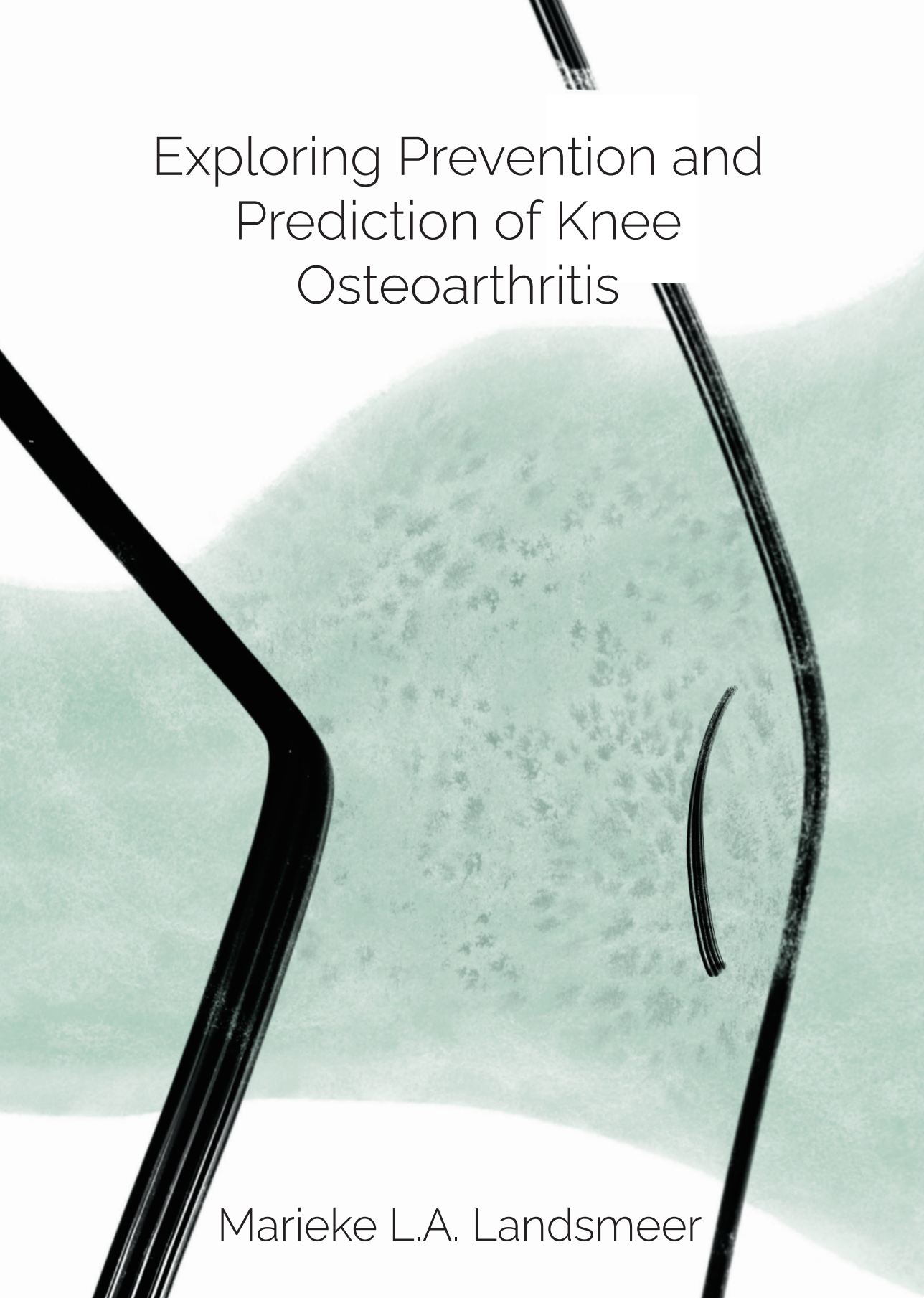
Exploring Prevention and Prediction of Knee Osteoarthritis



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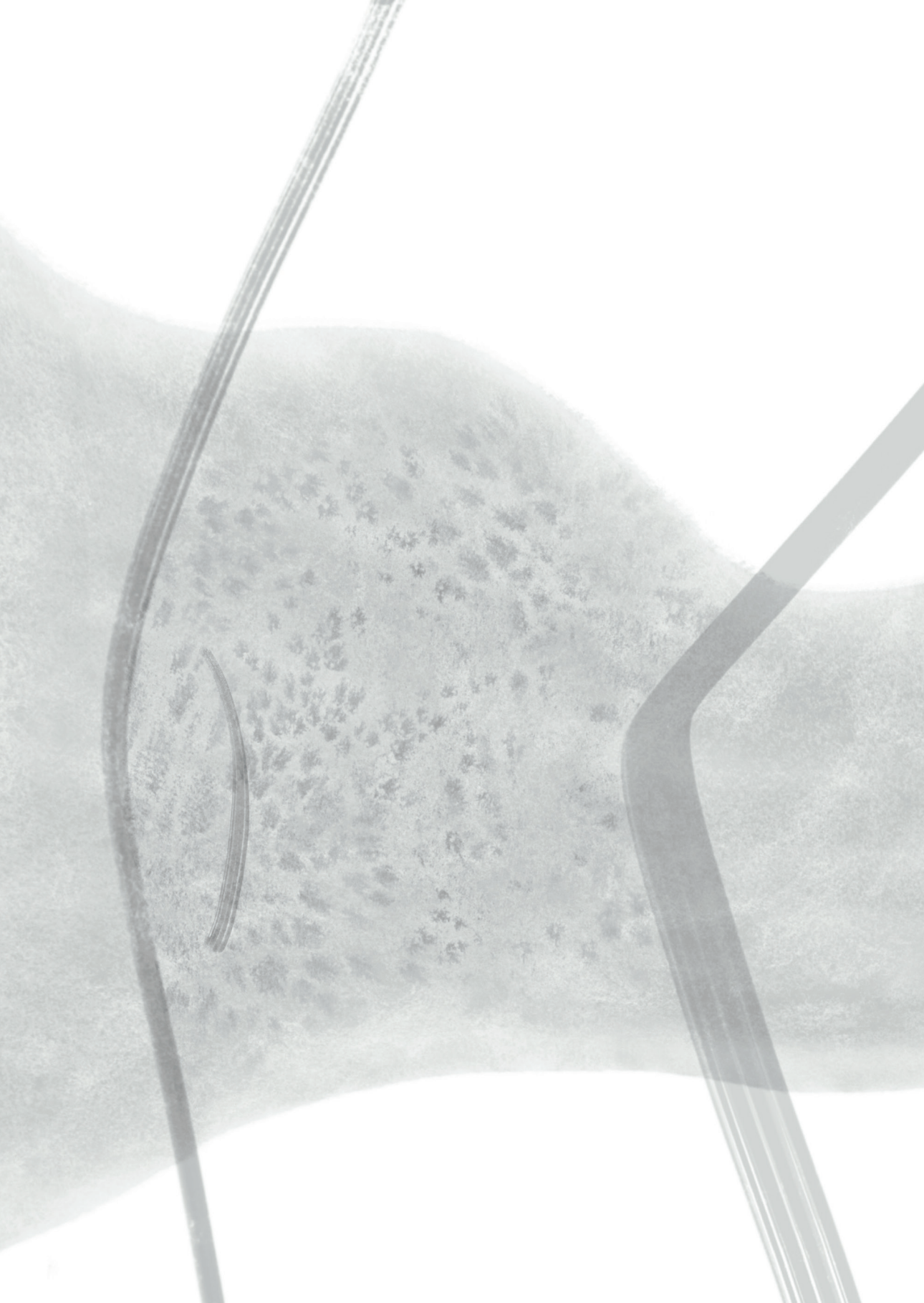

Chapter 1

General introduction

OSTEOARTHRITIS

Worldwide, osteoarthritis (OA) is the most frequent form of arthritis[1], with an estimated global prevalence of 303 million people in 2017 according to the Global Burden of Disease Study[2]. From 60 years of age, symptomatic OA is affecting about 10% of men and 18% of women[3]. It is a debilitating condition characterized by pain, joint inflammation and joint stiffness, resulting in a substantial degree of physical disability and a lower health-related quality of life[4]. In recent estimates of years lived with disability, OA is estimated to be the 12th leading cause of years lived with disability globally[5]. The economic costs of OA, including direct healthcare costs such as joint replacements and indirect costs due to losses in productivity, are considerable; 1% to 2.5% of the gross domestic product for westernized countries[6]. In the Netherlands, OA was the second most prevalent disease in 2015 (after low back and neck complaints) with 1.2 million people suffering OA. Trends are predicting an increase to more than 2.2 million people in 2040. Moreover, OA is estimated to increase from the 10th to the 3rd leading cause of years lived with disability in the Netherlands in 2040[7].

OA is characterized by changes to the structure of the entire synovial joint: loss of cartilage, subchondral bone sclerosis, synovial inflammation, osteophyte formation and changes to menisci, ligaments, capsule and periarticular muscles, resulting in structural and functional "joint failure"[8] (Fig 1). When severe enough, these pathological changes eventually result in changes on radiographs, which show narrowing of joint space, osteophytes and sometimes changes in the subchondral bone[8, 9]. However, the presence of OA on radiographs is not always concordant with the presence of other structural changes and related symptoms[10]. Although the interphalangeal joints of the hand are most commonly affected radiographically, their involvement is mostly asymptomatic, while knee OA, the second commonly involved joint, is most of the time symptomatic and responsible for 83% of the burden of disease from OA overall[4, 11]. The work in this thesis focuses on knee OA.

PREVENTION OF KNEE OA

At present, there are no curative therapies for knee OA[12]. Hence, management is focused on controlling pain, reducing functional limitation, improving health-related quality of life and, in case of end-stage disease, joint replacement surgery[13, 14]. This approach is only moderately effective. Moreover, pharmacological methods do have their side effects, such as acetaminophen-induced hepatotoxicity and NSAID-induced gastrointestinal and cardiovascular toxic effects[15]. Outcomes from total joint replacement surgery are also not optimal; up to 25% of patients complains of pain and disability 1 year after well performed surgery[16]. Also, prostheses have a finite lifespan[17]. In the light of the increase in knee OA prevalence in the coming decades, primary prevention strategies, defined as measures aiming to prevent the development of definite structural or clinical knee OA in subjects free of the disease, are highly necessary[18].

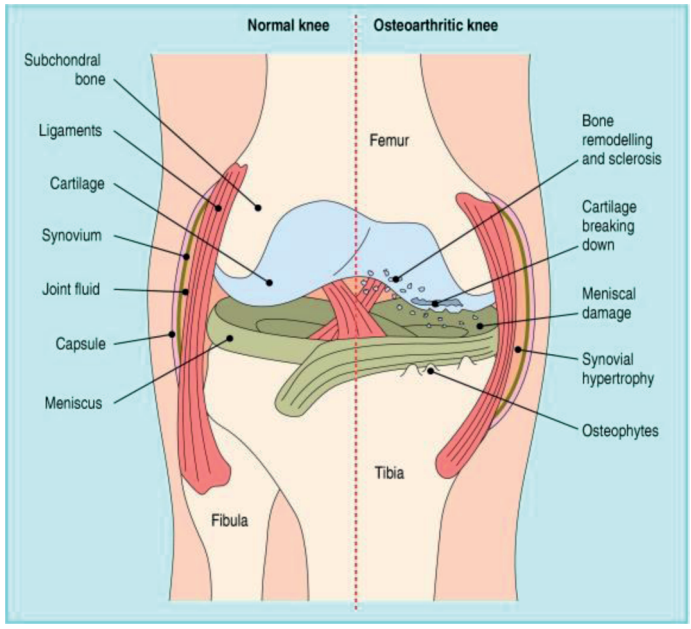

Fig 1. Pathogenic features consistent with osteoarthritis[8] *(with consent by the authors).*

Risk factors: identifying those at highest risk of knee OA

The identification of risk factors and the distinction between modifiable and non-modifiable risk factors is crucial for the selection of targets for primary prevention[17]. Risk factors for knee OA can be broadly separated into systemic and local factors[19]. A large systematic review and meta-analysis, including 46 studies, reported the following main factors associated with the development of knee OA in those aged 50 years and over: overweight, obesity, female gender, age and self-reported previous knee injury[20]. Table 1 shows an overview of the most commonly studied risk factors for knee OA and their pooled odds ratios.

Increasing age and female gender can be seen as systemic risk factors. Knee OA prevalence increases steeply with age starting at around age 50 to 55, especially in women[1, 12, 21]. A multitude of factors is thought to be responsible for this association, including cartilage senescence, muscle weakness, neurosensory failure and ligamentous laxity[12]. In addition, the increasing incidence of OA with age is also a result of the cumulative exposure to various risk factors during life[16]. The gender difference between men and women in the prevalence of knee OA, starts after the age of 50 years, exactly when estrogen levels in women drop down. Therefore, the association is thought to be related to the reduction in serum estradiol concentrations occurring in perimenopausal and postmenopausal women, although the exact mechanism is still unresolved, despite many studies[12].

Overweight/obesity was long seen as a pure local risk factor, since mechanical overload on the knee joints activates catabolic processes in chondrocytes, resulting in cartilage degeneration[22]. However, it has been shown that obesity is also a risk factor for OA in non-weight-bearing joints, like hands and wrists[23], indicating that it effects joint tissues also systemically. A role for inflammatory adipokines production from adipose tissue has been suggested, causing a low-grade inflammation in all joints[22].

Table 1. Most studied risk factors for incident knee OA (selected from[20]).

Risk factor	Number of studies	Number of participants	Pooled odds radio (95% CI)
Overweight	22	398,251	1.98 (1.57 – 2.20)
Obesity	22	401,119	2.66 (2.15 – 3.28)
Self-reported previous knee injury	13	27,326	2.83 (1.91 – 4.19)
Female gender	11	28,133	1.68 (1.37 – 2.07)
Heberden's nodes	6	5232	1.30 (0.90 – 1.87)
Age	19	-*	-*

* Although all studies were in agreement that increasing age was a significant risk factor for onset of knee OA, creating a pooled OR was not possible as the studies used a range of different age categorizations[20].

As shown by the study of Silverwood[20], self-reported previous knee injury is the most potent risk factor for the development of knee OA, acting locally by adversely affecting joint biomechanics and causing a local inflammatory response[12]. This injury, mostly caused by sport but also by occupational activities such as kneeling and lifting, increases the risk of knee OA by almost three times[20]. The risk is even higher when based on confirmed tissue injury, such as anterior ligament or meniscal tear[24, 25].

Obviously, ageing and female gender are non-modifiable risk factors. In theory, overweight/obesity and previous knee injury are modifiable. The contribution of these risk factors to the development of knee OA, based on the meta-analysis by Silverwood et al.[20], is as follows: for an estimated 5.1% of new knee OA patients, this is related to self-reported previous injury, 17.3% is related to obesity and 24.6% to being overweight or obese, the latter two largely depending on the country prevalence of obesity[16, 20]. This shows that reducing the prevalence of overweight/obesity has potentially more benefit than preventing knee injuries. As the most important modifiable risk factors for knee OA, overweight and obesity are key targets in knee OA prevention[26]. The present thesis focuses especially on overweight and obesity as modifiable risk factors.

In addition to the risk factors mentioned above, there are also other potential risk factors for the development of knee OA that are often mentioned in literature, such as heredity, low quadriceps muscle strength and knee malalignment[12, 27, 28]. For heredity it has been shown that, although a set of genetic abnormalities are found from large scale studies, none of the

separate genes was associated with a high risk of disease[12]. Varus alignment has been shown to increase the initial development of incident knee OA and is considered a moderate to strong risk factor[29]. Both varus and valgus malalignment are evident risk factors for the progression of knee OA[27]. Based on a systematic review, knee extensor muscle weakness is a weak risk factor for developing knee osteoarthritis[30].

Weight loss as prevention for knee OA

The epidemic of obesity is one of the most important health problems worldwide[31]. As described by the World Health Organization, the global prevalence of obesity has more than doubled since 1980[32]. In 2014, 11% of men and 15% of women aged 18 years and older were classified as obese (BMI ≥ 30 kg/m^2) and 39% of adults (38% of men and 40% of women) as overweight (BMI ≥ 25 kg/m^2)[32]. In the Netherlands, 37% of men and 26% of women above 18 years of age are estimated to be overweight and 11% of men and 14% of women to be obese[33].

Already in 1992, results from the Framingham study demonstrated that a 5.1 kg reduction in body weight over a 10-year period would decrease the risk in developing knee osteoarthritis by over 50% in women whose baseline body mass index (BMI) values were at least 25 kg/m^2 [34]. Felson et al. calculated from the Framingham osteoarthritis study that a reduction in body weight from the obese group (BMI ≥ 30 kg/m^2) to the overweight group (BMI ≥ 26 and < 30 kg/m^2) or from the overweight to the normal weight group (BMI < 26 kg/m^2) would decrease the rate of symptomatic knee OA by 21.4% in men and by 33% in women[35].

Despite this strong but indirect evidence, no studies had ever been performed that studied the direct preventive effects of weight loss on the incidence of knee OA. According to the Society for Prevention Research, it is essential to apply primary preventive measures in an early stage to those at high risk for disease development[36]. The preventive intervention itself should target modifiable risk factors. Therefore, in 2005, the PROOF study (PRevention of knee Osteoarthritis in Overweight Females), was launched[37]. The papers in this thesis are based on the PROOF study data.

The PROOF study

The PROOF study, the first randomized controlled trial in the prevention of clinical and radiographic knee OA, aimed to evaluate the preventive effects of a diet-and-exercise program and of oral crystalline glucosamine sulphate in women aged 50 - 60 years with a BMI ≥ 27 kg/m^2 and free of knee OA according to the clinical criteria of the American College of Rheumatology (ACR)[38]. The BMI cut-point of 27 kg/m^2 was chosen since there is a clear increase in incident knee OA beyond this point[39]. The participants of the PROOF study were recruited by 50 general practitioners in and around Rotterdam, the Netherlands, by sending an information letter to all women between 50 and 60 years registered at their

practice. All interested women with a self-reported BMI ≥ 27 kg/m^2 were contacted by phone to check all inclusion criteria[37].

The diet-and-exercise program, intended to reduce weight, was tailored made, meaning that no pre-defined scheme of diet and exercise was applied to the participants. Instead, a dietician who was trained in motivational interviewing made an individual plan regarding both diet and exercise in dialogue with each participant. This approach using motivational interviewing aimed to lead to a clinically significant amount of weight loss and to promote long-term weight loss maintenance for the participant[40]. A clinically significant amount of weight loss was defined as the loss of at least 5 kg or 5% of body weight, based on the Framingham results and since this amount of weight loss is associated with several other health benefits such as improvement of cardiovascular risk factors[41]. In addition to being randomized to the diet-and-exercise program or control group, participants were randomized to oral glucosamine sulphate or matching placebo. When the trial was designed, high dropout rates in the control group of the diet-and-exercise program were feared. To prevent this, the glucosamine sulphate vs. placebo intervention was introduced, to provide all participants with an intervention and hopefully avoid high dropout rates. In established knee OA patients, no efficacy of glucosamine had been proven in studies with adequate allocation concealment or in investigator-led studies[13]. However, literature suggested larger effects of glucosamine over placebo when used in an early phase of the disease and especially in the knee joint[42]. Moreover, side effects were similar to placebo, making it a safe and worthwhile preventive intervention[42, 43]. The glucosamine intervention lasted for 2.5 years. The tailor-made diet-and-exercise program intervention resulted in a duration that was different for each participant, but was maximized at 2.5 years.

During 2.5 years, every six months biometrical data was collected and questionnaires were filled in. In addition, at baseline and after 2.5 years radiographic and magnetic resonance imaging (MRI) data of both knees were collected. Prolongation of the follow-up time was initiated in order to evaluate long-term intervention effects. Therefore, all measurements were repeated after 4 years, resulting in a total follow-up time of 6.5 years. The primary outcome of the PROOF study was incidence of knee osteoarthritis after 2.5 years, defined as incidence of either Kellgren and Lawrence (KL)[44] ≥ 2, clinical knee OA (combined clinical and radiographic ACR criteria[38]) or joint space narrowing of ≥ 1.0mm in the medial or lateral compartment[37]. The 2.5-year follow-up results on the primary outcome measure showed no significant main effects of the diet-and-exercise program and the glucosamine sulphate intervention. However, due to an unexpected significant interaction between the two interventions, 4 instead of 2 groups had to be analyzed separately, resulting in slightly underpowered analyses[37].

Structural features of knee OA on MRI were one of the pre-specified secondary outcomes of the PROOF study after 2.5 years and will be evaluated in several chapters of this thesis. The

MRI assessment of the knee joint will be described more extensively in the next paragraph. The main outcome after 6.5 years was defined as incidence of clinical knee OA[38] and results of the interventions on this outcome will be presented as well in this thesis.

MRI assessment of the knee joint

The conventional imaging modality to visualize OA is radiography. It is the most commonly used and the first choice technique in clinical practice. However, joint space width between femur and tibia measured on a radiograph is only an indirect, surrogate measure of cartilage thickness[45]. Moreover, because OA development is a gradual process, radiographic features are late manifestations of the disease[8]. In the last years, MRI has become the most utilized and recommended imaging modality for diagnosis, monitoring and characterization of OA in scientific research[46]. It has the advantage of direct assessment of OA-related structures, such as cartilage, osteophytes, subchondral bone changes, meniscal abnormalities and extrusion, effusion and synovitis. In addition, MRI can show structural damage earlier than can be seen on radiographs, as MRI has shown to detect OA features in asymptomatic persons without radiographic knee OA[47]. In this way, MRI might be able to identify OA features at a pre-clinical stage. Another advantage of MRI is that it provides a three-dimensional view and that it visualize the whole joint, including patellofemoral abnormalities, while traditional radiographic definitions of knee OA focus on the projection of the tibiofemoral joint only. During the past years, OA features on MRI have been extensively studied and have improved the current understanding of OA pathogenesis[46]. For the PROOF study, the MRI OA knee scoring system (MOAKS) was used, employing 0/1 or 0 to 3 semi-quantitative scores for sub-regional pathologies[48]. Knowledge of the OA features on MRI in the PROOF population might provide a more detailed insight in the initial development of knee OA and in the preventive effects of the interventions in this high-risk population. In addition, in 2011 a Delphi consensus definition of MRI OA had been proposed based on the presence of specific combinations of MRI features[49]. In this definition, tibiofemoral MRI OA is defined as 'definite osteophyte' formation and full thickness cartilage loss or one of the latter and two or more of the following: bone marrow lesion or cyst, meniscal abnormalities, partial thickness cartilage loss, bone attrition. Patellofemoral MRI OA was defined as presence of a 'definite osteophyte' and partial or full thickness cartilage loss. The MOAKS features and the MRI definition will be used in this thesis.

PREDICTION OF KNEE OA

To be able to apply primary preventive strategies for knee OA, e.g. by general practitioners, it is important to identify those at the highest risk for disease development. Another important reason that necessitates the identification of subjects at highest risk of knee OA is to enrich study populations of preventive research studies for OA, as it is the most powerful to include

subjects in a study who are at highest risk of developing OA on a relatively short term[18]. Over the past few years, the identification of predictors of early OA development has been the focus of much research. Beside the use of clinical signs and symptoms to predict OA, imaging markers and biochemical markers are investigated as early disease markers of knee OA[50].

Signs and symptoms

The diagnosis of knee OA is often preceded by symptoms over a period of years before the appearance of OA features on plain radiographs occurs[51]. This offers the potential for earlier diagnosis. Patient-reported information or information obtained from physical examination might be an effective and inexpensive method, especially in the primary care setting, to identify those who will develop symptomatic radiographic knee OA in the (near) future. For instance, potential early patient-reported symptoms and signs from physical examination could be combined with established risk factors, such as age, BMI and previous injury, to improve the prediction of knee OA. Since prevalence of prodromal signs might be low in this early phase, multivariable models are needed as they might improve the individual risk prediction[51].

Biochemical markers

Biochemical markers can be measured in blood, urine or synovial samples and are structural molecules or fragments linked to cartilage, bone or synovium[50]. An important characteristic of a biochemical marker for prediction research should be its ability to detect very early (signs of) OA, before symptomatic or radiographic disease is present[52]. One of the biochemical markers recently developed, is the Coll2-1NO_2 peptide that represents the combination of collagen type II degradation products (Coll2-1) and reactive nitrogen and oxygen species (RNOS), NO and O_2[53]. It can be measured systemically in urine or serum. Collagen type II is one of the most important components of cartilage. In addition, elevated production of RNOS has been observed in chronic inflammatory conditions, including established OA[53]. As a low grade chronic inflammation has been suggested to be involved in the development of OA[54], it seems worthwhile to explore the potency of Coll2-1NO_2 in detecting disease activity in pre-symptomatic and pre-radiographic knee OA. The potency of urinary Coll2-1NO_2 peptide will be evaluated in this thesis.

MRI markers

Bone marrow lesions, meniscal damage, synovitis and cartilage damage on MRI have all been shown to be associated with the development of radiographic knee OA in several studies[55]. As evidence is accumulating that these lesions are not incidental, it is likely that these MRI features are not only risk factors, but may represent early signs of OA[56]. Recently it has been shown that among persons at higher risk for knee OA but with normal knee radiographs

(KL 0), cartilage damage, bone marrow lesions and meniscal damage improved the prediction of incident radiographic knee OA above known established risk factors such as age, BMI, gender etc.[56]. The feature of synovitis on MRI has not been evaluated yet in risk prediction models for incident knee OA, while there is increased evidence that synovial inflammation and the resultant pro-inflammatory mediators play a key role in the OA pathology, with effect on articular cartilage[57]. The underlying mechanisms are complex: it is thought that degraded cartilage can initiate synovial inflammation, but that in early OA the synovium itself induces the production of catabolic and pro-inflammatory mediators which in turn leads to an increase in cartilage degradation[57, 58]. Several studies have shown that synovitis is an independent risk factor for incident radiographic knee OA[59, 60] and for radiographic and symptomatic progression[61, 62]. However, the usefulness of synovitis on MRI as a clinically relevant predictive tool in a high-risk population without clinical knee OA as the PROOF study population, is currently unknown and will be evaluated in this thesis.

