evaluating the effects of exercise in knee OA

As exercise therapy cannot be blinded the observed clinical effects may be facilitated by effects associated with attention through study participation (7). In the evaluation of the effects of exercise therapy in knee OA, patient reported outcome measures (PROMs) and performance-based tests are recommended and frequently used (13, 228, 229). However, PROMs and performance-based tests are affected by patient-provider interaction (230, 231), which is an important source of bias when the intervention cannot be blinded. Furthermore, the validity of performance-based tests (e.g. muscle strength) may be challenged by pain or fear of pain during

the test (232, 233). This means that a high degree of participant cooperation is necessary, which increases the risks of measurement bias and underlines the need for objective and sensitive measures in the evaluation of the underlying effects of exercise.

DCE-MRI is such an objective method and its use is novel within OA research on exercise. The use of DCE-MRI in the studies comprising this PhD is a major strength. The results of Study I-III suggest that DCE-MRI assessed perfusion of knee structures may be potential objective markers on the effect of exercise therapy in knee OA.

Placebo and blinding in exercise trials

There are well-documented strong placebo effects on patient-reported outcomes in knee OA (141, 234) which there in general is not controlled for in exercise studies with the comparator often being a no treatment control group (7). Thus it is difficult to distinguish patient-reported effects of placebo from biased reporting (234) and it is not possible to determine the exact magnitude of the beneficial effects of the intervention.

Hence, there is a need of future double-blinded placebo-interventions in therapeutic exercise studies in knee OA in order to reduce the therapeutic impact of participating in a supervised therapeutic exercise intervention (235). A randomised, double-blinded placebo controlled trial on the effect of physiotherapy (consisting of exercise, massage, taping, and mobilisation) to knee OA did not find any differences in pain improvement and disability between the intervention and placebo group (236). These results underline the impact of placebo on patient-reported outcomes in exercise interventions.

10. CONCLUSIONS

The aim of this PhD project was to investigate the effects of exercise therapy on perfusion in knee joint related tissues and to investigate associations to pain in patients with knee OA. The three studies comprising this PhD have extended the current knowledge on tissue perfusion and exercise therapy in knee OA and the aim has been reached.

In Study I and II the results indicate that more widespread and increased muscle perfusion is beneficial in knee OA as it associates with reduced pain. Exercise therapy increased muscle perfusion compared to a no-attention control group in knee OA in Study II. Thus, higher muscle perfusion may contribute to the pain-relieving effects of exercise and be a potential objective marker on the effects of exercise in knee OA.

The results of Study III suggest that exercise therapy yields adverse effects on synovitis in the anterior part of the knee and reduce pain when compared to a no-attention control group in knee OA. The pain-reducing effect of exercise therapy was not associated with the changes in synovitis or BML.

The overall conclusion of this PhD is that exercise therapy yields increased muscle perfusion that correlates with reduced pain, but is paralleled by adverse effect on synovitis in the anterior part of the knee when compared to a no-attention control group in patients with knee OA.

11. PERSPECTIVES FOR FUTURE RESEARCH

The results from Study II and III confirmed that exercise therapy reduces pain in knee OA with increased muscle perfusion being associated with reduced pain. In order to clarify the role of muscle perfusion in the pain-reducing effect of exercise therapy, future studies focusing specifically on increasing muscle perfusion would indicate if the pain-perfusion relationship can be optimised and subsequently optimise exercise therapy.

As the effect of exercise therapy on knee OA symptoms is known to decline after cessation of exercise therapy (7, 12) patients in clinical practice are recommended to continue exercise at a regular basis in order to maintain the improvements. However, the results of Study III suggest potential undesirable pro-inflammatory effects of exercise therapy. This gives an implication for future investigations of the long-term consequences of exercise therapy on structural disease progression in knee OA as the evidence in this area is sparse (7).

The lack of association between changes in synovitis and changes in pain in Study III indicate that pain-reductions cannot be explained by changes in synovitis. As pain perception is influenced by multiple factors, future exercise therapy studies could benefit from evaluating both joint tissues and include psychological aspects such as depression, mood, anxiety and self-efficacy on which exercise therapy also has an impact (10, 79, 237).

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