SCIENTIFIC RESEARCH AND CLINICAL PRACTICE OF KNEE OA IN PRIMARY CARE

The aim of studying early clinical symptoms and biochemical and MRI markers of early knee OA is to identify high-risk subjects. Early detection is necessary to be able to offer preventive interventions to those at highest risk. In addition, it can optimize preventive trials in OA research, as it enables to enrich the study sampling of trials[51]. The latter is not directly applicable to the practical work of the general practitioner, while at the same time most patients with knee OA will be managed in primary care. He or she has a crucial role in prevention and early intervention of chronic diseases on an individual (patient) level[63]. Therefore, running high quality preventive trials in OA research is of great importance, since they can supply the general practitioner with evidence-based information about effective preventive interventions for knee OA. MRI markers are therefore important measures since they improve understanding of early development of OA and improve the evaluation of early preventive interventions before (late) radiographic disease develops. Further, the use of easy applicable diagnostics that can be used directly by the general practitioner would be of great help for the prediction and selection of those at highest risk to develop disease in primary care. Besides the use of clinical risk factors to predict knee OA, biochemical markers in urine or serum are another set of promising markers, that are in potential easy applicable for a general practitioner[52]. A disease so common in primary care with such great impact on pain and function, deserves the attention of both researchers and general practitioners.

OVERALL AIM AND OUTLINE OF THE THESIS

The overall aim of this thesis is twofold: investigate prevention and prediction of knee OA.

The main objectives of this thesis were as follows: 1) investigate the 2.5-year effects of the PROOF study interventions and the 2.5-year effects of weight change on knee OA features on MRI; 2) assess the long-term effects of the PROOF study interventions on incidence of clinical and radiographic knee OA; 3) evaluate the potency of the urinary Coll2-1NO_2 biochemical marker in detecting early knee OA; 4) investigate prediction of incident knee pain and incident knee OA using clinical signs and symptoms and MRI OA features as predictors.

The results described in this thesis are based on the PROOF study population. The first chapters focus on prevention of knee OA. The effects of the PROOF interventions and the effects of particular weight change patterns on incidence and progression of MRI knee OA features after 2.5 years are evaluated in **Chapter 2 and 3**. **Chapter 4** evaluates the impact of differences in body weight over ±15 years prior to inclusion into the PROOF study on the prevalence of midlife MRI knee OA. The long-term effects of the PROOF study interventions on incident clinical and radiographic knee OA are reported in **Chapter 5.** The next chapters are related to prediction of knee OA. The association between urinary Coll2-1NO_2 and incident clinical and radiographic knee OA after 2.5 years is evaluated in **Chapter 6**. **Chapter 7** describes the development of a prediction model for incident frequent knee pain and knee OA with clinical risk factors obtained from questionnaires and physical examination. **Chapter 8** evaluates and compares the predictive value of synovitis on MRI, patient-reported swelling and patient-reported morning stiffness of the knee on incident clinical and radiographic knee OA after 2.5 and 6.5 years. **Chapter 9** reflects on the main findings of the preceding chapters, as well as their limitations. Furthermore, implications for future research and clinical practice are discussed.

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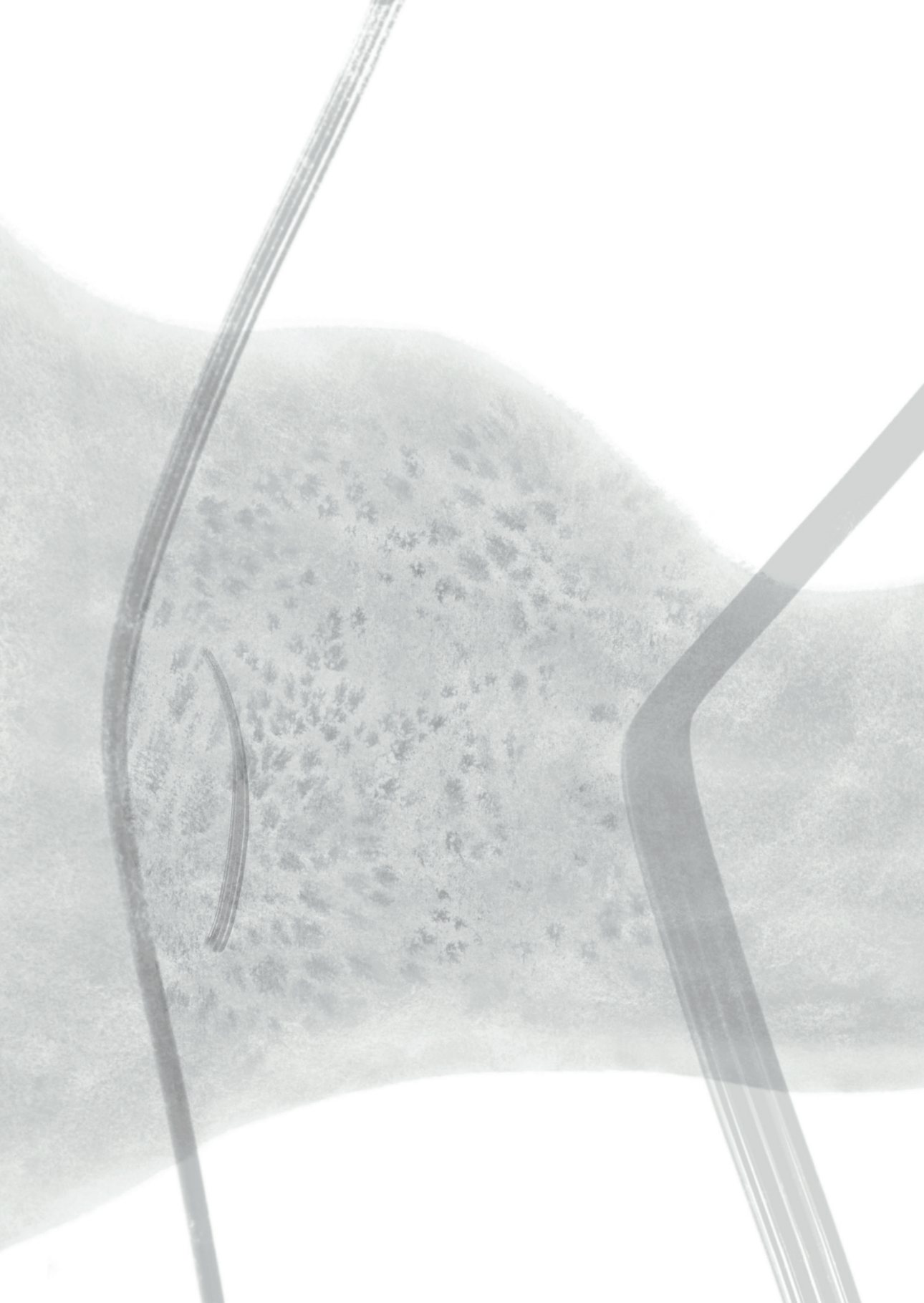

Chapter 2

Reducing progression of knee OA features assessed by MRI in overweight and obese women: secondary outcomes of a preventive RCT

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ABSTRACT

Objective

To evaluate the preventive effects of a randomized controlled trial on progression of Magnetic Resonance Imaging (MRI) features of knee osteoarthritis (OA) in overweight and obese women.

Design

In a 2 x 2 factorial design, 2.5 years effects of a diet and exercise program and of glucosamine sulphate (double-blind, placebo-controlled) were evaluated in 407 middle-aged women with body mass index (BMI) ≥ 27 kg/m^2 without clinical signs of knee OA at baseline (ISRCTN 42823086). MRIs were scored with the MRI Osteoarthritis Knee Score (MOAKS). Progression was defined for bone marrow lesions (BMLs), cartilage defects, osteophytes, meniscal abnormalities and meniscal extrusion. Analyses on knee level were performed over the four intervention groups using adjusted Generalized Estimating Equations.

Results

687 knees of 347 women with mean age 55.7 years (±3.2 SD) and mean BMI 32.3 kg/m^2 (±4.2 SD) were analyzed. Baseline prevalence was 64% for BMLs, 70% for cartilage defects, 24% for osteophytes, 66% for meniscal abnormalities and 52% for meniscal extrusions. The diet and exercise program + placebo intervention showed significantly less progression of meniscal extrusion compared to placebo only (12% vs 22%, OR 0.50, 95% CI [0.27–0.92]). The interventions did not result in significant differences on other OA MRI features.

Conclusions

In subjects at high risk for future knee OA development, a diet and exercise program, glucosamine sulphate and their combination showed small and mainly non-significant effects on the progression of OA MRI features. Only progression of meniscal extrusion was significantly diminished by the diet and exercise program.

INTRODUCTION

Knee osteoarthritis (OA) is one of the leading causes of global disability[1], affecting about 10% of men and 13% of women aged > 60 years[2]. Due to the aging population and global epidemic of obesity, the prevalence of symptomatic knee osteoarthritis is likely to rise rapidly, with associated burden for society[1]. Current treatment options can diminish symptoms such as pain and disability, but a curative treatment is not available[3]. Increasing focus on preventive interventions should therefore be highly considered[4, 5].

To meet these demands, the results of the first preventive trial in osteoarthritis research were published recently[6]. The PROOF study (PRevention of knee Osteoarthritis in Overweight Females) evaluated the preventive effects of a diet and exercise program and of oral crystalline glucosamine sulphate on the incidence of knee OA in overweight and obese middle-aged women, without diagnosed knee OA at inclusion. With 2.5 years follow-up, the interventions showed no significant preventive effects on the primary outcome measure, incidence of clinical and radiographic knee OA. Only in a post-hoc analysis with additional data, crystalline glucosamine sulphate with or without the diet and exercise program reduced minimum joint space narrowing of the medial tibiofemoral (TF) compartment[7].

Because OA development is a gradual process and radiographic features are late manifestations, MRI features of OA may provide more direct insight in early joint changes[8]. MRI has shown to be more sensitive compared to Kellgren and Lawrence (K&L)[9] grading on posterior-anterior flexed knee radiographs in detecting structural knee OA[10] and is able to detect early OA features in asymptomatic persons without radiographic knee OA[11]. We hypothesized that, compared to clinical and radiographic criteria, MRI would provide more detailed insight in the initial development of knee OA and in the preventive effects of the interventions in this high-risk population. Therefore, the secondary outcome of the PROOF study was pre-defined as the effects of the interventions on OA MRI features[6]. The aim of the present study was to evaluate the preventive effects of a diet and exercise program and of oral glucosamine sulphate on the progression of knee OA MRI features in overweight and obese women between 50 and 60 years, without clinical knee OA at baseline.

METHODS

Study design, setting and population

A description of the design and results of the PROOF study (ISRCTN 42823086) has been published previously[6, 7, 12]. This randomized controlled trial evaluated the preventive effects of a diet and exercise program and of oral glucosamine sulphate (double-blind, placebo-controlled) on the development of knee OA in 407 middle-aged (50 - 60 years) women with body mass index (BMI) ≥ 27 kg/m^2, in a 2 x 2 factorial design with 2.5 years follow-up.

Participants were recruited by their general practitioner (GP) and had to be free of clinical knee OA (clinical American College of Rheumatology (ACR)-criteria[13]). They had to master the Dutch language and had to be free of severely disabling co-morbidities, free of inflammatory rheumatic diseases, not under treatment of a physical therapist or GP for knee complaints, not using walking aids, not using oral glucosamine for the last 6 months and free of contraindications for MRI. The Institutional Review Board of Erasmus MC University Medical Center Rotterdam approved the study. All participants gave written informed consent prior to baseline measurements.

Randomization and interventions

In this 2 x 2 factorial design, eligible patients were randomly assigned to either the intervention group of the diet and exercise program or to the control group and to either daily 1500mg oral crystalline glucosamine sulphate or to placebo. The description of the diet and exercise program, aimed to achieve weight loss in the intervention group, has been presented elsewhere[12]. It provided individual consultations by dieticians trained in Motivational Interviewing[14], who gave tailor-made advices for diet and physical activity. Participants were invited to participate in different physical exercise classes of low impact sports, such as Nordic walking, dancing and aqua jogging. These weekly 1-hour classes were supervised by a local physical therapist and offered during 20 weeks, spread over half a year period. Participants in the control group were not offered an intervention, but for ethical reasons, they were not actively discouraged to lose weight themselves. Crystalline glucosamine sulphate and placebo were provided by Rottapharm Madaus, Monza, Italy (not involved in any way in study design, data collection and statistical analysis) and identical in appearance, smell and taste; subjects and research staff were blinded for allocation. All women were asked to consume one sachet (1500mg powder) per day during the complete 2.5 years of follow-up. During home visits by a research assistant every six months, unused study medication was retrieved and the participants were provided with new supply.

Questionnaires and physical examination

At baseline, participants filled in a questionnaire to record demographic characteristics such as age, postmenopausal status, ethnicity and clinical characteristics such as history of knee injury, physical activity (measured with the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH))[15] and knee complaints ("did you experience knee pain in the past 12 months?"). Body weight, body height and presence of Heberden's nodes on both hands were assessed with a standardized physical examination by a research nurse at baseline and 2.5 years.

Radiography

Posterior-anterior radiographs of both knees were taken at baseline and 2.5 years, using the semi-flexed metatarsophalangeal (MTP) view[16]. K&L grading[9] and medial knee alignment[17] was scored on both radiographs at once (sequence known) by a trained researcher blinded for clinical outcomes and treatment assignment (MR and JR respectively). Normal alignment was defined as angles between 182° and 184°, valgus and varus alignment were defined as angles > 184° and < 182° respectively[18]. The reproducibility of K&L grading (kappa 0.6) and knee alignment (kappa 0.7) was assessed by the independent scoring of a random subset of 20% of the radiographs by a second blinded researcher (JR or MR).

MRI acquisition and assessment

MRIs of both knees were made at baseline and 2.5 years on a 1.5 Tesla scanner. The MRI protocol included coronal and sagittal non-fat suppressed proton density weighted sequences (slice thickness 3.0 mm/slice gap 0.3 mm), a coronal T2 weighted Spectral Presaturation by Inversion Recovery (SPIR) sequence (slice thickness 5.0 mm/slice gap 0.5 mm), an axial dual spin-echo sequence (slice thickness 4.5 mm/slice gap 0.5 mm) and a sagittal 3D water selective (WATS) sequence with fat saturation (slice thickness 1.5 mm). Baseline and follow-up MRIs were scored at once (sequence known) by two blinded researchers (JR human movement scientist, PvdP radiology trainee) using the semi-quantitative MRI Osteoarthritis Knee Score (MOAKS)[19]. They evaluated the following OA features: bone marrow lesions and cysts (BMLs), cartilage defects, osteophytes, meniscal abnormalities and meniscal extrusion. We defined meniscal abnormalities as meniscal morphologic abnormalities (tears, maceration, hypertrophy and cysts) and (degenerative) signal abnormalities. Meniscal extrusion was defined separately from meniscal abnormalities. Anterior, medial and lateral extrusion was scored on a 0 - 3 scale for the medial and lateral meniscus, where grade 0 = < 2 mm, grade 1 = 2–2.9 mm, grade 2 = 3–4.9 mm and grade 3 = > 5 mm. For implementing the MOAKS adequately, the two researchers were trained under supervision of an experienced musculoskeletal radiologist (EO: 10 years of experience with musculoskeletal MRI in clinical and research settings). This training has been described in detail previously[20]. The change of the individual OA MRI features was scored using the recently proposed definitions for longitudinal evaluation of OA MRI features (see Appendix table 1)[20], in which the average prevalence-adjusted bias-adjusted kappa (PABAK) values per feature showed 'substantial' to 'nearly perfect agreement' (range 0.77 – 0.88, observed agreement 89 - 94%)[20]. For the present study, the subregional change scores (1 for progression, -1 for improvement and 0 for no change) were summed over the different MOAKS subregions into an overall measure of change per feature. The summed change scores per feature were dichotomized into progression versus no progression (change score ≥ 1 = progression, change score < 1 = no progression). The tibiofemoral (TF) and patellofemoral (PF) joint were combined for the assessments, as well as the medial and lateral meniscus.

Outcome measures

The outcome measures of this study were pre-defined secondary outcome measures of the original PROOF study. They were defined as the effects of the four intervention groups (diet and exercise program control + placebo group, diet and exercise program control + glucosamine sulphate group, diet and exercise program intervention + placebo group and diet and exercise program intervention + glucosamine sulphate group) on the progression of the following OA MRI features: BMLs, cartilage defects, osteophytes, meniscal abnormalities and meniscal extrusion.

Statistical Analysis

Participants with an available MRI at baseline and 2.5 years of one or both knees were included and analyzed on the basis of a modified 'intention to treat' (ITT) approach, i.e. including all women with available MRIs. Descriptive data were presented as mean ± standard deviation (SD) or as numbers (percentages). Because of a significant interaction between both interventions on the primary outcome (clinical and radiographic knee OA) of the original PROOF study, described extensively in an earlier publication[6], the secondary outcome analyses were performed conform the approach for the primary outcome, over the four separate groups. Subjects in the diet and exercise program control + placebo group were defined as reference. Differences in baseline variables among the groups were analyzed with one-way analysis of variance or with the chi-squared test. We performed uni- and multivariable regression analyses on knee level with Generalized Estimating Equations (GEE), taking into account the association between two knees within one person. Firstly, the unadjusted effects on progression of OA MRI features were determined for the four groups. Secondly, the analyses were adjusted for the presence of the corresponding baseline MRI feature and for possible baseline differences. Since the outcome measure of this paper differs from the primary outcome of the PROOF study, we performed a sensitivity analysis to examine the interaction between the two interventions on the progression of MRI features. In case of no significant interaction, the effects of the two interventions were additionally analyzed with GEE (unadjusted and adjusted). For explorative reasons, we evaluated the progression rates within the four separate groups for the medial and lateral TF joint and the PF joint separately. Statistical analyses were performed with SPSS 21.0 (Chicago, IL). P values of less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

Of the 407 women, 60 (14.7%) were lost to follow-up for current analyses. The main reason was no further time available or interest in the study (48 women, 80%). Other reasons (12 women, 20%) were claustrophobia (3 women), unattainability (6 women) and insufficient

MRI quality (1 woman). Two persons deceased during follow-up (death not related to study). Additionally, seven knees were excluded for analyses due to a recent severe knee trauma (n = 1), a prosthetic knee replacement (n = 1) or to inability or unwillingness to continue MRI scanning of the second knee (n = 5). This resulted in the analysis of 687 knees of 347 women. Comparison of baseline characteristics (table 1) between missing and non-missing knees showed a significantly lower prevalence of any cartilage defect in the missing knees (58.3% vs 70.1%, p = 0.020). Mean age was 55.7 ± 3.2 years and mean BMI was 32.3 ± 4.2 kg/m^2. K&L ≥ 2 was present in 6% of the knees. Prevalence of OA MRI features ranged from 24% to 70%. Statistically significant baseline differences between the intervention groups were found for the presence of BMLs (p = 0.015), cartilage defects (p = 0.003) and meniscal extrusion (p = 0.049). After 2.5 years, both progression of BMLs and cartilage defects was found in 30% of 687 knees, progression of osteophytes was found in 17%. Progression of meniscal abnormalities and meniscal extrusion was found in 28% and 17% respectively.

Table 1. Distribution and mean (± SD) of baseline characteristics among the randomized intervention arms.

		Diet & exercise program				**P-value**
		Control		**Intervention**		
	All	**Placebo**	**Glucosamine**	**Placebo**	**Glucosamine**	
Baseline characteristics						
N - subjects	347	87	82	87	91	
Age (yr)	55.7 ± 3.2	55.7 ± 3.3	55.7 ± 3.1	55.7 ± 3.2	55.6 ± 3.0	0.999
BMI (kg/m^2)	32.3 ± 4.2	32.8 ± 4.5	31.9 ± 3.9	32.5 ± 4.4	32.2 ± 3.8	0.575
Postmenopausal status	236 (68)	62 (71)	56 (68)	59(68)	59 (65)	0.776
Physical activity score (SQUASH)*	6915 ± 3614	7074 ± 3544	7187 ± 3699	6856 ± 4002	6573 ± 3221	0.706
N - knees	687	172	164	171	180	
Heberden's nodes	177 (26)	44 (26)	42 (26)	55 (32)	36 (20)	0.092
K&L 0	340 (49)	92 (53)	78 (48)	82 (48)	88 (49)	0.638
K&L 1	300 (44)	71 (41)	77 (47)	75 (44)	77 (43)	
K&L ≥ 2	43 (6)	7 (4)	9 (5)	12 (7)	15 (8)	
Varus alignment	267 (39)	74 (43)	68 (41)	61 (36)	64 (36)	0.406
Mild symptoms	213 (31)	54 (31)	53 (32)	59 (35)	47 (26)	0.502
History of knee injury	94 (14)	28 (16)	23 (14)	17 (10)	26 (14)	0.444
BMLs**	436 (64)	97 (56)	118 (72)	102 (60)	119 (66)	**0.015**
Cartilage defects	481 (70)	107 (62)	126 (77)	111 (65)	137 (76)	**0.003**
Osteophytes	164 (24)	41 (24)	43 (26)	39 (23)	41 (23)	0.874
Meniscal abnormalities***	452 (66)	113 (66)	112 (68)	109 (64)	118 (66)	0.883
Meniscal extrusions	359 (52)	104 (60)	89 (54)	80 (47)	86 (48)	**0.049**

SD = standard deviation.

*Higher scores represent higher physical activity. **BMLs = Bone marrow lesions. *** Meniscal abnormalities: tears, maceration, hypertrophy, cysts and (degenerative) signal abnormalities. Bold indicates p-value < 0.05.

Intervention effects of the four groups on progression of MOAKS features

Table 2 shows the ORs of the intervention effects for the four groups. The diet and exercise program intervention + placebo group showed statistically significantly less progression of meniscal extrusion compared to the reference group (12% vs 22%, adjusted OR 0.50 [0.27 – 0.92]). The other intervention groups did not demonstrate any statistically significant differences in progression of all of the other OA MRI features.

Table 2. Odds ratios from Intention To Treat analyses for the four randomized groups on progression of OA MRI features (TF and PF joint combined).

	Intervention	n/total knees (%)	OR (unadjusted)	95% CI	OR (adjusted)*	95% CI
Progression BMLs	DEP control/ placebo	53/172 (31)	1	reference	1	reference
	DEP control/ glucosamine	47/164 (29)	0.90	0.54 – 1.50	0.76	0.45 - 1.28
	DEP intervention / placebo	43/171 (25)	0.75	0.44 – 1.27	0.73	0.43 – 1.23
	DEP intervention/ glucosamine	62/180 (34)	1.15	0.69 – 1.90	1.09	0.65 – 1.82
Progression cartilage defects	DEP control/ placebo	49/172 (28)	1	reference	1	reference
	DEP control/ glucosamine	53/164 (32)	1.21	0.72 – 2.05	1.06	0.62 – 1.81
	DEP intervention / placebo	50/171 (29)	1.06	0.63 – 1.78	1.08	0.64 – 1.81
	DEP intervention/ glucosamine	53/180 (29)	1.05	0.64 – 1.74	1.02	0.61 – 1.70
Progression osteophytes	DEP control/ placebo	33/172 (19)	1	reference	1	reference
	DEP control/ glucosamine	30/164 (18)	0.94	0.50 – 1.76	0.72	0.38 – 1.37
	DEP intervention / placebo	24/171 (14)	0.71	0.37 – 1.34	0.68	0.34 – 1.33
	DEP intervention/ glucosamine	32/180 (18)	0.90	0.49 – 1.67	0.88	0.46 – 1.67
Progression meniscal abnormalities	DEP control/ placebo	51/172 (30)	1	reference	1	reference
	DEP control/ glucosamine	46/164 (28)	0.91	0.55 – 1.51	0.88	0.53 – 1.47
	DEP intervention / placebo	43/171 (25)	0.80	0.49 – 1.30	0.81	0.50 – 1.33
	DEP intervention/ glucosamine	52/180 (29)	0.96	0.60 – 1.56	0.97	0.60 – 1.56
Progression meniscal extrusions	DEP control/ placebo	37/172 (22)	1	reference	1	reference
	DEP control/ glucosamine	32/164 (20)	0.87	0.48 – 1.55	0.81	0.44 – 1.47
	DEP intervention / placebo	20/171 (12)	**0.47**	**0.26 – 0.85**	**0.50**	**0.27 – 0.92**
	DEP intervention/ glucosamine	25/180 (14)	0.58	0.32 – 1.05	0.56	0.31 – 1.03

Bold indicates p-value < 0.05. BMLs = bone marrow lesions, DEP = diet and exercise program, OR = odds ratio, CI = confidence interval. *Adjusted for baseline presence of the corresponding OA MRI feature and baseline differences.

Interaction and effects of the two interventions on progression of MOAKS features

In contrast to the paper on the primary outcome of the PROOF study[6], there was no statistically significant interaction between the two interventions on progression of any of the different MRI features (p-values ranged from 0.06 – 0.88). Therefore, the effects of the two interventions were additionally analyzed (table 3). The diet and exercise program interven-

tion group demonstrated significantly less progression of meniscal extrusion compared to the control group (13% vs 21%, adjusted OR 0.59 [0.38 – 0.91]. The diet and exercise program intervention did not affect the progression of the other MRI features in comparison to the control group. Glucosamine had no preventive effect on the progression of any of the different MRI features compared to placebo.

Table 3. Odds ratios from Intention To Treat analyses for the diet-and-exercise program intervention and the glucosamine versus placebo intervention on progression of OA MRI features (TF and PF joint combined).

	Intervention		n/total knees (%)	OR (unadjusted)	95% CI	OR (adjusted)*	95% CI
Progression BMLs	DEP	Control	100/336 (30)	1	reference	1	reference
		Intervention	105/351 (30)	0.99	0.69 – 1.43	1.04	0.72 – 1.49
	GSvP	Placebo	96/343 (28)	1	reference	1	reference
		Glucosamine	109/344 (32)	1.18	0.82 – 1.70	1.07	0.74 – 1.55
Progression cartilage defects	DEP	Control	102/336 (30)	1	reference	1	reference
		Intervention	103/351 (29)	0.96	0.68 – 1.37	1.02	0.71 – 1.45
	GSvP	Placebo	99/343 (29)	1	reference	1	reference
		Glucosamine	106/344 (31)	1.10	0.77 – 1.55	1.00	0.70 – 1.43
Progression osteophytes	DEP	Control	63/336 (19)	1	reference	1	reference
		Intervention	56/351 (16)	0.83	0.53 – 1.31	0.92	0.57 – 1.49
	GSvP	Placebo	57/343 (17)	1	reference	1	reference
		Glucosamine	62/344 (18)	1.08	0.69 – 1.70	0.96	0.60 – 1.53
Progression meniscal abnormalities	DEP	Control	97/336 (29)	1	reference	1	reference
		Intervention	95/351 (27)	0.92	0.66 – 1.30	0.95	0.68 – 1.33
	GSvP	Placebo	57/343 (17)	1	reference	1	reference
		Glucosamine	57/344 (17)	1.05	0.74 – 1.47	1.02	0.72 – 1.44
Progression meniscal extrusions	DEP	Control	69/336 (21)	1	reference	1	reference
		Intervention	45/351 (13)	**0.57**	**0.37 – 0.87**	**0.59**	**0.38 – 0.91**
	GSvP	Placebo	94/343 (27)	1	reference	1	reference
		Glucosamine	98/344 (28)	0.99	0.64 – 1.51	0.92	0.60 – 1.42

Bold indicates p-value < 0.05. BMLs = bone marrow lesions, DEP control = diet and exercise program control group, DEP intervention = diet and exercise program intervention group, GSvP = Glucosamine versus placebo intervention, OR = odds ratio, CI = confidence interval. *Adjusted for baseline presence of the corresponding OA MRI feature and baseline differences.

Explorative analyses

Progression rates in the medial and lateral TF joint and the PF joint are presented in table 4. Progression rates ranged from 1% in the diet and exercise program intervention + placebo group for lateral meniscus extrusion to 26% in the diet and exercise program intervention + glucosamine group for PF BMLs. Overall, progression rates seemed to be higher in the medial than in the lateral TF joint. For cartilage defects and BMLs, the highest progression rates were found in the PF joint.

Table 4. Progression of MOAKS features within the randomized groups for the medial and lateral TF joint and the PF joint over 2.5 years.

		Diet & exercise program			
		Control		Intervention	
	All	Placebo	Glucosamine	Placebo	Glucosamine
N - knees	687	172	164	171	180
Progression BMLs					
Medial TF joint (%)	63 (9)	24 (14)	16 (10)	11 (6)	12 (7)
Lateral TF joint (%)	42 (6)	11 (6)	6 (4)	11 (6)	14 (8)
PF joint (%)	151 (22)	37 (22)	38 (23)	30 (18)	46 (26)
Progression cartilage defects					
Medial TF joint (%)	59 (9)	14 (8)	19 (12)	14 (8)	12 (7)
Lateral TF joint (%)	41 (6)	8 (5)	10 (6)	10 (6)	13 (7)
PF joint (%)	158 (23)	38 (22)	39 (24)	39 (23)	42 (23)
Progression osteophytes					
Medial TF joint (%)	79 (11)	21 (12)	20 (12)	18 (11)	20 (11)
Lateral TF joint (%)	38 (6)	11 (6)	8 (5)	7 (4)	12 (7)
PF joint (%)	53 (8)	12 (7)	16 (10)	10 (6)	15 (8)
Progression meniscal abnormalities					
Medial meniscus (%)	146 (21)	38 (22)	36 (22)	35 (20)	37 (21)
Lateral meniscus (%)	77 (11)	23 (13)	14 (9)	15 (9)	25 (14)
Progression meniscal extrusion					
Medial meniscus (%)	99 (14)	32 (19)	28 (17)	19 (11)	20 (11)
Lateral meniscus (%)	22 (3)	8 (5)	7 (4)	1 (1)	6 (3)

TF = tibiofemoral, PF = patellofemoral, BMLs = Bone marrow lesions.

DISCUSSION

Summary

This study evaluated the preventive effects of a tailored diet and exercise program and of oral crystalline glucosamine sulphate on progression of OA MRI features over 2.5 years among overweight and obese middle-aged women without clinical knee OA at baseline. The diet and exercise intervention in combination with placebo resulted in significantly less progression of meniscal extrusion compared to placebo only. Also, when analyzing both interventions separately, the diet and exercise intervention showed a significant preventive effect on progression of meniscal extrusion. Progression of the other MRI features was not significantly influenced by glucosamine sulphate, the diet and exercise program, or their combination.

Context and comparison with existing literature

Our baseline results showed a considerable amount of OA MRI features in this high-risk group of women without clinical knee OA. Other MRI studies have analyzed pre-osteoarthritic populations[11, 21-23], but only the study by Sowers et al. was performed in a cohort of women only[23]. The percentages of cartilage lesions in these studies varied from 57 to 81%[11, 21-23], comparable to the amount of lesions in our population (70%). The amount of BMLs ranged between 39 and 75%[11, 21, 22], which is similar to the amount in our study (64%). Only compared to Sowers et al[23], BMLs were more prevalent in our study (64% compared to 39%). This difference is likely due to higher age and BMI in our study and to differences in the semi-quantitative scoring. Sowers et al. did not score bone marrow cysts, while MOAKS scores both bone marrow lesions and cysts. Further, the women in our study showed fewer osteophytes compared to the Framingham Osteoarthritis Study[11] and the Multicenter Osteoarthritis Study (MOST)[21] (24% compared to 74% in the Framingham cohort and almost 100% in MOST). This might be due to differences in age and differences in the semi-quantitative scoring. MOST scored mild osteophytes while we only scored osteophytes grade ≥ 2 as definite osteophyte. Meniscal extrusions (52%) and abnormalities (66%) were more prevalent in our study than in other studies among pre-OA subjects (18% - 24%)[11, 22]. This is likely due to a higher BMI in our study (32.3 kg/m^2 vs 26.7 kg/m^2 – 27.9 kg/m^2), an association that has been found previously in studies evaluating BMI and meniscal abnormities and extrusion[24-26].

Our results showed a lack of significant differences in all outcome measures, except for the progression of meniscal extrusion. Both the analysis of the four separate groups as the two intervention groups showed significantly less progression of meniscal extrusion in the diet and exercise intervention group with or without placebo, compared to the controls. Although the number of knees with progression of meniscal extrusion was relatively low, the relative change was large. The intervention group with or without placebo showed almost half the amount of progression compared to controls. This preventive effect was no longer significant when the diet and exercise intervention was combined with glucosamine (adjusted OR 0.56 [0.31 – 1.03]). In contrast to the interaction on the primary outcome of the PROOF study, there was no significant interaction between the two interventions on the progression of MRI features. Therefore, this finding cannot be explained by such a mechanism and this result is not well understood. Separately, glucosamine did not have a preventive effect on progression of meniscal extrusion.

The non-significance in the diet and exercise group on the four other outcome measures can most reasonably be explained by low adherence for the diet and exercise program and only a mild weight loss. The retention rates for follow-up measurements were high (85%), but only 28% of the initial 203 randomized women in the PROOF study were compliant to the diet and exercise program (≥ 6 dietary consultations and ≥ 7 exercise classes) and showed a weight

loss of 1.4 ± 5.2kg versus 0.0 ± 6.7 kg in the control group (p = 0.01)[6]. Although mean attended dietician consultation was 6.9 ± 4.9 and mean attended physical activity class was 7.3 ± 6.3, the amount of attendance varied widely[12]. Instead of strictly dictating the participants about their exercises and diet, the intervention was based on a pragmatic approach in order to simulate everyday clinical practice, but the lack of strict and continued controls might have negatively influenced the adherence rates.

The diet and exercise program showed a preventive effect on the progression of meniscal extrusion. The underlying mechanism causing extrusion is largely unknown, but is often a sign of meniscus degradation and considered as the end result of pre-existing meniscal damage[27]. Exercise programs can increase upper leg muscle strength and improve knee stability[28, 29], which might both have protective effects on the rate of meniscal extrusion. In addition, physical exercise and a weight lowering diet have local and systemic anti-inflammatory effects[30, 31]. A lower inflammatory joint status may prevent that prevalent meniscal damage like tears, (degenerative) signal abnormalities and maceration results in (end-stage) meniscal extrusion[32].

We have taken into account all levels of extrusion (MOAKS 1 – 3) and not only pathologic extrusion (MOAKS ≥ 2), with the aim to detect all progression in these women without established knee OA. Whether less progression of meniscal extrusion reduces the development of knee OA cannot be concluded from this study. Systematic reviews among knee OA patients showed that meniscal damage (extrusion/maceration) was a prognostic factor for radiographic knee OA but not for clinical knee OA[33, 34]. Both these findings were based on limited evidence and more studies are definitely needed. Longitudinal studies in subjects with and without knee OA showed that meniscal extrusion was an independent predictor of cartilage loss[24, 35-38], due to altering of the load bearing, shock absorbing and stability function of the meniscus[39]. Recently, a narrative review has described the influence of joint inflammation on the pathway from meniscal lesions to osteoarthritis[32] and suggested that joint inflammation has, either direct (meniscal damage) or indirect (obesity or ageing), an important additional negative effect on the rate at which meniscal extrusion leads to cartilage degradation. In this light, influencing joint inflammation through a diet and exercise program might be a worthwhile target in the prevention of knee OA development. The clinical and radiographic long-term follow-up data of the present population (currently being collected) might provide insight whether less progression of meniscal extrusions will result in less clinical and radiographic knee OA.

Strengths and limitations

This study has a number of limitations. Firstly, instead of a true ITT analysis, we used a modified ITT analysis, since only women with baseline and follow-up MRIs available were included. Secondly, the progression of MOAKS features is based on recently developed

definitions of longitudinal change per subregion. These are the only developed definitions, but have not been validated yet against clinical and other structural outcomes[20]. In addition, certain feature grades within the MOAKS reflect a wide range of severity[19]. As a result, within-grade progression may remain unnoticed when using the proposed progression definitions. Moreover, we summed all progression scores of the different subregions to score the change of the specific MOAKS feature for the whole knee. Consequently, detailed information about the number of affected regions or the degree of change per subregion is not visible anymore. Also, some subregions might be more at risk for developing progression of certain MOAKS features than others[40]. Therefore, we evaluated progression rates within the randomized groups for the medial and lateral TF joint and PF joint separately. Given the low progression rates within these compartments (especially lateral), effect differences between intervention groups were not statistically tested. However, these explorative results suggest that progression rates for the different features in the medial knee compartment are lower among the women in the diet and exercise program intervention + placebo group compared to the controls. Furthermore, they show that the overall progression rates of cartilage defects and BMLs are at least twice as high in the PF joint compared to the medial and lateral TF joint. These observations suggest that the PF joint is predominantly affected in overweight and obese women at risk for knee OA.

Another limitation is represented by the significant baseline differences between groups for the prevalence of OA MRI features. These were evident not only for meniscal extrusions, but especially for BMLs and cartilage defects. Although our statistical analysis adjusted the data for this imbalance, these early MRI features have been shown to predict a greater risk for OA progression[41] and it is therefore unknown whether this influenced the results for both of the interventions.

Furthermore, we are aware of the relatively large number of analyses performed, especially when testing the four different groups. This has resulted in an increased family-wise error rate. This probability might be decreased by the fact that the effects for the two interventions are in line with the results of the four groups, but still a type-I error cannot be fully neglected. However, hopefully the detailed description of these secondary outcome measures will be of valuable input for the design of future preventive OA trials.

As discussed, the poor adherence rate and only mild weight loss may have been improved when more continued contacts were offered during the diet and exercise intervention. Although the approach simulated everyday clinical practice, we recommend more strictly regulated contacts when starting a weight loss intervention in overweight and obese women, to prevent low compliance rates in future preventive studies.

Finally, despite the fact that the included women were free of clinical knee OA at initial screening, 43 of 687 knees (6%) had K&L grade ≥ 2 at baseline. As a very pragmatic design

was chosen, with high comparability to clinical practice, these 47 knees were included in the analyses. When we performed the analyses including only knees with K&L grade ≤ 1, the obtained results did not change (data not shown).

Conclusions and implications

This study of overweight and obese middle-aged women without clinical knee OA showed a high prevalence of OA MRI features. In this population at high risk of knee OA development, a diet and exercise program only showed a significant effect on the progression of meniscal extrusion; subjects randomized to a diet and exercise program intervention had less progression of meniscal extrusion compared to controls. Glucosamine sulphate or the combination of glucosamine sulphate and the diet and exercise program did not show preventive effects on progression of any of the MRI features under investigation. Follow-up data of the present population need to confirm whether the women with less progression of meniscal extrusions will subsequently develop less clinical and radiographic knee OA.

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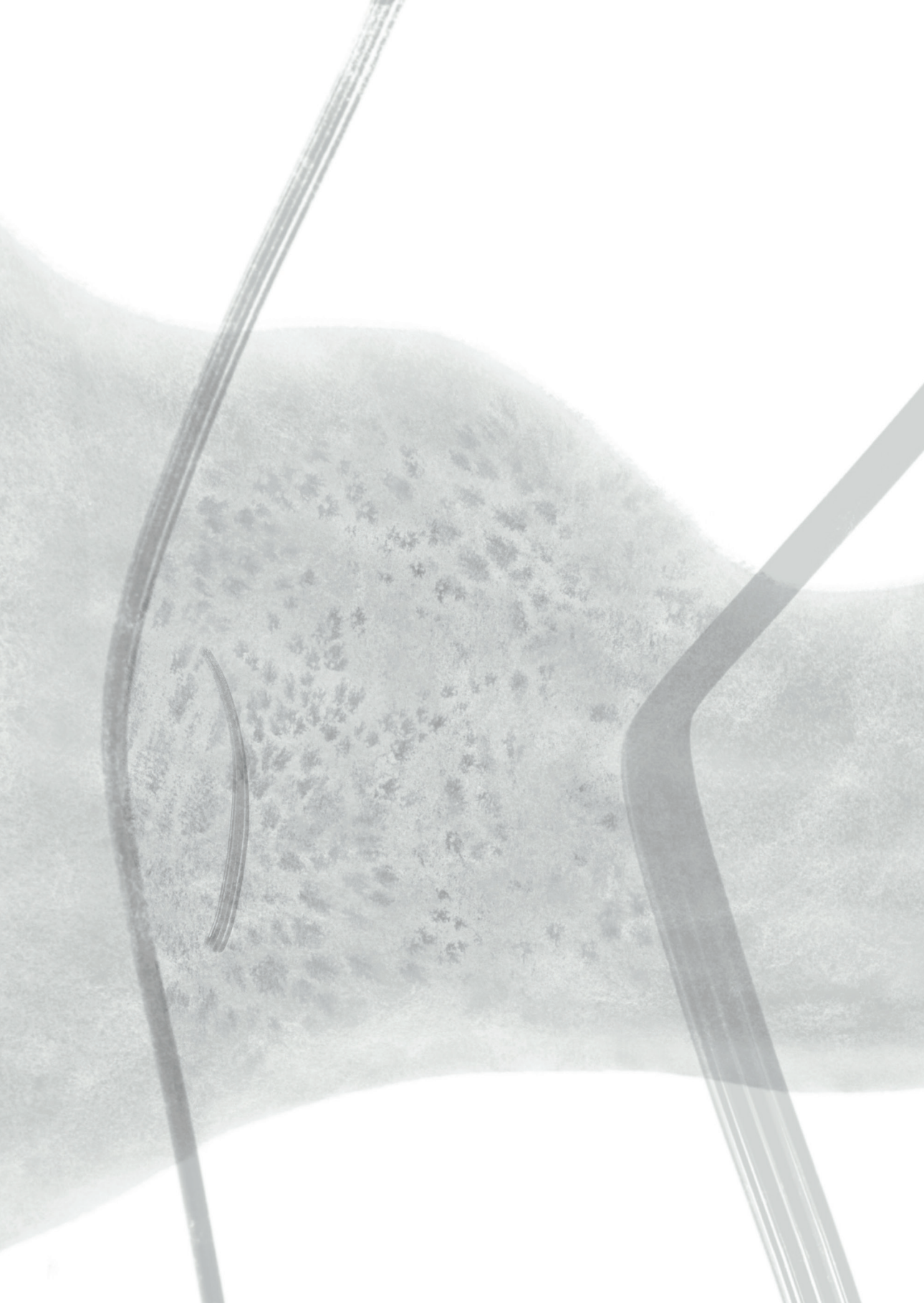

Chapter 3

Effect of weight change on progression of knee OA structural features assessed by MRI in overweight and obese women

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ABSTRACT

Objective

To evaluate the effects of weight change on progression of knee osteoarthritis (OA) structural features by magnetic resonance imaging (MRI) in overweight and obese women without clinical knee OA.

Design

347 participants from the Prevention of Knee Osteoarthritis in Overweight Females (PROOF) study were classified with latent class growth analysis into a subgroup with steady weight (n=260; +0.1±4.0kg, +0.2±4.4%), weight gain (n=43; +8.6±4.0kg, +9.8±4.1%) or weight loss (n=44; -9.0±7.2kg, -9.8±7.5%) over 2.5 years. Baseline and follow-up 1.5T MRIs were scored with MRI Osteoarthritis Knee Score (MOAKS) for progression of bone marrow lesions (BMLs), cartilage defects, osteophytes, meniscal abnormalities, meniscal extrusion and synovitis. Associations between subgroups and change in MRI features at knee-level were assessed using adjusted Generalized Estimating Equations.

Results

687 knees from 347 women (median age 55.2 years, interquartile range (IQR) 5.5, median BMI 31.2kg/m^2, IQR 5.3) were analyzed. Progression of synovitis was 18% in the weight gain versus 7% in the stable weight subgroup (OR 2.88; 95%CI 1.39–5.94). The odds for progression of patellofemoral (PF) BMLs and cartilage defects increased with 62% (OR 1.62; 95%CI 0.92–2.84) and 53% (OR 1.53; 95%CI 0.92–2.56) in the weight gain versus the stable weight subgroup.

Conclusions

In overweight and obese women, progression of synovitis increased more than 2.5 times in a weight gain compared to a stable weight subgroup over 2.5 years. Large effect sizes were also found for the difference in progression of PF BMLs and PF cartilage defects between the weight gain and stable weight subgroup.

INTRODUCTION

The epidemic of obesity is one of the most important health problems worldwide [1]. As described by the World Health Organization, the global prevalence of obesity has more than doubled since 1980 [2]. In 2014, 11% of men and 15% of women aged 18 years and older were classified as obese (body mass index (BMI) ≥ 30 kg/m^2) and 39% of adults (38% of men and 40% of women) as overweight (BMI ≥ 25 kg/m^2) [2]. Overweight and obesity are established risk factors for incident clinical and radiographic knee osteoarthritis (OA) [3, 4]. The high prevalence of knee OA has important negative impact on public health and economics [5]. As the most important modifiable risk factors for knee OA, overweight and obesity are key targets in knee OA management and prevention [6].

Weight reduction can reduce pain and physical disability in overweight and obese persons with knee OA, as shown in two systematic reviews [7, 8]. The effect of weight loss in knee OA subjects on structural damage, in terms of joint space width, remains to be elucidated [9], but evidence from the Framingham Osteoarthritis Study suggests that weight loss may prevent the onset of symptomatic and radiographic knee OA in subjects without knee OA [10]. Likewise, the first preventive trial in OA research, the Prevention of Knee Osteoarthritis in Overweight Females (PROOF) study [11], showed that weight reduction of ≥ 5 kg or ≥ 5% body weight reduces the risk of incident Kellgren and Lawrence (K&L) grade ≥ 2 knee OA over 30 months [12].

In recent years, magnetic resonance imaging (MRI) has been shown to be more sensitive to structural joint abnormalities compared to conventional radiography and may provide more direct insight in joint changes, especially in the early phases [13]. Several studies have evaluated the effects of weight loss and weight gain on MRI features in combined populations of subjects with or at risk for clinical knee OA [14-17]. Bucknor et al found evidence that four years weight gain increased progression of cartilage lesions, meniscal lesions and bone marrow edema compared to stable weight subjects [14]. Also, Guimaraes showed that subjects who gained weight had increased progression of meniscal lesions compared to stable weight subjects over 48 months[17]. While Guimaraes et al did not find differences in progression of meniscal tears between weight loss and stable weight groups[17], Gersing et al showed less progression of cartilage degeneration over four years weight loss compared to stable weight groups [16]. Another study found that substantial weight loss over 12 months was protective on the biochemical composition of cartilage (dGEMRIC) and reduced cartilage thickness losses [15]. Others did not find differences in bone marrow lesions (BMLs), synovitis and cartilage damage and thickness between subjects with (≥20%) and without weight loss [18]. Weight change studies in populations that included only subjects without clinical knee OA at baseline are scarce. One such study among community-based obese adults showed that 1% weight change (gain or loss) was associated with 0.2% change in cartilage volume; however, these results were applicable only for those with medial meniscal tears [19].

The method to assess weight change in most studies is the percentage weight change over time relative to baseline. As a disadvantage, subjects with fluctuations in BMI during follow-up were not distinguished from those with steady weight loss. Recently, Latent Class Growth Analysis (LCGA) successfully identified three subgroups with different weight change during 2.5 years within the PROOF Study [20].

Therefore, the aim of the present study was to assess 2.5 years changes in different OA structural features assessed on MRI, using three distinct weight change subgroups of middle-aged overweight and obese women without clinical knee OA at baseline.

PATIENTS AND METHODS

Study design, setting and population

For the present study, we used data from the PROOF study (ISRCTN 42823086). The description of the trial design and first results have been published previously [11, 21]. This 2.5 years follow-up study evaluated the preventive effects of a diet and exercise program and of oral crystalline glucosamine sulphate (double-blind and placebo-controlled) on the development of knee OA, in a 2x2 factorial design. All women aged between 50 and 60 years and with a BMI ≥ 27 kg/m^2 were contacted by their general practitioner (GP). They had to be free of knee OA according to the clinical criteria of the American College of Rheumatology (ACR) [22]. They had to master the Dutch language and had to be free of major co-morbidities, free of inflammatory rheumatic diseases, not under treatment of a physical therapist or GP for knee complaints, not using walking aids, not using oral glucosamine for the last 6 months and free of contraindications for MRI. The description of the diet and exercise program, aimed to achieve weight loss in the intervention group, has been presented elsewhere [21]. The Institutional Review Board of Erasmus MC University Medical Center Rotterdam approved the study. All participants gave written informed consent prior to baseline measurements.

Evaluation of changes in body weight over time

For the present study, we used three weight change subgroups, identified previously with LCGA using six-monthly weight data [20]. This three-group model showed the best fit to the data according to objective parameters and had the best usefulness of the latent classes [20]. Accordingly, LCGA was capable to identify homogeneous subgroups in the larger heterogeneous population based on individual response patterns of the participants, so that individuals within a subgroup were more similar than individuals between subgroups [23]. The women in the present study were classified into a subgroup of relatively unchanged weight (n = 260), a subgroup representing subjects who steadily gained weight (n = 43) and a subgroup of women who steadily lost weight over time (n = 44) (Figure 1) [20]. By using LCGA, participants with highly fluctuating weight changes around zero were treated similarly to

participants who remained steady around zero. The weight change after 30 months in the stable weight subgroup was minimal with 0.1 ± 4.0kg (+0.2 ± 4.4%, range -19.7 - +8.6kg) weight gain, weight change in the weight gain subgroup was 8.6 ± 4.0kg (+9.8 ± 4.1%, range +1.10 - +21.8kg) weight gain and in the weight loss subgroup this was 9.0 ± 7.2kg (-9.8 ± 7.5%, range -24.3 - +8.7kg) weight loss ($P < 0.001$).

Clinical and radiographic assessment

At baseline all subjects filled in a questionnaire to record demographics, self-reported body weight around their 40th year of age, history of knee injury and 'mild knee symptoms' (defined as having any knee pain in the last 12 months). Baseline body weight and height were assessed with a standardized physical examination by a research assistant at the research center. A standardized semi-flexed posteroanterior radiograph of both knees was taken according to the metatarsophalangeal protocol [24]. The K&L classification [25] was assessed on all knee radiographs. All measurements were repeated after the 2.5 years of follow-up of the PROOF study, body weight was recorded every six months.

MRI acquisition and assessment

An MRI of both knees was made at baseline and 2.5 years on a 1.5 Tesla scanner (Philips or Siemens). The Philips MRI protocol included coronal and sagittal non-fat suppressed proton density weighted sequences (slice thickness 3.0 mm/slice gap 0.3 mm), a coronal T2 weighted Spectral Presaturation by Inversion Recovery (SPIR) sequence (slice thickness 5.0 mm/slice gap 0.5 mm), an axial dual spin-echo sequence (slice thickness 4.5mm/slice gap 0.8 mm), and a sagittal 3D water selective (WATS) sequence with fat saturation (slice thickness 1.5 mm). Appendix 1 provides the protocol of both scanners. Baseline and follow-up MRIs were scored in one session (sequence known) by two trained and blinded researchers (JR and PvdP) using the semi-quantitative MRI Osteoarthritis Knee Score (MOAKS) [26]. The following OA-features were evaluated: BMLs, cartilage defects, osteophytes, meniscal abnormalities, meniscal extrusion and synovitis. We defined meniscal abnormalities, separately from meniscus extrusion, as meniscal morphologic abnormalities (tears, maceration, hypertrophy and cysts) and (degenerative) signal abnormalities. For the purpose of adequate implementation of MOAKS, an extensive training [27] for the two researchers was organized under supervision of an experienced musculoskeletal radiologist (EO: > 12 years of experience with musculoskeletal MRI in clinical and research settings). The change of the individual features over 2.5 years was scored using the recently proposed definitions for longitudinal evaluation of MOAKS, in which the average prevalence adjusted bias adjusted kappa (PABAK) values per feature showed 'substantial' to 'nearly perfect' agreement (range 0.77 – 0.88, observed agreement 89% - 94%) [27]. Appendix 2 shows the definitions of change, which we applied to the present study [27]. For the present study, the subregional change scores (1 for incidence/progression, -1 for improvement and 0 for no change) were summed over

the different MOAKS subregions into an overall score per feature. The summed change scores per feature were dichotomized into progression versus no progression (change score ≥ 1 = progression, change score < 1 = no progression) for the tibiofemoral (TF) and patellofemoral (PF) joint separately. In addition, effusion-synovitis was scored 0-3 according to the distension of the joint capsule as 1 = small, 2 = moderate and 3 = large. Hoffa-synovitis is scored 0-3 according to the amount of hyperintensity signal in Hoffa's fat pad as 1 = mild, 2 = moderate, 3 = severe. To create a sum of the amount of synovitis, we added the score of the two measures, creating a 0-6 score. Change in synovitis was dichotomized into progression versus no progression (change score ≥ 1 = progression, change score < 1 = no progression). In accordance with the definitions for longitudinal evaluation of MOAKS [27], the incidence of a MOAKS feature in the present study was also defined as 'progression'.

Outcome measures

The primary outcome measure was defined as the progression over 2.5 years of the following OA MRI features: BMLs, cartilage defects, osteophytes, meniscal abnormalities, meniscal extrusion and synovitis (effusion- and Hoffa-synovitis combined). BMLs, cartilage defects and osteophytes were assessed in the TF and PF joint separately. Meniscal abnormalities and meniscal extrusion were assessed for the medial and lateral meniscus together. The secondary outcome measure was defined as the progression over 2.5 years of BMLs, cartilage defects and osteophytes in the medial and lateral TF joint separately, the progression of meniscal abnormalities and meniscal extrusions for the medial and lateral meniscus separately and of effusion-synovitis and Hoffa-synovitis separately.

Statistical analysis

Participants with available body weight data and available MRI of one or both knees at baseline and 2.5 years were included. The analyses were performed on knee level. Descriptive data were presented as mean ± standard deviation (SD), as median (interquartile range (IQR)) for a non-normal distribution, or as frequencies. First, unadjusted associations between the weight change subgroups and the primary outcome were analyzed using generalized estimating equations (GEE) for a binomial outcome, which takes into account the correlation of measurement between two knees within one subject. The subgroup with stable weight was defined as reference group. Next, the associations were adjusted for baseline BMI, injury and mild knee symptoms, covariates which are likely to affect both weight change and MRI feature progression. Preliminary analyses of these covariates were conducted to ensure no violation of the assumption of multicollinearity. Tolerance values were > 0.95, indicating no multicollinearity. In addition, for each MRI feature outcome, adjustment was made for the presence of that MRI feature at baseline. Also, the analyses were adjusted for K&L classification (0 vs. ≥ 1) and performed irrespective of the original trial interventions of the PROOF study and therefore adjusted for the randomization groups. Results from the GEE analyses

were presented in odds ratios (ORs) with 95% confidence intervals (CI). As an explorative analysis, we evaluated the progression rates within the three weight change subgroups for the medial and lateral TF joint, the medial and lateral meniscus and for effusion- and Hoffa-synovitis separately. Analyses were performed with SPSS 21.0 (Chicago, IL). P values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

Of 407 women, 60 (15%) were not available for current analyses. The main reason was no further time available or no interest in the study (45 women, 75%). Other reasons (25%) were claustrophobia (3 women), unattainability (9 women) and insufficient MRI quality (1 woman). Two persons deceased during follow-up. Additionally, 7 unilateral knees were excluded for analysis due to a recent severe knee trauma (n = 2) or the inability or unwillingness to continue MRI scanning of the second knee (n = 5). This resulted in the analysis of 687 knees of 347 women. Comparison of the baseline characteristics between included and non-included knees showed a statistically significantly higher prevalence of injury (94/687 (14%) vs. 7/127 (6%), p = 0.01), PF BMLs (341/687 (50%) vs 36/127 (28%), p = 0.03) and PF cartilage defects (408/687 (59%) vs 46/127 (36%), p = 0.04) in the included knees. Table 1 shows the distribution and medians of baseline characteristics for the three weight change subgroups.

Table 1. Baseline characteristics of the study participants.

	All	Stable weight	Weight gain	Weight loss
N - subjects	347	260	43	44
Age (years, IQR)	55.2 (5.5)	55.3 (5.8)	55.1 (4.9)	55.0 (5.0)
BMI (kg/m^2, IQR)	31.2 (5.3)	31.1 (5.1)	31.0 (4.2)	33.1 (6.6)
BMI at age 40 years (kg/m^2, IQR)	26.6 (4.6)	26.4 (4.2)	28.1 (5.2)	26.3 (5.3)
DEP control + placebo (%)	87 (25)	62 (24)	15 (35)	10 (23)
DEP control + glucosamine (%)	82 (24)	59 (23)	15 (35)	8 (18)
DEP intervention + placebo (%)	87 (25)	66 (25)	7 (16)	14 (32)
DEP intervention + glucosamine (%)	91 (26)	73 (28)	6 (14)	12 (27)
N - knees	687	514	85	88
K&L grade 0 (%)	340 (49)	259 (50)	39 (46)	42 (48)
K&L grade 1 (%)	300 (44)	229 (45)	31 (36)	40 (45)
K&L grade 2 (%)	40 (6)	21 (4)	15 (18)	4 (5)
K&L grade 3 (%)	3 (0.4)	3 (1)	0 (0)	0 (0)
Mild knee symptoms* (%)	215 (31)	152 (30)	28 (33)	35 (40)
History of knee injury (%)	94 (14)	63 (12)	15 (18)	16 (18)
Varus alignment (%)	267 (39)	189 (37)	42 (49)	36 (41)

Table 1. Baseline characteristics of the study participants. (continued)

	All	Stable weight	Weight gain	Weight loss
Bone marrow lesions and cysts				
TFJ (%)	227 (33)	170 (33)	24 (28)	33 (38)
Medial (%)	165 (24)	122 (24)	18 (21)	25 (28)
Lateral (%)	87 (13)	67 (13)	7 (8)	13 (15)
PFJ (%)	341 (50)	258 (50)	44 (52)	39 (44)
Cartilage defects				
TFJ (%)	282 (41)	218 (42)	26 (31)	38 (43)
Medial (%)	222 (32)	168 (33)	25 (29)	29 (33)
Lateral (%)	116 (17)	92 (18)	8 (9)	16 (18)
PFJ (%)	408 (59)	312 (61)	54 (64)	42 (48)
Osteophytes				
TFJ (%)	128 (19)	96 (19)	18 (21)	14 (16)
Medial (%)	108 (16)	80 (16)	17 (20)	11 (13)
Lateral (%)	61 (9)	46 (9)	8 (9)	7 (8)
PFJ (%)	89 (13)	63 (12)	8 (9)	18 (20)
Meniscal pathologies (medial and/or lateral) (%)	452 (66)	346 (67)	55 (65)	51 (58)
Medial (%)	405 (59)	315 (61)	49 (58)	41 (47)
Lateral (%)	165 (24)	126 (25)	17 (20)	22 (25)
Meniscal extrusions (medial and/or lateral) (%)	359 (52)	268 (52)	44 (52)	47 (53)
Medial (%)	353 (51)	266 (52)	42 (49)	45 (51)
Lateral (%)	43 (6)	28 (5)	8 (9)	7 (8)
Synovitis (Effusion and/or Hoffa)	101 (15)	74 (14)	14 (16)	13 (15)
Effusion-synovitis	85 (12)	59 (11)	13 (15)	13 (15)
Hoffa-synovitis	19 (3)	18 (4)	1 (1)	0 (0)

'Stable weight' = subgroup of relatively unchanged weight participants (0.1 ± 4.0 kg); 'Weight gain'= subgroup of subjects who steadily gained weight (8.6 ± 4.0 kg); 'Weight loss'= subgroup of subjects who steadily lost weight (9.0 ± 7.2 kg). IQR = interquartile range; BMI = body mass index; DEP = Diet and Exercise Program; K&L = Kellgren and Lawrence classification; TFJ = tibiofemoral joint; PFJ = patellofemoral joint; *Mild knee symptoms defined as having any knee pain in the last 12 months.

Effect of weight change subgroups on progression of semi-quantitative OA MRI features

Progression rates of the OA MRI features for the TF and PF joint, meniscus and of synovitis with corresponding ORs for the weight change subgroups are presented in table 2. Progression ranged from 7% in the stable weight subgroup for PF osteophytes and synovitis, to 31% in the weight gain subgroup for PF cartilage defects. There was a statistically significant increase in synovitis progression in the weight gain (18%) versus the stable weight subgroup (7%) (adjusted(a)OR 2.88; 95% CI 1.39 – 5.94). The odds for progression of PF BMLs and PF cartilage defects increased with 62% (aOR1.62; 95% CI 0.92–2.84) and 53% (aOR1.53; 95% CI 0.92–2.56) respectively, in the weight gain versus the stable weight subgroup.

Table 2. Progression of OA MRI features over 2.5 years for the weight change subgroups. Subjects with stable weight were used as reference.

	Weight subgroup	N (%)	OR	95% CI	aOR*	95% CI
Bone marrow lesions and cysts						
TFJ	Stable (n = 514)	70 (14)	1	Reference	1	Reference
	Gain (n = 85)	11 (13)	0.94	0.50 – 1.80	0.92	0.46 – 1.85
	Loss (n = 88)	13 (15)	1.11	0.59 – 2.12	0.96	0.47 – 1.97
PFJ	Stable	108 (21)	1	Reference	1	Reference
	Gain	25 (29)	1.59	0.89 – 2.84	1.62	0.92 – 2.84
	Loss	18 (20)	1.00	0.54 – 1.84	1.18	0.63 – 2.18
Cartilage defects						
TFJ	Stable	67 (13)	1	Reference	1	Reference
	Gain	10 (12)	0.89	0.45 – 1.74	0.87	0.44 – 1.73
	Loss	10 (11)	0.90	0.45 - 1.80	0.73	0.36 – 1.47
PFJ	Stable	117 (23)	1	Reference	1	Reference
	Gain	26 (31)	1.48	0.89 – 2.47	1.53	0.92 – 2.56
	Loss	15 (17)	0.73	0.43 – 1.25	0.73	0.41 – 1.29
Osteophytes						
TFJ	Stable	66 (13)	1	Reference	1	Reference
	Gain	13 (15)	1.22	0.64 – 2.36	1.07	0.54 – 2.11
	Loss	15 (17)	1.41	0.64 – 3.11	1.30	0.56 – 3.02
PFJ	Stable	34 (7)	1	Reference	1	Reference
	Gain	9 (11)	1.65	0.68 – 4.02	1.46	0.58 – 3.69
	Loss	10 (11)	1.81	0.78 – 4.20	1.14	0.43 – 3.02
Meniscal abnormalities						
Medial and/or lateral	Stable	142 (28)	1	Reference	1	Reference
	Gain	25 (29)	1.08	0.61 – 1.89	1.05	0.59 – 1.85
	Loss	25 (28)	1.09	0.61 – 1.97	1.00	0.54 – 1.86
Meniscal extrusions						
Medial and/or lateral	Stable	87 (17)	1	Reference	1	Reference
	Gain	16 (19)	1.13	0.61 – 2.11	1.04	0.55 – 1.98
	Loss	11 (13)	0.72	0.36 – 1.44	0.79	0.38 – 1.61
Synovitis (Effusion/Hoffa)	Stable	36 (7)	1	Reference	1	Reference
	Gain	15 (18)	**2.84**	**1.39 – 5.82**	**2.88**	**1.39 – 5.94**
	Loss	8 (9)	1.36	0.58 – 3.21	1.31	0.52 – 3.31

For definitions of stable weight, weight gain and weight loss, see table 1. TFJ = tibiofemoral joint; PFJ = patellofemoral joint; N = number of knees with progression; aOR = adjusted odds ratio. *Adjustments are made for baseline body mass index (kg/m2), mild knee symptoms, injury, Kellgren & Lawrence score (0 vs. ≥ 1), presence of MRI feature at baseline and randomized groups of the PROOF study. Bold indicates p-value < 0.05.

Explorative analysis

Table 3 presents the progression rates for the weight change subgroups in the medial and lateral TF joint, in the medial and lateral meniscus and for effusion- and Hoffa-synovitis. Progression rates ranged from 1% for Hoffa-synovitis to 21% for medial meniscal pathologies. Overall, progression rates were highest in the medial TF joint and medial meniscus and higher for effusion-synovitis than for Hoffa-synovitis. Since absolute progression numbers

per weight change subgroup were low, no further analysis was performed to assess the effect of weight change on progression of the medial and lateral features and separate synovitis features.

Table 3. Progression of OA MRI features for the three weight change subgroups in the medial and lateral tibiofemoral joint (TFJ),the medial and lateral meniscus and for effusion- and Hoffa-synovitis over 2.5 years.

	All	Stable weight	Weight gain	Weight loss
N - knees	687	514	85	88
Progression BMLs				
Medial TFJ (%)	63 (9)	46 (9)	7 (8)	10 (11)
Lateral TFJ (%)	42 (6)	31 (6)	5 (6)	6 (7)
Progression cartilage defects				
Medial TFJ (%)	59 (9)	47 (9)	7 (8)	5 (6)
Lateral TFJ (%)	41 (6)	30 (6)	5 (6)	6 (7)
Progression osteophytes				
Medial TFJ (%)	79 (11)	56 (11)	11 (13)	12 (14)
Lateral TFJ (%)	38 (6)	26 (5)	5 (6)	7 (8)
Progression meniscal pathologies				
Medial (%)	146 (21)	106 (21)	22 (26)	18 (20)
Lateral (%)	77 (11)	62 (12)	5 (6)	10 (11)
Progression meniscal extrusions				
Medial (%)	99 (14)	75 (15)	16 (19)	8 (9)
Lateral (%)	22 (3)	15 (3)	3 (4)	4 (5)
Progression synovitis				
Effusion-synovitis	55 (8)	33 (6)	14 (16)	8 (9)
Hoffa-synovitis	6 (1)	5 (1)	1 (1)	0 (0)

For definitions of stable weight, weight gain and weight loss, see table 1. BMLs = bone marrow lesions.

DISCUSSION

Summary

Three weight change subgroups within a high-risk group of middle-aged overweight and obese women without clinical knee OA at baseline were analyzed over 2.5 years for the effects on the progression of different OA structural features on MRI. Progression of synovitis was increased by more than 2.5 times in the weight gain subgroup compared to the stable weight subgroup. Although not statistically significant, large effect sizes were also found for the difference in progression of PF BMLs and PF cartilage defects between the weight gain and stable weight subgroup.

Context and comparison with existing literature

The women in the present study were free of clinical knee OA and the fast majority (93%) was without radiographic knee OA. Nevertheless, there was a substantial amount of OA MRI features present at baseline. High prevalence of structural features are also seen in other studies that evaluated high-risk populations [28-31]. Likewise, in our study, a high prevalence of BMLs, cartilage defects (41% in TF and 59% in PF) and meniscal lesions (66% abnormalities and 52% extrusions) was found, which might be due to the overweight and obese participants (median BMI 31.2kg/m^2). The association between BMI and prevalence and severity of cartilage and meniscal lesions in subjects without radiographic knee OA has previously been shown by data of the Osteoarthritis Initiative (OAI) [14, 32, 33]. The prevalence and progression rates of cartilage defects and BMLs in the present study were the highest in the PF joint. Although the TF joint is the joint most studied in OA research, the importance of the PF joint has already been shown in the early '90s [34] and recently strengthened within the Rotterdam study [35], the Framingham Osteoarthritis study [36] and the Cohort Hip and Cohort Knee study [37]. When assessing the medial and lateral compartment separately, more progression was found in the medial compartment. This is likely attributable to differences in load distribution; the highest compressive loads during daily activity are transmitted to the medial compartments of the knee [38].

In the present study we found a statistically significant increased progression of synovitis in the weight gain subgroup compared to the women with stable weight. Although the synovitis score was based on effusion-synovitis and on Hoffa-synovitis, results are mainly due to effusion-synovitis. Obesity as a risk factor is considered to induce a local and systemic low-grade inflammation [39]. The increased progression of synovitis in the weight gain subgroup seems to support this evidence. The larger increase in synovitis in the weight gain subgroup is an important finding, since synovitis is seen as a characteristic feature in OA onset [40]. In addition synovitis may even be an independent cause of radiographic OA onset and structural progression[41]. Although counterintuitive, we did not find a statistically significant effect of weight loss on the progression of synovitis. The stable weight subgroup was intuitively chosen as reference group. However, also when using the weight gain subgroup as a reference, we did not find statistically significant effects between the weight loss and weight gain subgroup (aOR 0.49, 95% CI 0.16 – 1.47). Future (preventive) studies among high-risk subjects are necessary to evaluate our findings.

With the use of LCGA, we were able to objectively classify individuals into subgroups based on individual response patterns over time since LCGA is able to use all the available information about inter-individual differences in change over time [23]. The model with the three latent classes was the model with the best fit and with the best usefulness of the latent classes. This model resulted in a large stable weight subgroup and two smaller weight change

subgroups. However, other models with more or less latent classes did not fit to the data or had too small subgroups, making them not useful for statistical analyses [20].

The progression rates of PF BMLs and PF cartilage defects were not statistically different between the weight gain and stable weight subgroup, although there was an almost 1.4-fold increase in the weight gain subgroup (29% for PF BMLs and 31% for PF cartilage defects) compared to the stable weight subgroup (21% and 23% respectively). Additionally, the progression of PF cartilage defects and meniscal extrusions was about 1.3-fold decreased in the weight loss subgroup (17% for PF cartilage defects and 13% for meniscal extrusions) compared to the stable weight subgroup (23% and 17% respectively). When obtaining larger subgroups by combining the stable weight and weight gain subgroup (n = 599 knees), differences in progression rates with the weight loss subgroup (n = 88 knees) were not statistically significant (data not shown). However, the differences might be clinically relevant and suggest that with larger subgroups of 'gainers' and 'losers' and with a longer follow-up, statistically significant detrimental and preventive effects might be found. In addition, when using the weight gain subgroup as reference, we did find a statistically significant difference for PF cartilage defects, with a 52% decrease in the odds of progression of PF cartilage defects in the weight loss group versus the weight gain group (aOR 0.48, 95% CI 0.24 – 0.97, p-value 0.04). This supports the study from Gersing et al in data from the OAI, that also found less progression of patellar cartilage changes in participants with weight loss [16].

The selection of women in the present study were overweight or obese for many years, as demonstrated by the self-reported weight data at age 40. Since the weight loss subgroup had a median BMI of 26.3 (IQR 5.3)kg/m^2 at age 40, high weight exposure time was at least ± 15 years. The effect of prolonged exposure to high BMI on development of knee OA has been previously described by Wills et al [42] and recently by Singer et al [43]. Although we found a mean weight loss of 9.0 ± 7.2 kg in the weight loss subgroup, a more extreme weight loss might be necessary to minimize the articular damage in those women with prolonged high weight exposure. On the other hand, it might be possible that long exposure to high BMI has hindered weight loss to result in more statistically and clinically significant changes in structural OA features, besides the small effect found for PF cartilage defects. In that case, it emphasizes the importance of weight control throughout entire life and suggests that the optimal timing for a more effective primary prevention intervention, aimed to reduce weight or to prevent weight gain, should start long before the age of 50 years.

Strengths and limitations

This study assessed weight changes on multiple OA MRI features in women without clinical knee OA. It is the first study in OA research that used LCGA to obtain distinct and most objectively acquired subgroups of weight change in relation to structural progression.

Probability of correct group allocation was ≥ 88%, which suggests that classification of the majority of the participants was correct [20].

This study has also some limitations. As mentioned previously, the small samples in the subgroups reduce statistical power. Also, the observation period may need to be longer. Further, MOAKS may not be sensitive enough to measure changes over 2.5 years in a relatively healthy and young cohort as the PROOF study. The original MOAKS scoring system has been developed for the assessment of disease status and the definition for change over time was not described in the original paper. We applied a recently proposed definition for the longitudinal change of the different MOAKS features, but it has not been validated yet against clinical and other structural outcomes [27]. Certain grades of the structural features of MOAKS reflect a wide range of severity. For instance, grade 2 for size and thickness of cartilage loss is defined as 10-75% loss of the subregion [26]. As a result, within-grade progression may remain unnoticed when using the proposed progression definitions of MOAKS features. For future studies, it might be interesting to use also quantitative compositional MRI techniques, which may be more sensitive.

Furthermore, our data showed that the prevalence of injury, PF BMLs and PF cartilage defects was higher in the knees included in the analyses compared to those excluded due to missing data. Less healthy knees might react differently to weight changes, but since we adjusted for these three factors, impact on the results will be limited.

Conclusion and implications

A high prevalence of OA MRI features was found in a high-risk group of middle-aged women, without clinical knee OA at baseline. With LCGA, objective weight change subgroups over 2.5 years were obtained. A more than 2.5-fold increase in progression of synovitis was found in women with weight gain compared to those with stable weight. Although not statistically significant, large effect sizes were also found for the difference in progression of PF BMLs and cartilage defects between the weight gain and stable weight subgroups. Since knee OA is responsible for a major burden of disease, effective preventive strategies and treatments are of utmost importance [6]. Whether weight loss, or at least the prevention of weight gain, is able to prevent (progression of) structural knee OA, remains a key topic for further research. The information provided by this study can be used well to design future confirmatory studies, whereby lessons can be learnt with regards to observation period, sample size and MRI definitions for change.

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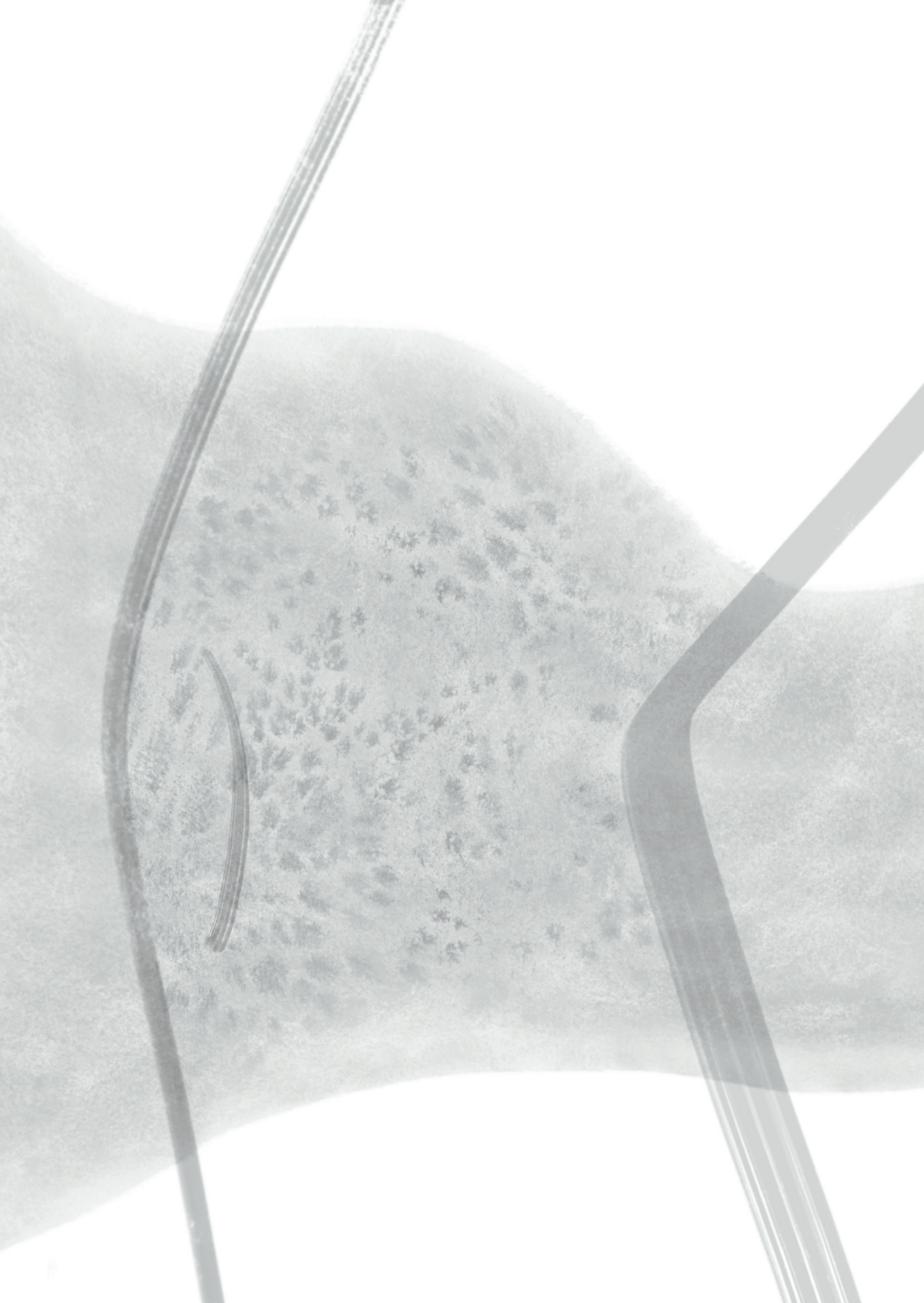

Chapter 4

Exploring body weight change over time on the risk of middle-age knee osteoarthritis on magnetic resonance imaging

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Submitted

ABSTRACT

Objective

Exploring the effect of body weight over time on midlife knee osteoarthritis (OA) prevalence on Magnetic Resonance Imaging (MRI).

Design

The population consisted of 375 overweight and obese women, aged 50 to 60 years, without clinical knee OA (PROOF study; ISRCTN 42823086). Current weight status was classified in overweight (27≤BMI<30kg/m^2) and obesity (BMI≥30kg/m^2). Retrospectively, BMI at 40 years was classified into normal weight (BMI<25kg/m^2), overweight (25≤BMI<30kg/m^2) and obesity (BMI≥30kg/m2). Baseline MRIs were scored using the MRI Osteoarthritis Knee Score to define MRI OA. Weight status groups at both time-points were compared for MRI OA prevalence.

Results

127 women (34%) had current overweight and 248 (66%) obesity. Mean age was 55.7±3.2 years. At 40 years, 20% had normal weight, 55% overweight and 25% obesity. MRI OA prevalence was 23% among women currently overweight and 35% among women currently obese. 23% increase in MRI OA in women who changed from normal weight at age 40 to current obesity was found. Women obese at both time-points demonstrated a 44% increase.

Conclusions

MRI OA prevalence was substantial in middle-aged overweight and obese women without clinical knee OA. Higher body weight at 40 years resulted in higher MRI OA prevalence ±16 years later.

INTRODUCTION

Being overweight or obese results in an increased risk for knee osteoarthritis (OA) development[1, 2]. In fact, a high body mass index (BMI) is the most important modifiable risk factor for knee OA[1, 3, 4]. A recent systematic review by Daugaard et al. suggested a beneficial effect of weight loss on cartilage composition in early knee OA[5]. The only preventive trial in OA research available is the PROOF study (ISRCTN 42823086)[6]. This study used a high risk group of middle-aged (50-60 years) overweight (BMI≥27kg/m^2) women to test the preventive effects of a lifestyle intervention, aimed to reduce body weight, and oral glucosamine sulphate on future knee OA development. Despite the fact that these women were free of clinical knee OA and 94% of the knees had Kellgren and Lawrence (KL)[7] grade <2 ('no radiographic knee OA'), magnetic resonance imaging (MRI) showed a substantial prevalence of OA features[8]. These are found to be associated with incident frequent knee complaints and seen as early signs of OA[9]. The PROOF study has shown that an increase of body weight (8.6±4.0kg) over 30 months follow-up, increased the progression of inflammation markers on MRI more than 2.5 times compared to stable weight[10]. No other significant effects were found on other OA features. Moreover, neither a decrease of body weight (9.0±7.2 kg) affected the 30 months progression on MRI[10]. The high baseline prevalence of OA features gives rise to the question what the impact is of weight status earlier in life. Since the population reported a mean BMI of 27.4±4.2kg/m^2 around 40 years, ±16 years before the start of the trial, it was suggested that the timing for a preventive weight loss strategy had possibly already passed[10].

The present study aimed to further explore the effects of differences in body weight in the ±16 years prior to inclusion into the PROOF study on the prevalence of baseline MRI knee OA. For clinical purposes, the current study focuses not on single OA features but on MRI OA according to the proposed multi-structural definition by Hunter et al[11].

METHOD

Study population

For the PROOF study (ISRCTN 42823086)[6], all registered women aged 50-60 years at 50 general practitioners in the area of Rotterdam, the Netherlands, were invited. Those interested to participate and with a self-reported BMI≥27kg/m^2, were screened by phone. Inclusion criteria were: no knee OA according to the clinical criteria of the American College of Rheumatology[12], mastering the Dutch language, free of major co-morbidities, free of inflammatory rheumatic diseases, not under treatment of a physical therapist or GP for knee complaints, not using walking aids, not using oral glucosamine for the last 6 months and free of contraindications for MRI. The Medical Ethics committee of Erasmus MC University

Medical Center Rotterdam approved the study and all participants gave written informed consent prior to baseline measurements.

Data collection

At baseline, participants filled-in a questionnaire for demographic data, knee complaints ("did you experience knee pain in the past 12 months?"), current body weight and body weight at 40 years. Body weight and height in standing position without shoes were measured at baseline examination by a research assistant and BMI (kg/m^2) was calculated. A posterior-anterior semi-flexed radiograph of both knees was obtained, using the semi-flexed metatarsophalangeal view[13], and scored using KL criteria[7]. An MRI of both knees was made at baseline on a 1.5 Tesla scanner (Philips or Siemens). The MRI protocols included coronal and sagittal non-fat suppressed proton density weighted sequences, a coronal T2 weighted sequence, an axial dual spin-echo sequence and a sagittal 3D water selective (WATS) sequence with fat saturation (Appendix 1). MRIs were scored by trained and blinded researchers using the semi-quantitative MRI Osteoarthritis Knee Score[8,14]. Next, OA was defined in the tibiofemoral and patellofemoral compartments of all knees using the MRI OA definition published by Hunter et al. (Appendix 2)[11]. Per individual, MRI OA was defined as MRI OA in ≥1 out of 4 compartments (2 compartments per knee).

Body weight assessment

Current body height combined with self-reported body weight at 40 years, was used to calculate BMI at 40 years. To adjust for self-reported body weight, body weight at 40 years was adjusted for over- or underestimation of the own body weight at baseline (difference between self-reported and objectively measured). BMI at 40 years was classified into normal (BMI<25kg/m^2), overweight (BMI≥25and<30kg/m^2) and obesity (BMI≥30kg/m^2). Due to the inclusion criteria, current BMI was classified into overweight (BMI≥27 and <30kg/m^2) and obesity (BMI≥30kg/m^2)[6]. Next, a descriptive analysis was performed. Combinations of BMI group at 40 years and current BMI group were compared for the prevalence of MRI OA, both uni- and bilateral, and for the amount of involved knee compartments. Women with available MRI, baseline and 40 years BMI, were selected for the present study.

RESULTS

Of the 407 women included in the PROOF study, 375 were available for the present analysis, with mean age 55.7±3.2 years. Current weight status was overweight for 127 (34%) and obese for 248 (66%) women. At 40 years, 20% had normal weight, 55% overweight and 25% obesity. Of those currently obese, 11% had normal weight, 52% overweight and 37% obesity at 40 years. Of those currently overweight, 39% had normal weight, 61% overweight and < 1% obesity at 40 years (Figure 1).

Prevalence of MRI OA was 23% (17% unilateral and 6% bilateral) among women currently overweight and 35% (23% unilateral and 11% bilateral) among women currently obese. Table 1 presents the baseline prevalence of MRI OA, the prevalence of unilateral and bilateral MRI OA and the percentage of women with 2 or more affected compartments for the different combinations of weight status groups. The lowest prevalence was found for women with current overweight and normal BMI at age 40. A 23% increase in MRI OA in women who changed from normal weight at age 40 to current obesity was found. Women who were obese at both time-points demonstrated an increase of 44%.

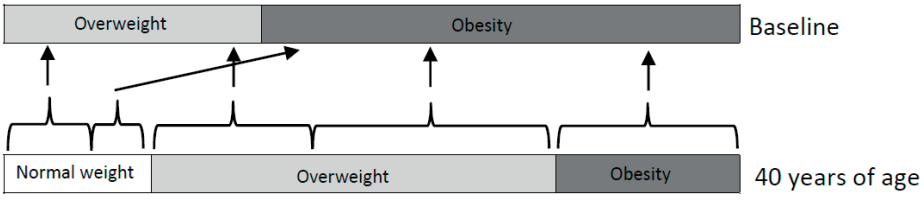

Figure 1. Current (baseline) and 40-years BMI groups and their course*^
*the one woman currently overweight that had obesity at 40 years was omitted for clarity reasons. ^The length of the different parts of the bars is scaled to the corresponding percentages.

Table 1. Prevalence of MRI OA and number of affected compartments for different weight status groups.

BMI group at 40 years	Current BMI group	N*	Prevalence of MRI OA overall (uni-/bi-lateral/2+ compartments)
Obesity	Obesity	92	44% (25% / 19% / 23%)
Overweight	Obesity	130	32% (25% / 7% / 9%)
Normal weight	Obesity	26	23% (15% / 8% / 12%)
Overweight	Overweight	77	27% (22% / 5% / 12%)
Normal weight	Overweight	49	16% (10% / 6% / 6%)

N = number of women; *the one women currently overweight that had obesity at 40 years was omitted for clarity.

DISCUSSION

This study described the effect of differences in body weight change on the prevalence of midlife MRI knee OA. In this population of middle-aged overweight and obese women without clinical knee OA, the prevalence of MRI knee OA was substantial. Women who changed from normal weight at age 40 to overweight after ±16 years had the lowest increase in MRI OA, while women who were obese at both time points showed the highest increase. Women in the highest 40-years BMI group showed the highest prevalence of MRI OA at baseline.

A remarkable difference between the groups with both obesity and the group currently obese and overweight at 40 years, is the prevalence of bilateral knee OA (19% vs. 7%) and the number of women with ≥2 affected knee compartments (23% vs. 9%). Since the likelihood for knee pain is associated with the severity and the amount of structural defects[15], subjects in the obesity group (currently and at 40 years) might be at greater risk for (future) knee pain than the difference in overall prevalence of MRI OA (44% vs. 32%) may suggests.

One could argue that a weight loss strategy around 40 years of age might be more effective regarding prevention of knee OA; compared to a 44% increase in MRI OA in those obese at 40 years, the women with overweight at 40 years demonstrated an increase of 30% and those with normal weight an increase of 18%. Thus, after ±16 years the prevalence of MRI OA was 2.4 times higher among obese women compared to those with normal weight at 40 years. While the prevalence of MRI OA was the lowest in those with normal weight at 40 years, the mean weight increase over ±16 years (17.0±7.3kg) was much higher in this group compared to those with overweight (11.9±7.6kg) or obesity (7.5±13.1kg) at age 40 years ($p < 0.001$). This confirms previous findings that exposure-time is the main way in which BMI influences the risk on knee OA and supports the importance of weight control throughout life as primary prevention[16]. Due to the lack of a current control group with normal weight and the lack of weight information at earlier time periods, inferences cannot be made about the optimal timing of a preventive weight loss strategy.

Another limitation of this study is the retrospective evaluation of weight at age 40, but records from previous medical visits were not available. Further, the MRI OA definition is based on a Delphi exercise and requires further assessment[11]. Up to now, this is the only definition currently available.

In conclusion, comparing groups of women with different body weight change over ±16 years, those with higher BMI at 40 years show higher MRI knee OA prevalence at middle-age. Body weight reduction starting at least around the age of 40 years might be more effective to prevent knee OA than it would be in the age range of 50 to 60 years, where radiographic and clinical knee OA usually develops.

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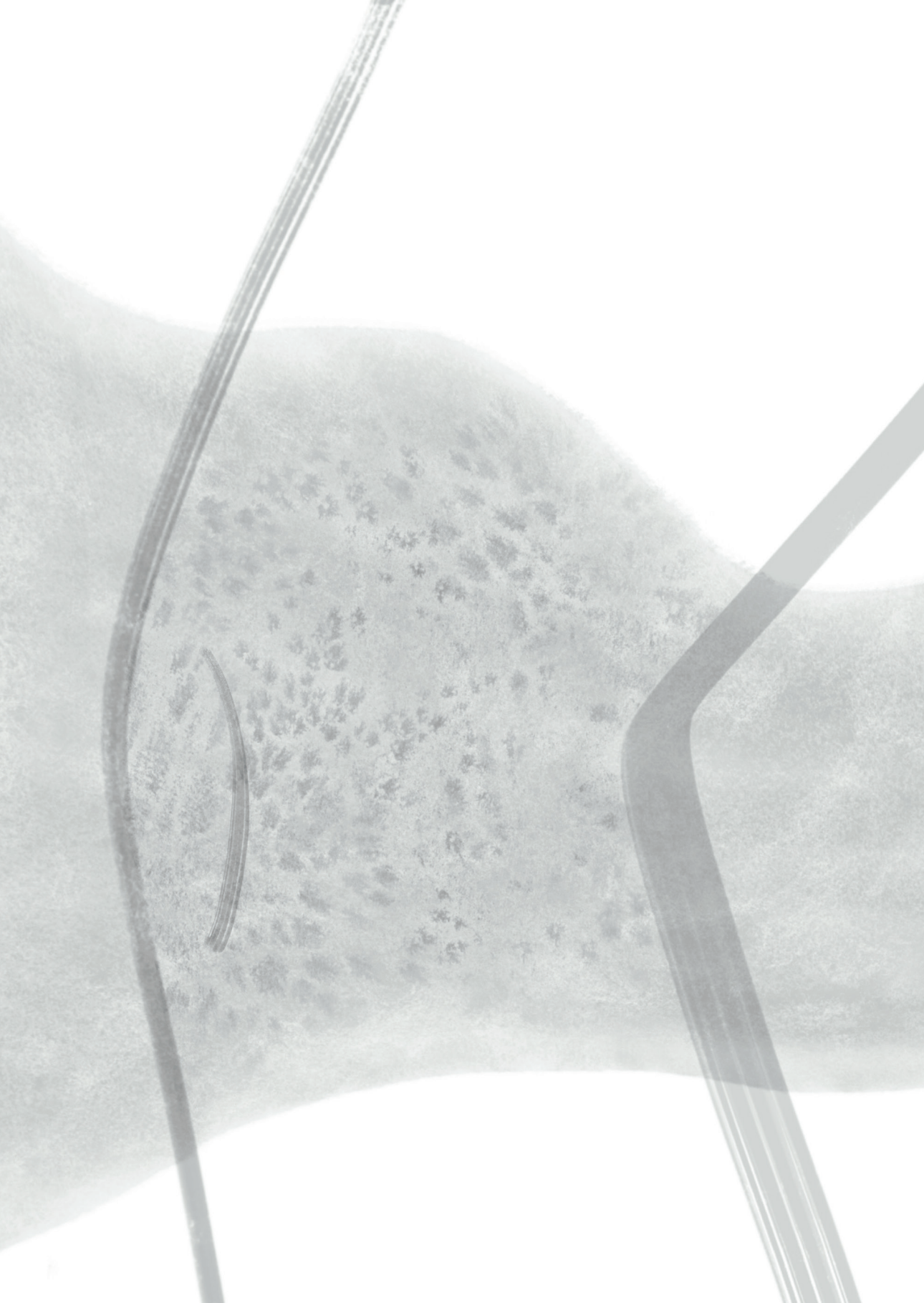

Chapter 5

Long-term effects of a lifestyle intervention and oral glucosamine sulphate in primary care on incident knee OA in overweight women

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ABSTRACT

Objectives

The present study was designed to evaluate the effect of a lifestyle intervention aimed to reduce body weight and of oral glucosamine sulphate on the incidence of knee osteoarthritis (OA) after 6-7 years in a population of middle-aged, overweight women, without knee OA at baseline.

Methods

The Prevention of knee Osteoarthritis in Overweight Females study, ISRCTN42823086, was a randomized controlled trial with a 2 x 2 factorial design. Four hundred and seven women aged 50-60 years with a BMI of ≥27 kg/m^2 and free of knee OA were randomized.

Results

Four hundred and seventy-seven knees from 245 participants were available after a mean follow-up time of 6.6 years. Nineteen per cent of all knees showed incident knee OA. Both interventions showed no significant preventive effect on incident knee OA. Despite the fact that per protocol analyses showed greater differences between both groups for the lifestyle intervention, significance was not reached. A significant effect of losing ≥5 kg or ≥5% of baseline weight in the first 12 months on the incidence of knee OA according to the primary outcome was found (odds ratio = 0.10; 95% CI: 0.02, 0.41).

Conclusion

No significant preventive effect on incident knee OA of either the lifestyle intervention or the glucosamine intervention was found. As a proof of concept, the preventive effect of moderate weight loss in 1 year on the incidence of clinical knee OA is demonstrated. This trial provides important insights for future studies on the prevention of knee OA, which are currently lacking.

INTRODUCTION

The association between obesity and knee OA has been extensively described in the literature[1]. The majority of these studies have focused on obesity as a risk factor or weight loss as a treatment for knee OA in individuals with obesity[1-4]. Considering the increasing body of evidence stating that obesity is an important risk factor for knee OA, the options of primary prevention by weight loss should be investigated[5]. As early as 1992, results from The Framingham Study suggested a preventive approach to knee OA by weight loss[6]. Thereafter, few trials were specifically designed to study the preventive effect of weight loss on knee OA, despite recommendations in literature to design preventive trials[6-9]. Recent results of trials investigating the effect of weight loss on intermediate outcomes, such as cartilage thickness or chronic pain[10, 11], support the hypothesis that weight loss can prevent the development of knee OA, as suggested by Felson et al.[6]. Recommendations made in the literature regarding the design of a trial to investigate the preventive effect of weight loss on knee OA often include the following: a randomized design, a high-risk population of overweight, middle-aged participants without knee OA, a long follow-up period and clinical and radiographic outcome measures[6-9, 12].

In addition, recommendations have been made to study the efficacy of pharmacological substances, such as glucosamine[13]. A large review found an overall significant beneficial effect of glucosamine on pain and function of the knee in participants with established knee OA[14]. However, the heterogeneity of the included studies was very high[14]. Literature suggests that in patients in an earlier stage of disease, larger effects could be found[15]. Furthermore, in some studies, glucosamine has been shown to modify disease progression, raising the question of whether it would be more effective as a preventive intervention rather than as a treatment[5, 15]. The above-mentioned review found the safety of glucosamine to be equal to placebo, making a trial to investigate the preventive effect of glucosamine on the development of knee OA feasible[14].

The objective of the present study was to evaluate the long-term effectiveness of a tailor-made weight-loss intervention, using diet and exercise, and of oral glucosamine on the incidence of knee OA in a high-risk population of overweight, middle-aged women without knee OA at baseline. Previously, short-term results of the trial were published, showing no significant preventive intervention effects on knee OA[16, 17]. It was hypothesized that prolongation of the follow-up time could possibly result in greater effects. The present study focuses on the long-term effectiveness 6-7 years after randomization.

METHODS

The Prevention of knee Osteoarthritis in Overweight Females Study, of which the present study is a part, was approved by the Medical Ethics Committee of Erasmus MC in 2005 (Pre-

vention of knee Osteoarthritis in Overweight Females, ISRCTN42823086). All participants provided informed consent according to the Declaration of Helsinki. The present manuscript was prepared according to the CONsolidated Standards of Reporting Trials (CONSORT) Statement guidelines[18]. A full description of the study protocol has been published elsewhere[17]. In short, in a 2 x 2 factorial design, the preventive effect of both a diet and exercise programme (DEP) and of oral glucosamine sulphate (OGS) on the incidence of knee OA was investigated. For the DEP, the study was open labelled, whereas the glucosamine intervention was double blind and placebo controlled. Inclusion took place between 2006 and 2009. Inclusion criteria were as follows: female gender; aged 50-60 years; BMI of ≥27 kg/m^2; free of clinical ACR criteria for knee OA[19]; no contraindications for MRI; no rheumatic diseases; not using a walking aid; not under treatment for knee complaints; mastering the Dutch language; and no use of oral glucosamine during the past 6 months. All women who were willing to participate and who met all inclusion criteria were invited for baseline measurements and randomization. For both interventions, participants were randomized 1:1 using block size 20 in block randomization.

Measurements

At baseline, body weight, body height, knee pain upon pressure at the joint margins, warmth and crepitation of both knees and Heberden's nodes in both hands were recorded. Also, semi-flexed posterior-anterior knee radiographs were taken according to the MTP protocol[20]. These measurements were repeated after 2.5 years of follow-up and after 6-7 years of follow-up. The radiographs were scored using Kellgren and Lawrence (K&L) criteria[21]. All radiographs were scored by a trained researcher, blinded for treatment assignment and clinical outcomes. Inter-observer variability was determined by a second blinded researcher, who scored a subset of 20% of the radiographs. Digitally, medial knee alignment was measured, and varus alignment was defined as an angle < 178°.

Participants filled out a questionnaire every 6 months for the first 2.5 years and one after 6-7 years, recording number of days with knee pain, physical activity, co-interventions and quality of life. Physical activity was measured using the validated Short Questionnaire to Assess Health-enhancing physical activity questionnaire[22, 23]. Quality of life was measured using the validated EQ-5D EuroQol questionnaire[24]. In addition, participants filled in questions on knee complaints, menopausal status, comorbidities and filled in the Knee injury and Osteoarthritis Outcome Score questionnaire at baseline, 12 months and 2.5 and 6-7 years[25]. Mild knee symptoms were defined as having any knee pain in the past 12 months.

Participants were visited at home every 6 months for the first 2.5 years to measure body weight, to check the questionnaire for unanswered questions and to replace the batch of study drugs with a new one. The retrieved batch was used for objective calculation of compliance.

Interventions

Both interventions are described in detail elsewhere[16, 17]. In short, participants randomized to the DEP were referred to a local dietitian, agreements were made on frequency of visits, and personal goals regarding nutritional patterns and physical activity were set, using motivational interviewing[26]. In addition, participants were invited to participate in a series of 20 weekly physical exercise classes. These 1-h classes were supervised by a physiotherapist, were offered near participants' homes and were conducted in small groups of 12-15 participants. The goal of these classes was to regain pleasure in physical exercise and to find activities suited for long-term continuation. A wide variety of low-impact sports were offered. Participants in the control group did not receive this intervention, but were free to take any actions to improve their health independently.

Participants randomized to OGS were prescribed 1500 mg of oral crystalline glucosamine sulphate per day for 2.5 years. Participants in the control group received placebo. All study drugs were provided by Rottapharm Madaus (Monza, Italy). There was no involvement of Rottapharm Madaus in study design, data collection or statistical analyses. All participants and research staff were blinded for allocation during these 2.5 years. After the intervention ended, observation of participants continued for 4 years.

Statistical analyses

The primary outcome measure for the present study was the incidence of knee OA after 6-7 years, according to the combined clinical and radiological ACR criteria[19]. The secondary outcome measure was the incidence of knee OA after 6-7 years, defined as K&L grade 2 or higher. Analyses were performed at the knee level. OA was considered an irreversible process. Therefore, all knees that met ACR criteria at 2.5 or 6-7 years of follow-up were considered positive for knee OA for the primary outcome. Given that initial screening of inclusion criteria was done by telephone, it was expected that there would be a proportion of participants that met ACR criteria or showed K&L grade ≥2 for one or two knees at baseline already. These knees were excluded from the analysis.

Intention-to-treat (ITT) analysis served as the primary analysis. The intervention effect on the primary and secondary outcome measures was tested using generalized estimating equations (GEE), because this method takes the correlation of both knees of one participant into account. Effects were reported as odds ratios (ORs) with 95% CIs. First, the associations between known prognostic variables and the outcome were tested with univariate GEE analyses. Age, K&L grade ≥1 vs 0, varus alignment, mild knee symptoms, BMI, a history of knee injury, Heberden's nodes and postmenopausal status were tested accordingly. Next, all variables with a P-value <0.2 were analysed using multivariate GEE analysis. All variables with a P-value <0.05 in the multivariate model were adjusted for in the analyses testing the intervention effects. This was done separately for the primary and secondary outcome

measures. Additionally, all analyses were adjusted for follow-up duration in months, because follow-up time was not equal for all participants, owing to the long period of recruitment (July 2006 to May 2009). Using the GEE model, interaction between both interventions was assessed. In the event of significant interaction, all four groups would be assessed separately.

For the predefined per protocol (PP) analyses, participants who were compliant to the intervention were compared with the participants who were randomized to the control group. Compliance to the DEP intervention was defined as having visited the dietitian at least six times and having attended at least seven physical activity classes. Regarding the OGS intervention, an objective compliance calculation of ≥75% was used, which was assessed using the retrieved batches of study drugs.

As an explanatory analysis, incident knee OA was compared between participants who lost 5 kg or 5% of their baseline weight at 1 year of follow-up and participants who did not meet this predefined goal in the first year of the study. This outcome served as the primary outcome of the weight-loss intervention and was chosen for its associations with improvement of cardiovascular risk factors[16]. We hypothesized that achieving this goal in the period of 1 year could possibly be an easily achievable goal to recommend to patients in primary care in the context of preventing knee OA. Adjusting for follow-up duration and confounding factors, as in the ITT and the PP analyses, GEE was used for this analysis.

To estimate the effect of the missing data, multiple imputation was performed, as recommended in the literature[27]. Fifty imputed data sets were used. The method was set to automated selection of linear regression or predictive mean matching, maximal iterations were set to 20, and a maximum of 150 parameters per variable was used. All variables used in the GEE, including the outcome variables, were imputed and used as predictors. Both baseline characteristics and follow-up data were used as auxiliary variables.

As a sensitivity analysis, a worst-case scenario was explored according to literature recommendations[28]. We hypothesized that no intervention effect at all would be the worst-case scenario. Therefore, in all participants with missing data, the outcome was imputed evenly distributed over the two groups that were compared, resulting in an equal incidence of knee OA in both groups in all participants with missing data. The incidence found in the completers' analysis was used to impute these variables. Missing values in covariates used in the GEE model were imputed by the average value of the completers. The results of the sensitivity analysis were used to check the plausibility of the results produced by the multiple imputation model.

All analyses were performed using IBM SPSS statistics version 21 (SPSS Inc., Chicago, IL, USA). In all analyses, a value of $P < 0.05$ was defined as statistically significant.

RESULTS

Four hundred and seven women were randomized after 50 general practitioners contacted 6691 women. A full description of the selection process is published elsewhere[17]. Figure 1 shows the selection process and participants lost to follow-up with reasons. At baseline, knee OA data were available for 405 participants with 810 knees (99.5%). After 2.5 years, there were 356 participants for whom knee OA data were available on 712 knees (87.5%). After 6.6 years, 260 participants supplied knee OA data on 508 knees (62.4%). At baseline, 32 knees (4.0%) met ACR criteria and were excluded from analyses concerning the primary outcome. In addition, 51 knees (6.3%) showed K&L grade ≥2 and were excluded from analyses concerning the secondary outcome. As a result, knee OA data were available on 477 knees (58.9%) for the primary outcome measure, and for the secondary outcome measure knee OA data on 452 knees (55.8%) were available. Attrition rates were similar between both randomized groups of the DEP intervention: 27% in the intervention group vs 31% in the control group. For the OGS intervention, attrition rates were higher in the placebo group: 35%, vs 18% in the intervention group. Participants who completed the long-term follow-up had a lower baseline BMI (32.0 vs 33.0 kg/m^2) and more Heberden's nodes (18 vs 15%). Baseline knee OA incidence figures were similar between participants who completed the follow-up time and those who did not: 4 vs 4% for the primary outcome and 5 vs 8% for the secondary outcome.

Table 1 shows baseline characteristics on the 508 knees from 260 participants that were available after complete follow-up. Mean follow-up time was 6.6 (0.7) years. There were no significant differences in baseline characteristics between both groups for both interventions. In the multivariate analysis, three baseline characteristics were associated with the primary outcome: BMI, K&L grade ≥1 vs 0; and mild knee symptoms. Regarding the secondary outcome, BMI, K&L grade ≥1 vs 0 and a history of knee injury were associated with the outcome in a multivariate model. Consequently, adjustment of these variables was performed in all analyses. Of the participants randomized to the DEP, 32% were compliant to that intervention. For the OGS intervention, 65% of all participants were considered compliant.

Intervention effects

After 6.6 years, the overall incidence of knee OA according to the primary outcome was 19%. No significant interaction between both interventions was found. ITT analysis showed no significant difference in knee OA between randomized groups, for both the DEP intervention (OR = 0.86, 95% CI: 0.47, 1.54) and the OGS intervention (OR = 1.58, 95% CI: 0.86, 2.89). Regarding the secondary outcome, knee OA incidence was 14%, also with no differences between both groups for both interventions.

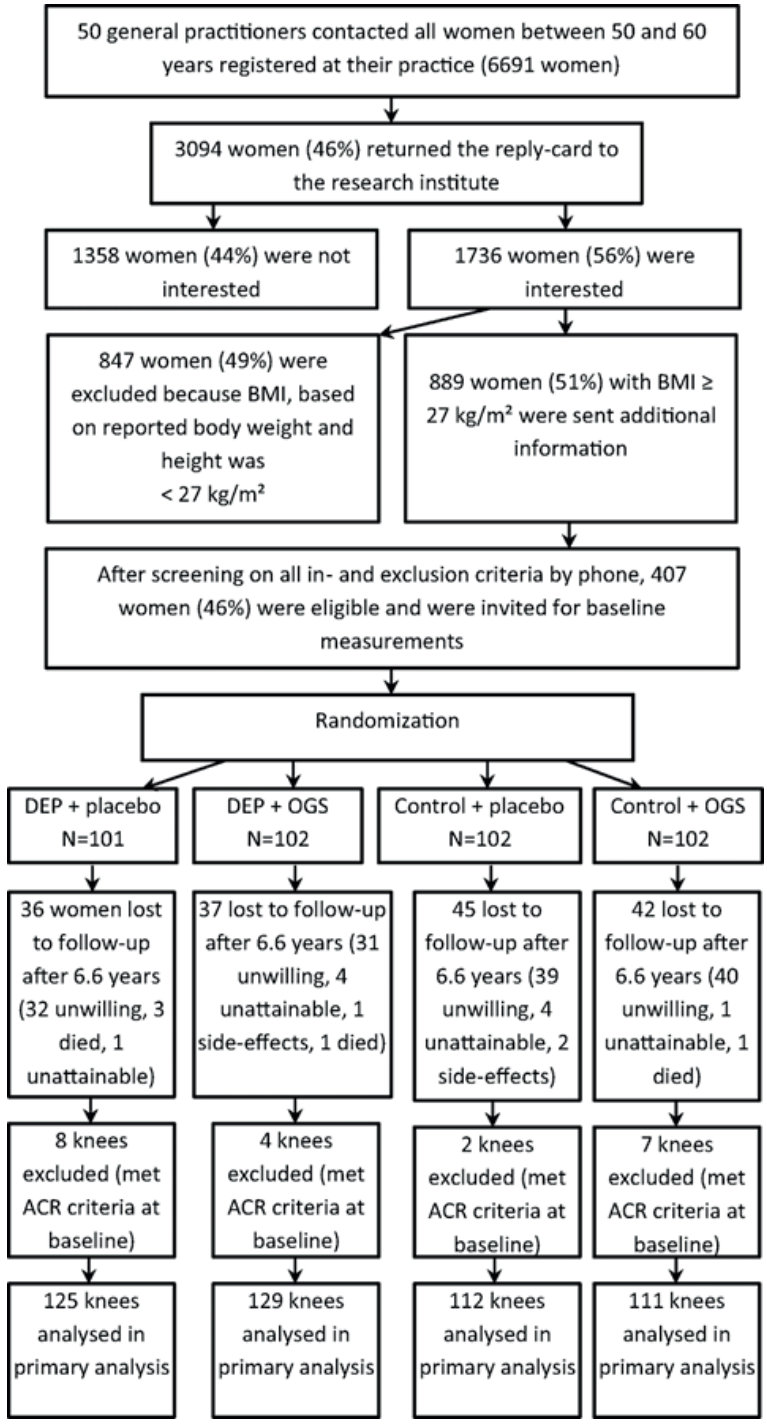

Figure 1. Flowchart of recruitment process.

Table 1. Means and distribution of prognostic variables.

	Diet and exercise program		Oral glucosamine sulphate	
	Control group	**Intervention group**	**Placebo**	**Glucosamine**
Baseline characteristics				
Subjects, n	122	138	130	130
Age, mean (S.D.), years	55.9 (3.2)	55.6 (3.2)	55.7 (3.2)	55.8 (3.1)
BMI, mean (S.D.), kg/m2	32.1 (4.1)	31.9 (3.9)	32.4 (4.2)	31.6 (3.6)
Postmenopausal status, %	73.7	65.4	70.4	68.3
Heberden's nodes, %	29.4	27.0	31.2	25.0
Knees, n	238	270	253	255
ACR[a], %	3.8	4.5	4.0	4.3
K&L grade, %				
Grade 0	49.6	48.5	49.4	48.6
Grade 1	47.0	44.8	44.6	47.1
Grade ≥2	3.4	6.7	6.0	4.3
Minimal JSW				
Medial, mean (S.D.), mm	4.8 (0.7)	4.7 (0.8)	4.7 (0.8)	4.8 (0.8)
Lateral, mean (S.D.), mm	6.2 (1.0)	6.2 (1.1)	6.1 (1.1)	6.2 (1.0)
Varus alignment, %	44.4	39.5	43.0	40.6
Mild symptomsb, %	32.4	32.3	32.8	31.9
History of knee injury, %	15.0	10.9	12.1	13.5

[a] Knee OA according to the ACR criteria.

[b] Mild symptoms defined as any pain in the concerned knee in the past 12 months. JSW: Joint Space Width; K&L: Kellgren and Lawrence.

Per protocol analysis showed greater intervention effects for the DEP than the ITT analysis, but they did not reach statistical significance, with ORs of 0.55 (95% CI: 0.23, 1.33) for the primary outcome and 0.39 (95% CI: 0.12, 1.29) for the secondary outcome. For the OGS intervention, effects in the PP analysis were not consistently greater. The intervention effect on the primary outcome did not change significantly, whereas the intervention effect on the secondary outcome changed in direction. ORs for the secondary outcome were 0.96 (95% CI: 0.48, 1.92) for the PP analysis and 1.26 (0.67, 2.39) for the ITT analysis. All incidence numbers and ORs obtained from ITT and PP analyses are presented in Table 2.

Exploratory analysis

Sixty-nine participants achieved the goal of losing 5 kg or 5% of their baseline body weight after 1 year of follow-up. These participants showed a lower incidence of knee OA after 6.6 years than participants who did not achieve this goal at 1 year of follow-up (7 vs 21%). Adjusted OR for the primary outcome was 0.10 (95% CI: 0.02, 0.41) and for the secondary outcome 0.28 (95% CI: 0.08, 0.94). Table 2 shows these ORs.

Table 2. Incidence figures and odds ratios on knee OA from intention-to-treat and per protocol analyses.

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted) [a] (95% CI)
Intention-to-treat analyses				
Diet and exercise program (n = 477: 254 vs 223)				
ACR criteria [b]	19	18	19	0.86 (0.47, 1.54)
K&L grades [c]	15	14	16	0.91 (0.48, 1.72)
Oral glucosamine sulphate (n = 477: 240 vs 237)				
ACR criteria	19	20	17	1.58 (0.86, 2.89)
K&L grades	15	15	14	1.26 (0.67, 2.39)
Per protocol analyses				
Diet and exercise program (n = 305: 82 vs 223)				
ACR criteria	18	13	19	0.55 (0.23, 1.33)
K&L grades	14	8	16	0.39 (0.12, 1.29)
Oral glucosamine sulphate (n = 413: 176 vs 237)				
ACR criteria	18	19	17	1.64 (0.86, 3.14)
K&L grades	13	12	14	0.96 (0.48, 1.92)
Exploratory analysis [d]				
Lost 5 kg or 5% in 1 year (n = 477: 69 vs 408)				
ACR criteria	19	7	21	**0.10 (0.02, 0.41)**
K&L grades	15	6	16	**0.28 (0.08, 0.94)**

Numbers are numbers of knees. Bold indicates p-value < 0.05.

[a] Generalized estimating equations adjusted for baseline differences and confounding factors.

[b] Knee OA according to the ACR criteria.

[c] Knee OA, defined as K&L grade ≥2.

[d] Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

Multiple imputation

Pooled ORs obtained from the imputed data sets showed no significant intervention effects. Incidence numbers were markedly higher than in the original data. In these analyses, both interventions showed greater effects in the PP analyses compared with the ITT analyses. The association between losing 5 kg or 5% baseline weight became less strong and non-significant. Table 3 shows all incidence numbers and ORs obtained from the multiple imputation data sets.

Sensitivity analysis

ORs obtained from the worst-case scenario were very similar to the completers' analysis (Table 4). Naturally, all effects decreased; all ORs moved closer to one. Regarding the DEP intervention, the OR for the primary outcome changed from 0.86 (0.47, 1.54) to 0.97 (0.65,

1.45) and OR for the secondary outcome changed from 0.91 (0.48, 1.72) to 0.95 (0.60, 1.49). Regarding the OGS intervention, OR for the primary outcome changed from 1.58 (0.86, 2.89) to 1.07 (0.71, 1.60) and OR for the secondary outcome changed from 1.26 (0.67, 2.39) to 1.03 (0.67, 1.60). None of the associations changed in direction, and none of the confidence intervals that included one in the completers' analysis became significantly different from one, or vice versa.

Table 3. Incidence figures and odds ratios after multiple imputation.

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted)[a]	95% CI
Intention-to-treat analyses					
Diet and exercise program					
ACR criteria[b]	31	29	33	0.84	0.49, 1.44
K&L grades[c]	28	26	31	0.83	0.45, 1.53
Oral glucosamine sulphate					
ACR criteria	31	32	30	1.07	0.63, 1.81
K&L grades	28	28	29	0.92	0.56, 1.52
Per protocol analyses					
Diet and exercise program					
ACR criteria	30	22	33	0.57	0.27, 1.22
K&L grades	27	18	31	0.47	0.19, 1.19
Oral glucosamine sulphate					
ACR criteria	28	25	30	0.81	0.44, 1.51
K&L grades	25	20	29	0.61	0.34, 1.10
Exploratory analysis[d]					
Lost 5 kg or 5% in 1 year					
ACR criteria	31	22	32	0.54	0.23, 1.31
K&L grades	28	20	30	0.56	0.22, 1.43

[a] Generalized estimating equations adjusted for baseline differences and confounding factors.

[b] Knee OA according to the ACR criteria.

[c] Knee OA, defined as K&L grade ≥2.

[d] Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

Table 4. Incidence figures and odds ratios after worst-case scenario.

	Incident knee OA, %	Incident knee OA intervention group, %	Incident knee OA control group, %	OR (adjusted)[a] (95% CI)
Intention-to-treat analyses				
Diet and exercise program				
ACR criteria[b]	19	19	19	0.97 (0.65, 1.45)
K&L grades[c]	14	14	14	0.95 (0.60, 1.49)
Oral glucosamine sulphate				
ACR criteria	19	20	18	1.07 (0.71, 1.60)
K&L grades	14	15	14	1.03 (0.67, 1.60)
Per protocol analyses				
Diet and exercise program				
ACR criteria	18	14	19	0.68 (0.35, 1.33)
K&L grades	13	10	14	0.55 (0.24, 1.27)
Oral glucosamine sulphate				
ACR criteria	19	21	18	1.19 (0.74, 1.89)
K&L grades	13	12	13	0.99 (0.57, 1.69)
Exploratory analysis[d]				
Lost 5 kg or 5% in 1 year				
ACR criteria	19	11	20	0.45 (0.23, 0.90)
K&L grades	14	8	15	0.43 (0.20, 0.93)

[a] Generalized estimating equations adjusted for baseline differences and confounding factors.
[b] Knee OA according to the ACR criteria.
[c] Knee OA, defined as K&L grade ≥2.
[d] Comparing the incidence of knee OA between participants who lost 5 kg or 5% of their baseline weight in the first year of follow-up *vs* all participants who did not lose this amount of body weight in the first year of follow-up. K&L: Kellgren and Lawrence; OR, odds ratio.

DISCUSSION

The present study presents the long-term results of the first preventive randomized controlled trial in knee OA. ITT analyses showed no significant effects of either the DEP or the glucosamine sulphate on the long-term incidence of knee OA according to ACR criteria. Also, no effects were found on the incidence of knee OA, defined as K&L grade ≥2. Per protocol analyses showed greater effects for the DEP, but significance was not reached. As a proof of concept, the present study demonstrated the preventive effect of losing 5 kg or 5% of baseline body weight in the first year of the study on the incidence of knee OA after 6.6 years.

The primary analysis of the present study is a completers' analysis. As a result, under- or over-estimation of the intervention effect could have occurred. Weight-loss studies often suffer

from high dropout rates, resulting in a wide variety of methods used to handle missing data[27]. Multiple imputation was recommended in the literature as the best method for handling missing data in obesity randomized controlled trials[27]. In the present study, however, multiple imputation led to markedly higher incidence numbers of knee OA. In the original data, the incidence was 19 and 14% for the primary and secondary outcome, respectively. In the multiple imputation data sets, these incidence numbers were 31 and 28%. These numbers are markedly higher than the range found in population-based cohorts[29-32]. Incidence numbers found in the completers' analyses were much more comparable to the incidence numbers reported in the literature, giving reason to question the reliability of the multiple imputation. When looking only at the participants with missing data on the outcome, the imputed incidence numbers were 50% for the primary and secondary outcome; more than double the incidence numbers in the completers' analysis. Moreover, some of the ORs obtained from the multiple imputation sets were outside of the range of the ORs found in the completers' analysis and the worst-case analysis. For instance, in the exploratory analyses, ORs from multiple imputation were closer to one than ORs from the worst-case scenario. Given that the worst-case scenario simulated the scenario of no intervention effect at all in participants with missing data, this would indicate that the preventive effect of losing 5 kg or 5% baseline weight reversed in the participants with missing data, and increased the risk of incident knee OA. To our knowledge, an association between weight loss and knee OA in this direction has not been found before. Therefore, results from the multiple imputation model were considered unreliable. A possible reason why the multiple imputation model did not result in more plausible incidence rates is the possibility that not all of the assumptions underlying multiple imputation were met, such as the missing data mechanism being random[33]. Additionally, large amounts of missing data, especially on the outcome variable, can result in unreliable results and can introduce bias not present in a completers' analysis[34].

The present study pioneered in the prevention of knee OA and is, to our knowledge, the first to investigate the prevention of knee OA with incidence of knee OA as the primary outcome[17]. Results presented from trials investigating the preventive effect of weight loss on intermediate outcomes indicated a high possibility of a preventive effect of weight loss on knee OA[10, 11]. The present study, however, failed to find a significant intervention effect. Two possible mechanisms could have caused underestimation of the intervention effect. First, weight loss in the control group was considerably higher than expected, possibly caused by a high baseline motivation to participate in a DEP[35]. As a result, the difference in weight loss between both groups was smaller than expected. Second, compliance rates were lower than expected. A mere 32% of all participants randomized to the intervention group were compliant to the intervention. Considering these possible reasons for underestimation of the intervention effect, in addition to the fact that per protocol analyses showed greater effects than ITT analyses, a true preventive effect of weight loss on incident knee OA should be considered, despite the lack of significant findings in the present study. For this reason, as

a proof of concept, the exploratory analysis was undertaken, which did show a significant effect of losing 5 kg or 5% of baseline body weight on incident knee OA in the completers' analysis. This finding is consistent with intervention effects found on weight loss and physical activity[35].

In conclusion, no long-term effectiveness in preventing incident knee OA of the DEP or of the OGS was found in the present study. However, the PP effects of the DEP intervention were greater than the ITT effects, indicating a possibility of a significant effect, if there had been higher compliance rates and a more representative control group. Exploratory analyses showed an association between losing 5 kg or 5% baseline weight and a considerable decrease in incident knee OA. This association indicates that weight loss could be a successful strategy in preventing knee OA in an overweight population, but needs further study. However, these conclusions should be interpreted with caution, because the large amount of missing data resulted in high uncertainty of the results. As illustrated in the present study, this problem cannot always be mitigated reliably through multiple imputation.

The present study provides important insights in the possibilities of preventing knee OA. A follow-up time of 6.6 years seems to be sufficient to study the development of knee OA, given the large differences in knee OA incidence between groups in the exploratory analyses. Future research should investigate further the preventive effect weight loss on incident knee OA. Adherence rates should be of the utmost importance when designing trials to investigate prevention of knee OA. Further individualization of the content of the lifestyle intervention offered could add to this cause. The present study illustrates the large consequences of missing data, resulting in high uncertainty about the validity and usefulness of conclusions drawn. Additionally, higher compliance rates should be given high priority, in order to achieve a clinically significant amount of weight loss in a considerable proportion of the study population. Weight loss remains challenging in the present population, but this study provides proof that the concept of preventing knee OA through weight loss is viable.

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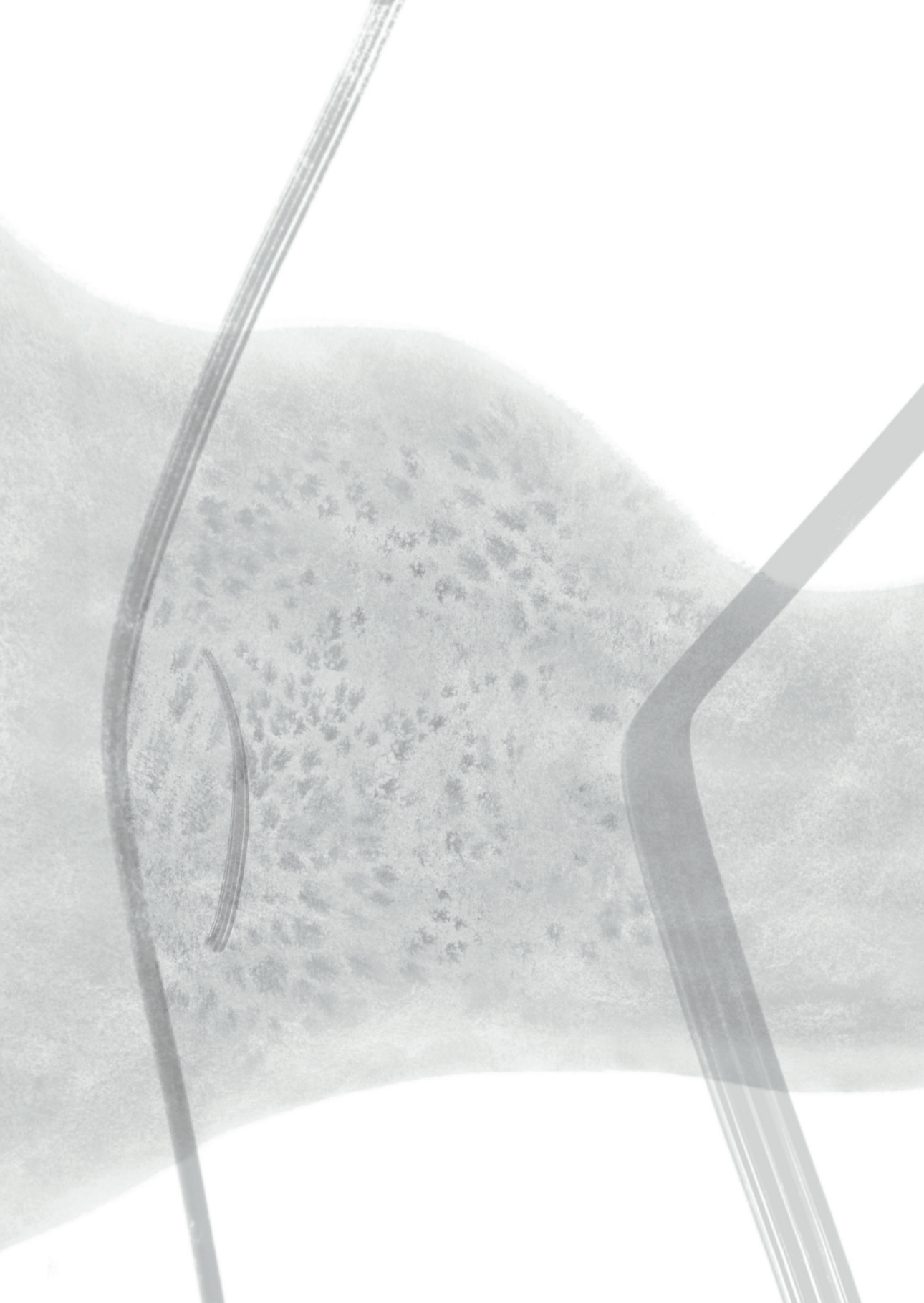

Chapter 6

Associations of urinary biomarker Coll2-1NO_2 with incident clinical and radiographic knee OA in overweight and obese women

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ABSTRACT

Objective

To investigate the association between urinary biomarker Coll2-1NO_2 (uColl2-1NO_2) and incident knee OA after 2.5 years follow-up in middle-aged overweight and obese women at high risk for knee osteoarthritis (OA).

Design

Data were used from PROOF, a randomized controlled trial with 2.5 years follow-up evaluating the preventive effects of a diet and exercise program and oral glucosamine sulphate (double blind and placebo controlled), on development of incident knee OA in women with body mass index ≥ 27 kg/m^2 without signs of knee OA at baseline. Baseline and 2.5 years uColl2-1NO_2 concentrations were assessed with ELISA. Primary outcome measure was incidence of knee OA in one or both knees, defined as incidence of either Kellgren & Lawrence grade ≥ 2, joint space narrowing of ≥ 1.0 mm or knee OA according to the combined clinical and radiographic ACR-criteria. We used binary logistic regression for the association analyses.

Results

254 women were available for analyses. At 2.5 years follow-up, incident knee OA was present in 72 of 254 women (28.3%). An inversed association was found between baseline uColl2-1NO_2 and incident knee OA at 2.5 years (OR 0.74, 95% CI 0.55 – 0.99). The concentration at 2.5 years and the change in concentration over 2.5 years did not show significant associations with the outcome.

Conclusions

In overweight and obese middle-aged women, not higher but lower baseline uColl2-1NO_2 concentration was significantly associated with an increased risk for incident knee OA. This interesting but counterintuitive outcome makes further validation of this biomarker warranted.

INTRODUCTION

Up to now there is no curative treatment for knee osteoarthritis (OA), only symptomatic treatment for pain and loss of function exists[1]. In this context it may be sensible to increase the focus on prevention of the initial development of knee OA[2]. In order to progress in this area we need to detect knee OA in an earlier, preclinical and preradiographic phase.

Currently, no sufficient tools for this aim exist. Plain knee radiography for measuring joint space width has a relatively large precision error and low sensitivity[3]. Magnetic resonance imaging (MRI) is more sensitive in detecting features of knee OA[4], but is not extensively applicable due to costs, long scan time and limited availability[1]. Given the limitations of imaging biomarkers for pre-clinical or pre-radiographic knee OA, biochemical markers are investigated as alternatives[5]. One of these, the Coll2-1NO_2 peptide, represents the combination of collagen type II degradation products (Coll2-1) and reactive nitrogen and oxygen species (RNOS), NO and O_2^-, and can be measured systemically in urine or serum[6]. Elevated production of RNOS has been observed in chronic inflammatory conditions, including established OA, but the effect of the preclinical and preradiographic phase of OA is still unknown[7]. As a low grade chronic inflammation has been suggested to be involved in the development of OA, before visible cartilage degeneration has occurred[8], we might hypothesize that elevated RNOS levels and thus elevated Coll2-1NO_2 concentrations could be measured in the pre-OA phase as well.

The aim of this study is therefore to explore the potency of Coll2-1NO_2 in detecting disease activity in preclinical and preradiographic knee OA, as earlier diagnosis of disease activity enables development of preventive therapies. We explored whether the baseline uColl2-1NO_2 concentration in subjects at risk for developing knee OA was associated with incident knee OA 2.5 years later. Additionally, we explored whether the concentration at 2.5 years was cross-sectionally associated and whether the change in concentration over 2.5 years was associated with incident knee OA.

METHOD

Study design, setting, and population

We used data from the PROOF study (Prevention of knee Osteoarthritis in Overweight Females, ISRCTN 42823086)[9]. The PROOF study is a randomized controlled trial, with a 2x2 factorial design and 2.5 years follow-up, which evaluates the preventive effects of a diet and exercise program (DEP) and of oral glucosamine sulphate, double blind and placebo controlled (GSvP), on the development of knee OA in overweight and obese middle-aged women. Inclusion criteria were age 50-60 years and BMI ≥ 27 kg/m^2 , as those are proven risk factors for knee OA[10, 11]. All participants were recruited by their General Practitioner

(GP) and had to be free of knee OA according to the clinical and radiographic criteria of the American College of Rheumatology (ACR)[12]. The participants had to master the Dutch language and had to be free of major co-morbidities, free of inflammatory rheumatic diseases, not under treatment of a physical therapist or GP for knee complaints, not using walking aids and not using oral glucosamine for the last 6 months. We treated data from PROOF as a pre-clinical OA cohort by adjusting analyses for the randomization groups. The Medical Ethics committee of Erasmus MC University Medical Center Rotterdam approved the PROOF study and all the participants gave written informed consent.

Radiography

Posterior-anterior radiographs of both knees were taken at baseline and at 2.5 years, using the semi-flexed MTP view[13]. A trained researcher blinded for clinical outcomes (MR) scored all radiographs, baseline and follow-up at once with known sequence using the Kellgren & Lawrence (K&L) criteria[14]. A random subset of 20% of the radiographs was independently scored by a second researcher (JR) blinded for clinical outcomes. The Cohen's kappa measure of agreement was moderate with a value of 0.6. Minimal joint space width was measured digitally in each tibiofemoral compartment, according to the method of Lequesne[15], using the average independent score of two researchers (JR and BdV), blinded for the clinical outcomes. Scores with a difference ≥ 2.0 mm between the researchers were re-evaluated in a consensus meeting. The inter-observer agreement for medial and lateral joint space narrowing was substantial with kappa values of 0.67 and 0.76, respectively. Medial anatomical knee alignment angle was assessed on knee radiographs as described previously[16]. Normal alignment was defined as angles between 182° and 184°, valgus and varus alignment were defined as angles > 184° and <182° respectively[17]. The test for reproducibility showed good agreement for alignment with kappa of 0.7[16].

Assessment of Coll2-1NO_2

uColl2-1NO_2 was determined at baseline and at 2.5 years in non-fasted, second morning void urine samples. The assessment in urine was based upon the qualification of the bio-marker according to the BIPED classification: Coll2-1NO_2 in urine is qualified as biomarker of prognosis[18]. A detailed description of the identification of Coll2-1NO_2 can be found in previous publications[18, 19]. In short, uColl2-1NO_2 concentration was assessed by enzyme-linked immunosorbent assay (ELISA) based on the method described by Rosenquist et al[20] using a polyclonal antibody against antigenic determinants of uColl2-1NO_2 according to the instructions of the manufacturer (Artialis s.a, Liège, Belgium). 150 µl of urine was needed for each sample. After thawing, total assay time was within a maximum of 3 hours. The precision of the immunoassay of Coll2-1NO2 in urine was previously established by Deberg et al[18] and demonstrated an intra-assay coefficient of variation (CV) of 8.3% and an inter-assay CV of 13.6%. In our study, uColl2-1NO_2 was measured in triplicate and two additional urine

samples were added on each plate as control. The inter-assay CVs for these two controls were respectively 9.6 and 11%.

uColl2-1NO_2 concentration was adjusted for urinary creatinine concentrations by expressing the results as nmol/mmol (nM/mM) creatinine. The creatinine was measured by the method of Jaffé[21] with the MicroVue Creatinine Assay Kit (Quidel, San Diego USA) on a MEGA autoanalyzer (Merck, Germany).

Questionnaires, physical examination and blood samples

At baseline all subjects filled in a questionnaire to record demographic (age, BMI, postmenopausal status, ethnicity) and clinical characteristics including questions on injury, physical activity (measured with the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH)[22]), knee complaints ("did you experience knee pain in the past 12 months?") and 'self-reported' OA in other joints. Body weight, body height, blood pressure, abdominal circumference, skin folds and Heberden's nodes on both hands were assessed at the research center. Non-fasted HbA1c concentration (mmol/mol) and total cholesterol concentration (mmol/L) were determined from blood samples taken at baseline.

Outcome

The primary outcome measure of this study was incidence of knee OA in one or both knees at 2.5 years. Incidence of knee OA was defined as either Kellgren & Lawrence (K&L) grade ≥ 2, joint space narrowing (JSN) of ≥ 1.0mm[23] or knee OA according to the combined clinical and radiographic ACR criteria (ACR knee OA). Secondary outcome measures were the separate clinical and radiographic definitions of the primary outcome.

Statistical analysis

For the present study, participants with available baseline and 2.5 years uColl2-1NO_2 concentrations and with a complete follow-up were included for analysis. Baseline characteristics were described as percentages for categorical/dichotomous data and as means ± standard deviation (SD) or medians (interquartile range, IQR) for continuous data.

For exploratory analyses, we conducted paired and independent-samples Student's t-tests with untransformed uColl2-1NO_2 data; The paired t-test to evaluate the difference between mean uColl2-1NO_2 at baseline and 2.5 years within the incident and non-incident knee OA women; The independent-samples t-tests to compare baseline-, 2.5 years- and change over 2.5 years- concentrations between the women with and without incident knee OA.

For the regression analyses of uColl2-1NO_2 with primary and secondary outcomes, uColl2-1NO_2 was logarithmically transformed to obtain normally distributed residuals. First, possible confounding variables and prognostic factors in the association of uColl2-1NO_2 with the primary and secondary outcomes were determined by univariable linear regression analyses.

The selection of the different demographic, metabolic, functional and radiographic variables was based on their possible relation with uColl2-1NO_2 and knee OA[10, 19, 24].Variables with a univariable p-value < 0.2 and with an r-value < 0.7 (cut-off point for multicollinearity) were adopted in a multivariable regression analysis (using the Enter method) to analyse significant associations with uColl2-1NO_2.

Subsequently, we analysed the association of uColl2-1NO_2 with the primary and secondary outcome measures. First, we determined the association of baseline uColl2-1NO_2 through binary logistic regression, using 3 different models. The first model was unadjusted, the second model was adjusted for age and BMI, as these are established risk factors for knee OA. The fully adjusted model 3 was adjusted for age, BMI, randomization groups (DEP, GSvP and their multiplicative interaction), possible confounders and prognostic factors from the multivariable analysis and for K&L grade at baseline (0 versus 1), as this has already been shown to be a prognostic factor for incident knee OA in the PROOF study[25] . Next, we analysed the cross-sectional associations of uColl2-1NO_2 with prevalent knee OA and secondary outcomes at 2.5 years to evaluate the diagnostic value of uColl2-1NO_2 . Finally, we analysed the association of the change in uColl2-1NO_2 concentration over 2.5 years, corrected for baseline concentration, with the primary and secondary outcomes. All analyses were performed with the three models.

To facilitate interpretation of the regression associations, uColl2-1NO2 was standardized into z-scores. Results for the regression analyses were presented as odds ratios per standard deviation (SD) increase in log uColl2-1NO_2 and their corresponding 95% confidence intervals. Statistical analyses were performed with SPSS 20.0 (Chicago, IL). A p-value < 0.05 was defined as statistically significant.

RESULTS

Characteristics of the study population

254 of 407 women with mean age of 55.8 years ± 3.19 and mean BMI of 31.0 kg/m^2 ± 3.97 were available for current analyses. The reasons for missing data were as follows: 1) unwilling to continue participation (28/407), 2) unattainable during follow-up (12/407), 3) no urine to the lab (8/407) 4) sample below the limit of detection of the test (61/407), 5) excluded based on K&L >=2 at baseline (42/407) and 6) deceased during follow-up (2/407). Analysis of the baseline differences between missing and non-missing subjects showed a statistically significant higher fat percentage (44.4% vs 43.0%), lower cholesterol concentration (5.9mmol/L vs 6.1mmol/L) and a higher percentage of varus alignment (55.7% vs 44.8%) in those missing. These differences did not seem to be relevant, as no correlation of these variables with Coll2-1NO_2 was found. Distribution, means and/or medians of baseline characteristics are displayed in table 1.

Table 1. Mean (± SD) or median (IQR) of baseline variables.

N-subjects	**254**
General	
Age (yr)	55.8 ± 3.19
Ethnicity	
Western	95.7%
Other	3.1%
Postmenopausal status	69.7%
Years postmenopausal	7.6 ± 5.3
Metabolic	
BMI (kg/m2)	31.9 ± 3.97
Weight (kg)	87.3 ± 12.7
Physical activity score (SQUASH)*	7058.3 ± 3672.4
Joint specific	
Heberden's nodes	27.2%
WOMAC (0 – 100)**	
Pain	6.2 ± 10.13
Function	6.2 ± 10.13
Stiffness	11.4 ± 17.0
K&L	
grade 0 bilateral	45.3%
grade 1 unilateral	22.4%
grade 1 bilateral	32.3%
Minimal JSW***	
medial (mm)	4.9 ± 0.7
lateral (mm)	6.1 ± 0.9
Varus alignment	
Unilateral	17.7%
Bilateral	26.8%
Mild symptoms	
Unilateral	25.6%
Bilateral	17.3%
History of knee injury	
Unilateral	17.7%
Bilateral	2.8%
Biomarker	
Mean uColl2-1NO$_2$/creatinine (nM/mM)	0.0330 ± 0.0165
Median uColl2-1NO$_2$/creatinine (nM/mM)	0.0313 (IQR 0.0220 – 0.0406)

SD = standard deviation. IQR = interquartile range. * Higher scores represent higher physical activity. ** Higher scores represent more pain/stiffness/worse function. *** JSW: joint space width.

Incident knee OA according to the primary outcome was found in 72/254 women (28.3%). Medial joint space narrowing (JSN) was found in 27/254 (10.6%), lateral JSN in 26/254 (10.2%), ACR defined knee OA in 20/254 (7,9%) and K&L grade ≥ 2 in 23/254 women (9.1%).

Exploratory associations between uColl2-1NO_2 and incident knee OA

Mean uColl2-1NO_2 concentration for the total study group was 0.033nM/mM creatinine ± 0.017 at baseline and 0.034nM/mM ± 0.017 at 2.5 years. The mean creatinine value of all samples was 7.69mM/L ± 4.36. Mean baseline uColl2-1NO_2 concentration was significantly lower in the women with incident knee OA as primary outcome after 2.5 years compared to the women without incident knee OA (0.029nM/mM ± 0.013 versus 0.034nM/mM ± 0.017, p = 0.03). The concentration at 2.5 years showed no significant difference between the women with and without incident knee OA (0.034nM/mM ± 0.018 versus 0.034nM/mM ± 0.017, p = 0.76). Although the change from baseline over 2.5 years within both groups was not significant, the change between both groups was. The mean increase in the women with incident knee OA was 0.005nM/mM ± 0.021 versus a mean decrease of 0.001nM/mM ± 0.020 in the women without incident knee OA (p = 0.04), see figure 1.

Baseline associations between uColl2-1NO_2 and incident knee OA

The variables ethnicity (Caucasian), weight, Heberden's nodes, SQUASH score and 'self-reported' OA in other joints were positively associated with uColl2-1NO_2. Age and years since menopause were negatively associated with uColl2-1NO_2. The variables BMI, waist circumference, fat percentage, total cholesterol, HbA1c, K&L grade 0 vs 1, knee alignment, mild knee symptoms and history of knee injury were not univariable associated with uColl2-1NO_2. In the multivariable regression analyses, none of the variables were significantly associated with uColl2-1NO_2.

The associations of baseline uColl2-1NO_2 with primary and secondary outcomes are displayed in table 2, showing a significant inversed association between baseline uColl2-1NO_2 and incident knee OA at 2.5 years, both in adjusted model 2 and 3 (OR 0.74, 95% CI 0.55-0.99 in model 3). No significant associations were found for the secondary outcomes.

Associations of uColl2-1NO_2 at 2.5 years and prevalent knee OA

The uColl2-1NO_2 concentration at 2.5 years did not show a significant cross-sectional association with prevalent knee OA (OR 1.03, 95% CI 0.77 – 1.37 in model 3) or with the separate outcome definitions, in any of the models (medial JSN: OR 0.93, 95% CI 0.63 – 1.38, lateral JSN: OR 0.88, 95% CI 0.57 – 1.34 , ACR knee OA: OR 1.39, 95% CI 0.82 – 2.37, and K&L ≥ 2: OR 0.92, 95% CI 0.57 – 1.47, all in model 3).

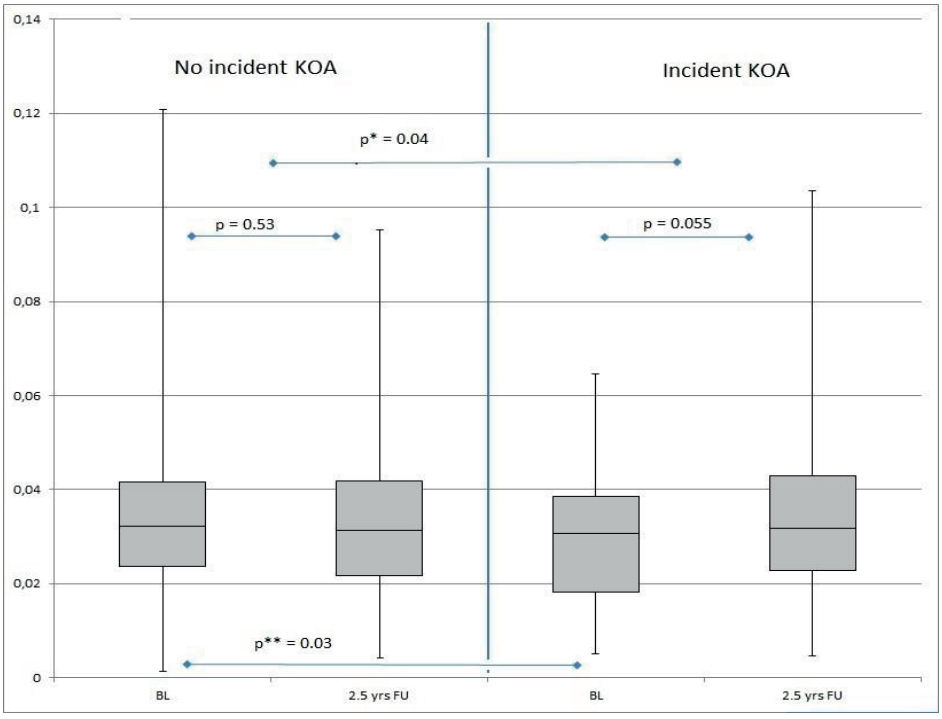

Figure 1. uColl2-1NO$_2$ (nM/mM) levels at baseline and 2.5 years follow-up for women without and with incident knee OA at 2.5 years, not adjusted for BMI, age, K&L grade (0 vs 1) and randomization groups. P-values obtained from paired t-tests, to evaluate the difference between mean uColl2-1NO$_2$ at baseline and 2.5 years within the incident and non-incident knee OA women. P-value* is obtained from unpaired t-test, to compare the change over 2.5 years in the women with and without incident knee OA. P-value** is obtained from unpaired t-test, to compare the baseline difference in women with and without incident knee OA. BL = Baseline, FU = Follow-up.

Change of uColl2-1NO$_2$ and incident knee OA

No significant association was found between the change in concentration over 2.5 years and incident knee OA (OR 1.10, 95% CI 0.81 – 1.48 in model 3), nor for the association with the separate outcome definitions, in any of the models (medial JSN: OR 0.94, 95% CI 0.62 – 1.41, lateral JSN: OR 0.88, 95% CI 0.57 – 1.36, ACR knee OA: OR 1.55, 95% CI 0.88 – 2.72, and K&L ≥ 2: OR 0.97, 95% CI 0.60 – 1.57, all in model 3).

Table 2. Multivariable adjusted association between uColl2-1NO$_2$ and adjusted variables age, BMI and K&L grade (0 vs 1) at baseline and overall incident knee OA and separate incidence definitions, at 2.5 years.

			uColl2-1NO$_2$		Age		BMI		K&L 0 vs 1	
	Cases (%)		**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Incident knee OA†	72/254 (28.3)	Model 1*	0.77	0.58 – 1.02		-		-		-
		Model 2**	**0.74**	**0.56 – 0.99**	1.03	0.94 – 1.22	**1.10**	**1.03 – 1.18**		-
		Model 3***	**0.74**	**0.55 – 0.99**	1.02	0.94 – 1.12	**1.09**	**1.01 – 1.17**	1.77	0.98 – 3.20
Medial JSN‡	27/254 (10.6)	Model 1*	0.99	0.65 – 1.49		-		-		-
		Model 2**	0.83	0.63 - 1.46	0.99	0.87 – 1.13	1.08	0.98 – 1.18		-
		Model 3***	1.00	0.61 - 1.49	1.00	0.88 – 1.13	1.06	0.96 – 1.16	1.37	0.58 – 3.23
Lateral JSN‡	26/254 (10.2)	Model 1*	0.95	0.63 – 1.44		-		-		-
		Model 2**	0.94	0.62 – 1.43	1.02	0.89 – 1.16	1.05	0.95 – 1.16		-
		Model 3***	0.95	0.63 – 1.43	1.02	0.89 – 1.17	1.10	0.99 – 1.22	**0.38**	**0.16 – 0.93**
ACR criteria‡	20/254 (7.9)	Model 1*	0.77	0.51 – 1.18		-		-		-
		Model 2**	0.72	0.47 – 1.12	0.96	0.83 – 1.12	**1.11**	**1.00 – 1.23**		-
		Model 3***	0.70	0.43 - 1.12	0.96	0.83 – 1.12	1.07	0.97 – 1.19	**7.87**	**1.74 – 35.55**
KL ≥ 2‡	23/254 (9.1)	Model 1*	0.83	0.55 – 1.25		-		-		-
		Model 2**	0.78	0.50 – 1.20	1.08	0.93 – 1.25	**1.18**	**1.08 – 1.30**		-
		Model 3***	0.74	0.47 - 1.18	1.07	0.93 – 1.24	**1.15**	**1.04 – 1.26**	**3.44**	**1.09 – 10.8**

Bold indicates p-value < 0.05

CI = confidence interval. OA = osteoarthritis

† Incidence of knee OA at 2.5 years: either Kellgren & Lawrence grade ≥ 2, joint space narrowing (JSN) of ≥ 1.0mm or knee OA according to the combined clinical and radiographic ACR criteria

‡ secondary outcomes: separate definitions of incidence of knee OA

* model 1: unadjusted

** model 2: adjusted for age and body mass index

*** model 3: adjusted for age, body mass index, randomisation groups, interaction between randomisation groups and K&L grade (0 vs 1) at baseline

DISCUSSION

This is the first study that assessed the uColl2-1NO$_2$ biomarker in a high-risk pre-OA cohort of middle-aged overweight and obese women. We found that a lower baseline uColl2-1NO$_2$ concentration was significantly associated with an increased risk of incident knee OA after 2.5 years. The cross-sectional association between uColl2-1NO$_2$ at 2.5 years and prevalent knee OA and the association between the change of uColl2-1NO$_2$ and incident knee OA were not statistically significant.

Context

Serum Coll2-1NO$_2$ was found to be significantly elevated in knee OA patients, compared to age-matched controls[19]. In another knee study, the one year uColl2-1NO$_2$ change from baseline, was shown to be predictive for radiographic medial joint space narrowing over 3 years[18]. Our study, unlike the others, was performed with patients at risk for knee OA instead of established knee OA.

Against our expectations, a lower baseline uColl2-1NO$_2$ concentration was found in the women who developed incident knee OA, compared to those who did not. In vitro studies[26-28] indicate that in the development of OA, besides catabolic inflammatory processes, compensatory anti-inflammatory mechanisms occur in an attempt by chondrocytes to restore cartilage homeostasis[27]. In vitro studies show that anti-inflammatory cytokine IL-10 can inhibit NO expression[28] and can antagonize chondrocyte apoptosis[26]. These studies might give some support for our, somewhat counterintuitive finding of lower baseline uColl2-1NO$_2$ formation. However, we can only speculate on the role of anti-inflammatory mechanisms, as this had not been studied comprehensively so far in the context of OA[29]. Moreover, some studies suggest that the anti-inflammatory response may never control the inflammatory response in OA completely[30]. We do not know how this balance is acting in the preclinical and preradiographic phase as studied in the present study. Besides, we might also hypothesize that subjects who develop OA have initially lower amounts of cartilage, which reduce the overall formation of uColl2-1NO$_2$.

We did perform our analyses on person level instead of knee level for different reasons. First, we had the aim to analyse the associations for women and not for knees. The biomarker was furthermore measured systemically and not locally. Moreover, a total of 72 women developed knee OA after 2.5 years follow-up, but only 14 of them had bilateral knee OA. As a result, this would not provide enough power to distinguish between uni- and bilateral knee OA. In ordinal regression analyses (data not shown) we found stronger, but not significant, associations for bilateral compared to unilateral knee OA.

In our exploratory analyses, we found a significant difference in change of uColl2-1NO$_2$ concentration over 2.5 years between incident and non-incident knee OA. Previously, Deberg

et al. suggested that uColl2-1NO_2 levels do not increase in preclinical and preradiographic OA phase, but later in OA development[18]. This is supported by the significant increase of uColl2-1NO_2 in women with incident knee OA compared to the women without knee OA development. This increase of uColl2-1NO_2 over time might be caused by the eventual failure of the above mentioned compensatory anti-inflammatory mechanisms during further development of knee OA. However, the significance is found only in our exploratory non-logarithmically transformed analyses.

In the 2.5 years cross-sectional data and in the change of uColl2-1NO_2 concentration over 2.5 years, the positive association with ACR knee OA was most pronounced, albeit not statistically significant. The absence of significance might be due to the small number of women who developed ACR knee OA (20/254, 7.9%) or the relatively short follow-up period of 2.5 years. The relation between (chronic) inflammation and knee pain[31, 32] and between (chronic) inflammation and osteophytes[33] as described in literature, seems to be reflected by this finding of a positive trend for the association between uColl2-1NO_2 (inflammatory marker) and ACR knee OA (pain and osteophytes).

Strengths and limitations

The major strength of this study is its focus on preclinical and preradiographic knee OA. Especially in high risk subjects there is a need for tools that could help detecting disease activity in this phase of knee OA. The assessment of the potency of the uColl2-1NO_2 biomarker in this study is contributing to fulfil this need.

We are aware of the relatively high number of analyses performed, resulting in an increased risk of a type I error. Nevertheless, given the exploratory nature of this study, these results should be seen as the first step in the validation of the uColl2-1NO_2 biomarker in high-risk pre-OA women.

One of the limitations of this study is that we could not undoubtedly exclude the presence of OA in other joints than the knee, which might have influenced the level of systemic uColl2-1NO_2. However, we have taken the presence of Heberden's nodes and the self-reported OA in other joints into account in our analyses. Choosing for self-reported OA is used in more studies[34, 35]. Moreover, the participants in the present study were asked to identify the location of their OA from a list of five (hip, ankle, hand, back/neck, other), which is known to improve the accuracy of self-reporting[36]. In this way we intended to correct as precisely as possible, making the results applicable to the knee joints.

Conclusions and implications

In this study of overweight and obese middle-aged women at risk for developing knee OA, lower baseline uColl2-1NO_2 levels were significantly associated with increased risk of overall incidence of knee OA 2.5 years later. These results might be caused by compensatory

mechanisms in the preclinical and preradiographic phase of the pathophysiologic process, lower NO production or an overall lower cartilage volume in people developing knee OA.

In the preclinical and preradiographic phase, distinguishing subjects who are at risk to develop definite knee OA from those who are not, has a high priority. It seems important to further validate the Coll2-1NO_2 biomarker and to increase our understanding of this very early phase of knee OA to enable development of preventive therapies for those subjects prone to develop knee OA.

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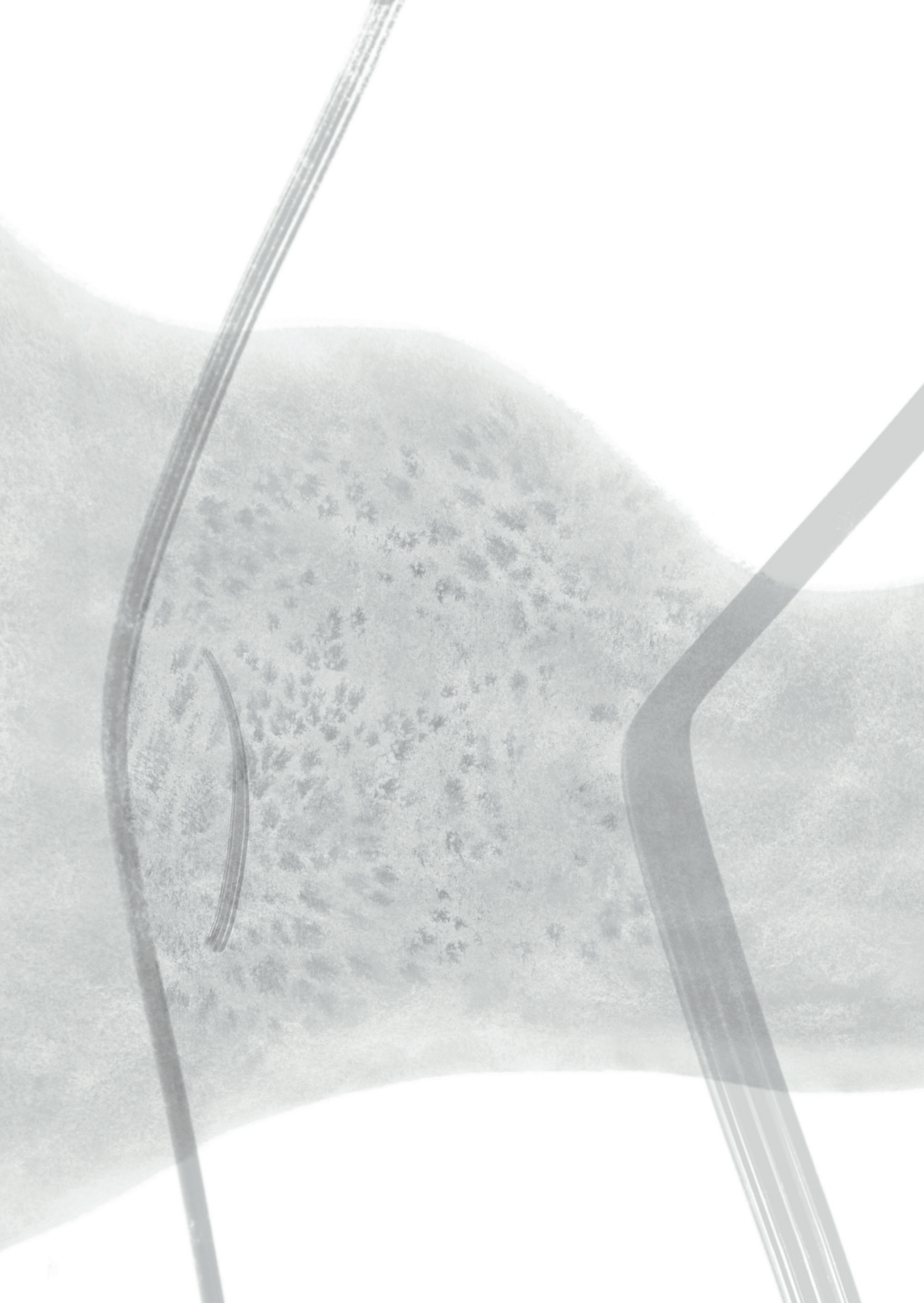

Chapter 7

Predicting knee pain and knee osteoarthritis among overweight women

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ABSTRACT

Background

There is a need for prediction of knee osteoarthritis (KOA) in general practice to motivate subjects for preventive therapies and optimize preventive trials.

Aim

To develop a prediction model, with questionnaire and physical examination variables, for incident frequent knee pain (FKP) and symptomatic KOA after 2.5 and/or 6.5 years among overweight and obese middle-aged women.

Design and setting

Models were developed in the Prevention of Knee Osteoarthritis in Overweight Females study (age 50 to 60 years, body mass index [BMI] ≥ 27 kg/m^2) (ISRCTN 42823086). FKP was defined as knee pain during most days in the past month. Symptomatic KOA was defined according to the combined (clinical and radiographic) American College of Rheumatology criteria.

Method

Multivariable analysis by backward stepwise deletion was performed for questionnaire and physical examination variables. The prediction model was externally validated in Rotterdam Study (RS)-III. Area under the curves (AUCs) of receiver operating characteristic were calculated.

Results

32% of 237 women (mean age 55.7 ± 3.2 years; mean BMI, 31.9 ± 3.8 kg/m^2) developed FKP and 30% developed symptomatic KOA. AUC of age and BMI was 0.63 (0.55 to 0.71) for incident FKP. The final model included age, BMI, mild knee symptoms, knee problems climbing stairs, morning stiffness, postmenopausal status, and heavy work. AUC was 0.71 (0.63 to 0.78). Results were similar for incident KOA. Applying external validation, similar results were observed in the RS-III.

Conclusion

In this study, easy-obtainable variables modestly improved the prediction of FKP and symptomatic KOA above age and BMI. To improve the identification of high-risk individuals, development of valid tests for other known risk factors, like meniscal damage, that are applicable in primary care, are urgently needed.

INTRODUCTION

Frequent knee pain (FKP) and knee osteoarthritis (KOA) are common complaints in general practice and constitute a substantial workload[1, 2]. Knee pain affects ±25% in those over 55 years; more women than men[1]. KOA is the most frequent diagnosis associated with FKP in older people[3].

In primary care, attention for prevention of diseases is common. Due to the large individual and socioeconomic burden of KOA and FKP, there is growing interest in early detection and prevention[1, 4]. Although overweight is one of the most important risk factors, also other factors might predict FKP or symptomatic KOA[5]. Identification of high-risk subjects is necessary for two reasons. First, to offer and motivate them for preventive strategies such as changes in lifestyle and in occupational habits. Heightening risk appraisals might change people's intentions and behavior, as shown in literature[6], without leading to unintended adverse effects[7, 8]. Second, identification is necessary to optimize preventive trials in OA research in a most cost-effective manner by including subjects in a study who are at highest risk of developing FPK/KOA on a short term.

Several studies have investigated prediction of KOA[9-11]. Zhang et al. developed a model with conventional and modifiable risk factors for the prediction of symptomatic radiographic KOA in a general population aged 40-70 years. This model, incorporating age, gender, body mass index (BMI), occupational kneeling/lifting, family history and knee injury, resulted in an AUC of 0.70[10]. Kerkhof et al. developed a model for the prediction of radiographic KOA in a general population aged 55 years and over, incorporating age, gender, BMI, questionnaire variables, genetic score, a urinary biomarker and radiographic signs of possible osteophytes (Kellgren and Lawrence (KL) grade 1[12]). Questionnaire variables, genetic score or a urinary biomarker did not improve prediction vs age, gender and BMI (AUC 0.66). The AUC increased to 0.79 by adding baseline KL 1[9]. Sharma et al. developed a model for prediction of radiographic KOA among persons at higher risk for KOA but with KL 0 in both knees (no radiographic KOA). They found that MRI lesions in tissues known to be involved in KOA improved prediction when added to models including age, gender, BMI, hand OA, injury, surgery, occupational activity and knee symptoms or function (AUC 0.84) [11].

For a general practitioner (GP) however, the use of easy-obtainable variables without the need for additional laboratory or radiologic assessments is highly preferable. It is known that the most important risk factors for incident knee pain/symptomatic KOA are older age, female gender and overweight/obesity. However, even in such a high-risk population, not all women develop KOA, as shown by the preventive trial of Runhaar et al.[13]. Therefore, it would be interesting to investigate predictors within such a high-risk group[14]. In addition, up to now, no studies have evaluated prediction of FKP, while this symptom is more important for patients than the underlying pathology (structural KOA)[15, 16].

Therefore, the objective of this study was to develop a risk prediction model for the development of FKP and symptomatic KOA in general practice among overweight middle-aged women incorporating questionnaire and physical examination variables.

METHOD

Study design and population

We used data from the PROOF study (PRevention of knee Osteoarthritis in Overweight Females)[13], a randomized controlled trial (RCT) in general practices in the Rotterdam area, the Netherlands. PROOF evaluated the preventive effects of a tailor-made diet and exercise program and of oral glucosamine sulphate versus placebo on the development of KOA over 2.5 years in 407 overweight and obese women of 50-60 years (ISRCTN 42823086)[13]. Post-hoc long-term outcome evaluation was performed over 6.5 years follow-up (mean 6.7±0.7 years). The Institutional Review Board of Erasmus University Medical Center approved the study and participants gave written informed consent. Participants were recruited by their GP by sending study information and a reply card to all registered women between 50 and 60 years. They had to be free of KOA according to the clinical ACR (American College of Rheumatology) criteria[17]. Further inclusion criteria were: BMI ≥27kg/m^2, no inflammatory rheumatic diseases, no severely disabling co-morbidities, not under treatment of a physical therapist or GP for knee complaints, not using walking aids, not using oral glucosamine for the last 6 months and mastering of the Dutch language. At baseline, participants filled in a questionnaire and underwent standardized physical examination at the research institute. Baseline posterior-anterior radiographs of both knees were taken using the semi-flexed metatarsophalangeal protocol[18]. Measurements were repeated after 2.5 and 6.5 years. Only women participating at 2.5 years were asked to participate at 6.5 years.

Risk factor assessment

Candidate predictors were selected on literature[14, 19-21] and expert recommendation. They had to be easy-obtainable through history taking or physical examination. We identified 16 relevant variables for the prediction of FKP (pain in or around one or both knees during most days in the past month[22]) and symptomatic KOA.

Questionnaire variables: Age, assessed by questionnaire. Postmenopausal status was defined after twelve consecutive months of amenorrhoea. Depression was defined when diagnosed with depression or having depressive complaints during the previous three months. Family history of OA (self-reported) was present when at least one first-degree relative had OA. Injury was defined when the women had ever visited a doctor for knee injury (no/yes). Physically demanding work was defined as doing heavy physical work "quite often" or "(almost) always". Mild knee symptoms were assessed using the question "Did you experience any

pain in or around your knee within the last 12 months?" (no/yes). Instability of the knee was assessed with the question "Did you experience a sensation of the knee giving way within the last 12 months?"(no/yes). 'Knee problems while climbing stairs' and 'knee problems while standing up from a chair', defined with the Knee Injury and Osteoarthritis Outcome Score (KOOS) subscale on 'Function, daily living'[23], were present when the women had any (seldom/sometimes/often/always) physical limitation due to her knee while climbing stairs or standing up from a chair respectively. Morning stiffness, evaluated with KOOS subscale on 'Stiffness'[23], was present when the women had moderate/much/very much knee joint stiffness after sleeping (versus no/little). Swelling of the knee, assessed with KOOS subscale on 'Symptoms', was present when the women had any swelling of the knee during the last week. The cut-off points for the KOOS variables were based on their distribution, with 10% as lower limit.

Physical examination variables: Body height in standing position without shoes and weight were measured at baseline examination; BMI was calculated (kg/m^2). Both hands were examined for Heberden's nodes (no/yes). Both knees were examined for pain at palpation of the medial and lateral joint line (no/yes) and tested for crepitus[21] during active flexion and extension of the knee (no/yes).

Outcome measure assessment

Outcome measures were incident FKP and incident symptomatic KOA after 2.5 and/or 6.5 years. FKP was assessed by questionnaire ("Did you experience pain in or around one or both knees during most days in the past month?" (no/yes)). Incident FKP after 2.5 and/or 6.5 years was defined when present at 2.5 and/or 6.5 years and not present at baseline. Symptomatic KOA was defined according to the combined (clinical and radiographic) ACR criteria[17]: FKP and a definite tibiofemoral(TF) osteophyte in the same knee and one of the following: age >50 years, morning stiffness <30 minutes, crepitus on active knee motion. Incident symptomatic KOA after 2.5 and/or 6.5 years was defined when present at 2.5 and/or 6.5 years and not present at baseline.

Statistical analysis

Analyses were done using the subject as unit of analysis. Descriptive data were presented as mean ± standard deviation(SD) or as counts (percentages). Age and BMI were kept continuous, other predictors were dichotomous. Preliminary analyses were conducted to ensure no violation of the multicollinearity assumption. Tolerance values were >0.6 for all predictors. For each outcome a binary logistic regression model was created with age and BMI (basic model). Next, all potential predictors were analysed in a multivariable logistic regression model for each outcome. Backward stepwise deletion based on the Wald test was applied using a P value of 0.20, to reduce the number of predictors in the final model. Since study participants were part of a randomized trial, analyses were additionally run with adjustments

for the original randomization groups and their interaction [13]. The Hosmer-Lemeshow χ^2 statistics for goodness-of-fit were used to compare observed and predicted risks. The explained variance was assessed by the Nagelkerke R^2. Discriminative ability was assessed with the AUC of the receiver operating characteristic. Analyses were performed with SPSS 21.0 (Chicago, IL). P values less than 0.05 were considered statistically significant.

External validation

For external validation of the final model, data from the Rotterdam Study (RS) were used. The RS is a population-based cohort study in the Netherlands that investigates determinants, incidence and progression of chronic disabling diseases in the elderly[24]. We used data of the RS-III-1 sub-cohort. Apart from 'physically demanding work', risk factor assessment was identical in the RS and in PROOF. In the RS 'physically demanding work' was defined as doing intense work (regularly lifting heavy objects at work) (no/yes) obtained with the Short QUestionnaire to Assess health-enhancing physical activity (SQUASH[25]). Women aged 50-60 years with BMI ≥27kg/m^2 were included for validation. Mean follow-up of RS-III-1 was 4.62 ± 0.56years[21]. The medical ethics committee of Erasmus University Medical Center approved the study and participants provided written consent.

RESULTS

Study population

36 of 407 women (9%) with baseline FKP were excluded for analyses. 134 of 371 women (36%) had missing data for baseline, 2.5 or 6.5 years FKP questions. Reasons were not completing the FKP questions (n=7), unattainability (n=8), no further time available or interest in the study (n=114). Five women died (not related to the study outcomes). For the prediction of symptomatic KOA, 24 women (6%) were excluded due to baseline symptomatic KOA. 148 of 383 women (39%) had missing data for FKP (n=134) or for radiography data (n=14).

Table 1 describes the baseline characteristics of both study populations. There were only 0.8% missing values of predictor variables. Drop-outs based on missing outcome had a lower presence of 'family history of OA' compared to those with complete outcome (35% versus 48% for FKP, 38% versus 49% for symptomatic KOA).

75 of 237 women (32%) developed FKP and 70 of 235 (30%) symptomatic KOA. Within those with incident FKP (n=75), 93% (n=70) had a TF osteophyte ipsilateral, hence also fulfilled the combined ACR criteria. Given the large overlap between the two cohorts, the results of the incident symptomatic KOA cohort will be presented in appendix table 1.

Table 1. Baseline characteristics.

	Study population for incident frequent knee pain^ (n = 237)	Study population for incident symptomatic knee OA^^ (n = 235)
Questionnaire variables		
Age (years) mean (SD)	55.7 ± 3.2	55.8 ± 3.2
BMI (kg/m^2) mean (SD)	31.9 ± 3.8	31.9 ± 3.8
Postmenopausal status, n (%)	159 (67)	159 (68)
Comorbidity of depression[1], n (%)	16 (7)	16 (7)
Family history of OA[2], n (%)	113 (48)	112 (48)
History of knee injury[3], n (%)	46 (19)	47 (20)
Physically demanding work[4,] n (%)	24 (10)	24 (10)
Mild knee symptoms[5], n (%)	98 (41)	100 (43)
Feeling of giving way[6], n (%)	35 (15)	37 (16)
Knee problems while climbing stairs[7], n (%)	20 (8)	20 (9)
Knee problems standing up from chair[7], n (%)	56 (24)	58 (25)
Morning stiffness[8], n (%)	29 (12)	29 (12)
Swollen knee[9], n (%)	27 (11)	27 (11)
Physical examination variables		
Heberden's nodes (in ≥ 1 finger), n (%)	65 (27)	64 (27)
Joint line tenderness (medial and/or lateral), n (%)	26 (11)	25 (11)
Crepitus during active motion, n (%)	134 (57)	135 (57)

n = number of women, SD = standard deviation, BMI = Body Mass Index, OA = Osteoarthritis

^Incident frequent knee pain after 2.5 and/or 6.5 years was defined when frequent knee pain was present at 2.5 and/or 6.5 years and when no knee pain was reported at baseline. Frequent knee pain was defined as self-reported pain in or around one or both knees during most days in the past month.

^^Incident symptomatic knee OA after 2.5 and/or 6.5 years was defined when incident symptomatic knee OA was present at 2.5 and/or 6.5 years and not present at baseline. Symptomatic knee OA was defined according to the clinical and radiographic ACR criteria : self-reported frequent knee pain and a definite osteophyte in the tibiofemoral (TF) joint of the same knee and one of the following: age > 50 years, morning stiffness < 30 minutes, crepitus on active motion of the knee.

[1] Comorbidity of depression was defined as being diagnosed with depression and/or currently under treatment.

[2] Present when at least one first-degree relative had OA.

[3] Present when visited a doctor for a knee injury.

[4] Doing heavy physical work "quite often" or "(almost) always".

[5] Pain in or around the knee within the last 12 months.

[6] The sensation of the knee giving way within the last 12 months.

[7] Defined with the Knee Injury and Osteoarthritis Outcome Score (KOOS) on physical functioning.

[8] Defined with the Knee Injury and Osteoarthritis Outcome Score (KOOS) on stiffness.

[9] Defined with the Knee Injury and Osteoarthritis Outcome Score (KOOS) on symptoms.

Risk prediction models

In the final model for incident FKP, the following predictors were selected based on our selection criteria: age, BMI, mild knee symptoms, knee problems while climbing stairs, morning stiffness, postmenopausal status and physically demanding work (table 2). When the analysis was additionally corrected for the original trial interventions and their interaction, minor non-relevant changes were found (data not shown).

Internal validation

Internal validation showed good calibration for the basic and final prediction model for incident FKP (table 2). The basic model showed an AUC of 0.63(0.55-0.71). Prediction improved in the final model to an AUC of 0.71(0.63–0.78).

Table 2. Multivariable models in prediction of incident frequent knee pain and internal validation (calibration and discrimination) of the risk prediction models.

	Basic model		**Backward model**	
Selected predictors*	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**
Age (years)	1.07 (0.98 - 1.16)	0.16	**1.15 (1.03 – 1.28)**	**0.02**
BMI (kg/m^2)	**1.09 (1.01 - 1.17)**	**0.02**	**1.13 (1.04 – 1.23)**	**0.004**
Mild knee symptoms[5]			1.74 (0.88 – 3.44)	0.12
Knee problems while climbing stairs[7]			**2.06 (1.03 – 4.12)**	**0.04**
Morning stiffness[8]			**3.03 (1.17 – 7.81)**	**0.02**
Postmenopausal status			0.57 (0.28 – 1.18)	0.13
Physically demanding work[4]			2.05 (0.72 – 5.83)	0.18
AUC of the model	0.63 (0.55 - 0.71)		0.71 (0.63 – 0.78)	
Calibration: Hosmer-Lemeshow p Value	0.72		0.93	
Variance explained (Nagelkerke)(%)	4.6		21.0	

OR = odds ratio; BMI = Body Mass Index; AUC = Area under the curve of the receiver operating characteristic. Bold indicates $p < 0.05$. *see Table 1 in main document for variable definitions.

External validation

In RS-III-1, 346 women were 50-60 years with BMI ≥27kg/ m^2. 236 of 346 women (68%) had data available for the prediction of FKP and 264 (76%) for the prediction of symptomatic KOA. In the FKP cohort (mean age 55.4±3.2 years; mean BMI 30.8±3.5kg/m^2), 41 (17%) developed FKP. In the symptomatic KOA cohort (mean age 55.5±3.3 years; mean BMI 30.9±3.6kg/m^2), 19 (7%) developed symptomatic KOA. The AUC for the prediction of FKP was 0.71(0.62 – 0.79) (table 3). For the prediction of symptomatic KOA, the AUC was 0.81(0.72 – 0.90) (Appendix table 2).

Table 3. External validation for the prediction of incident frequent knee pain in Rotterdam Study-III-1.

	Study population (n = 236)	
Selected predictors*	**OR (95% CI)**	**P Value**
Age (years)	1.01 (0.89 – 1.15)	0.88
BMI (kg/m^2)	1.08 (0.98 – 1.18)	0.13
Mild knee symptoms[5]	1.80 (0.84 – 3.87)	0.13
Knee problems while climbing stairs[7]	2.14 (0.95 – 4.82)	0.07
Morning stiffness[8]	1.65 (0.66 – 4.16)	0.29
Postmenopausal status	0.98 (0.36 – 2.69)	0.96
Physically demanding work**	1.14 (0.49 – 2.66)	0.76
AUC of the model	0.71 (0.62 – 0.79)	
Calibration: Hosmer-Lemeshow p Value	0.57	
Variance explained (Nagelkerke)(%)	12.2	

OR = odds ratio; BMI = Body Mass Index; AUC = Area under the curve of the receiver operating characteristic. *see Table 1 for variable definitions. **Defined as doing intense work (regularly lifting heavy objects at work).

DISCUSSION

Summary

We aimed to develop a risk prediction model for GPs with easy-obtainable predictors for incident FKP and incident symptomatic KOA among overweight and obese middle-aged women. A basic model, with only age and BMI had little discriminative power. With the variables age, BMI, mild knee symptoms, knee problems while climbing stairs, morning stiffness, postmenopausal status and physically demanding work, the AUC of the prediction model for incident FKP increased to 0.71(0.63-0.78). Similar results were found for the prediction model of incident symptomatic KOA.

Comparison with existing literature

Besides age and BMI, several variables were selected in the final prediction model for FKP, among which mild knee symptoms, problems while climbing stairs, morning stiffness and physically demanding work. These factors are also found in other studies: the Osteoarthritis Initiative (OAI) showed that pain during weight-bearing, knee-bending activities like climbing stairs, could be used to identify early OA[26]. Also, studies showed that increased risk of chronic knee pain was found among occupations that involve knee bending and heavy lifting[27, 28]. Recently, a proposal for classification criteria for early knee OA has been published, including stiffness as a symptom of early knee OA[29]. The incidence of developing FKP in the present study was 32%. This increases to 47% ('post-test') when knee problems while climbing stairs are present and to 52% when morning stiffness is present. None of the physical examination variables, except BMI, were selected in the final model. The only one study that evaluated physical examination variables for the prediction of KOA was the study

by Sharma et al. In their study, 'Heberden's nodes' was selected in their final prediction model by selection of univariable significance ($p<0.1$). Joint line tenderness and crepitus were not examined in their study[11]. Although our final model improved the basic model, the overall explained variance is still low and suggests that prediction of FKP (and symptomatic KOA) with easy-obtainable risk factors seems not yet clinically applicable.

Incidence rates in the present study are higher than in population-based cohorts[9, 30], but comparable to rates found among overweight subjects[31, 32]. In RS-III-1, incidence of FKP (17%) and symptomatic KOA (7%) was lower than in PROOF. This might be explained by lower baseline BMI and prevalence of mild knee symptoms in RS-III-1. Also, no X-rays were performed during an intermediate assessment, as done in PROOF after 2.5 years. Hence, incidence was based on the outcome after ±5 years. As seen in appendix table 2, we found a higher AUC in RS-III-1 (0.81[0.72 – 0.90]) than in the PROOF study (0.72[0.64 – 0.80]) for prediction of symptomatic KOA, due to the strong association between 'knee problems while climbing stairs' and incident symptomatic KOA (OR 4.47[1.31 – 15.23]) in RS-III-1. Also, the baseline prevalence of 'knee problems while climbing stairs' was higher in RS-III-1 (30%) than in PROOF (9%). This both resulted in better prediction within RS-III-1.

Strengths and limitations

With the PROOF study, the first preventive randomized controlled trial in KOA, the first steps in preventive research within a high-risk population for KOA, have been made[13]. The present study is directly applicable to GPs, since only easy-obtainable variables were used for the prediction models and also a symptom-only definition was used. Moreover, models were externally validated, which confirmed our results.

There are also some limitations. High numbers of lost to follow-up might have introduced selection bias. There were no significant differences in baseline variables compared to complete cases, except for a lower frequency of 'family history of OA' in drop-outs due to missing data (38% versus 49%). One could debate whether this difference is clinical relevant. An association between development of KOA and family history of OA has been described[33], however, others could not confirm this[34]. Overall, we estimated the possibility of selection bias as minimal. As a solution for the missing data, multiple imputation was considered. However, as described in a paper by Von Hippel et al., multiple imputation is only the solution when the independent variables are missing at random[35]. When the outcome is missing and the independent variables are complete, as in our study, the incomplete cases contribute no information to the outcome estimate and would only add noise to these estimates[35]. Secondly, a possible disadvantage of using an RCT for prediction modelling is that there are set points for follow-up measurements, in the present study after 2.5 and 6.5 years. In this way, no difference can be made between participants who develop knee pain after a short or after a longer follow-up time. Ultimately, there might have been more use from a model that

took "time to development of FKP/KOA" as the outcome of interest, but these data were not available. Further, the overlap between the prediction of both outcomes is large, since age >50 years and FKP are part of the definition of the combined ACR criteria[17]. To make prediction applicable to a GP's daily practice, we presented the symptom-only definition in the main text and the official definition for symptomatic KOA in the appendix. By analyzing data on subject level, with the aim to predict for a person and not for a knee, details on knee level are lost. As a consequence, it might be possible that presented symptoms were in the other knee than the outcome. Significant associations might be non-causal. Since proving causality is not the aim in prediction analysis[36], this seems not to affect results.

Implications for research and/or practice

Although incidence rates were relatively high after 6.5 years, the majority of women were free of FKP and symptomatic KOA. It might be worthwhile to obtain other variables that can discriminate in high-risk subjects. We did not include, for instance known risk factors for incident knee OA like malalignment, meniscal damage or effusion synovitis[37-39]. Those measures are obtained by radiologic assessments and not directly applicable in a GP's office. Surprisingly, no literature is available about the diagnostic accuracy of the clinical diagnosis of malalignment by a GP or the validity of knee specific questions for the diagnosis of synovitis. Diagnostic accuracy of the McMurray or Apley test for meniscal damage is small and not advised in general practice[40]. It seems necessary to improve the clinical diagnosis of the above risk factors in general practice or search for others.

This study showed that in middle-aged overweight women in general practice, the use of easy-obtainable risk factors contributed moderately to prediction of FKP and symptomatic KOA. Since the discriminative ability of the prediction models was moderate, the prediction of FKP and symptomatic KOA seems not yet clinically applicable.

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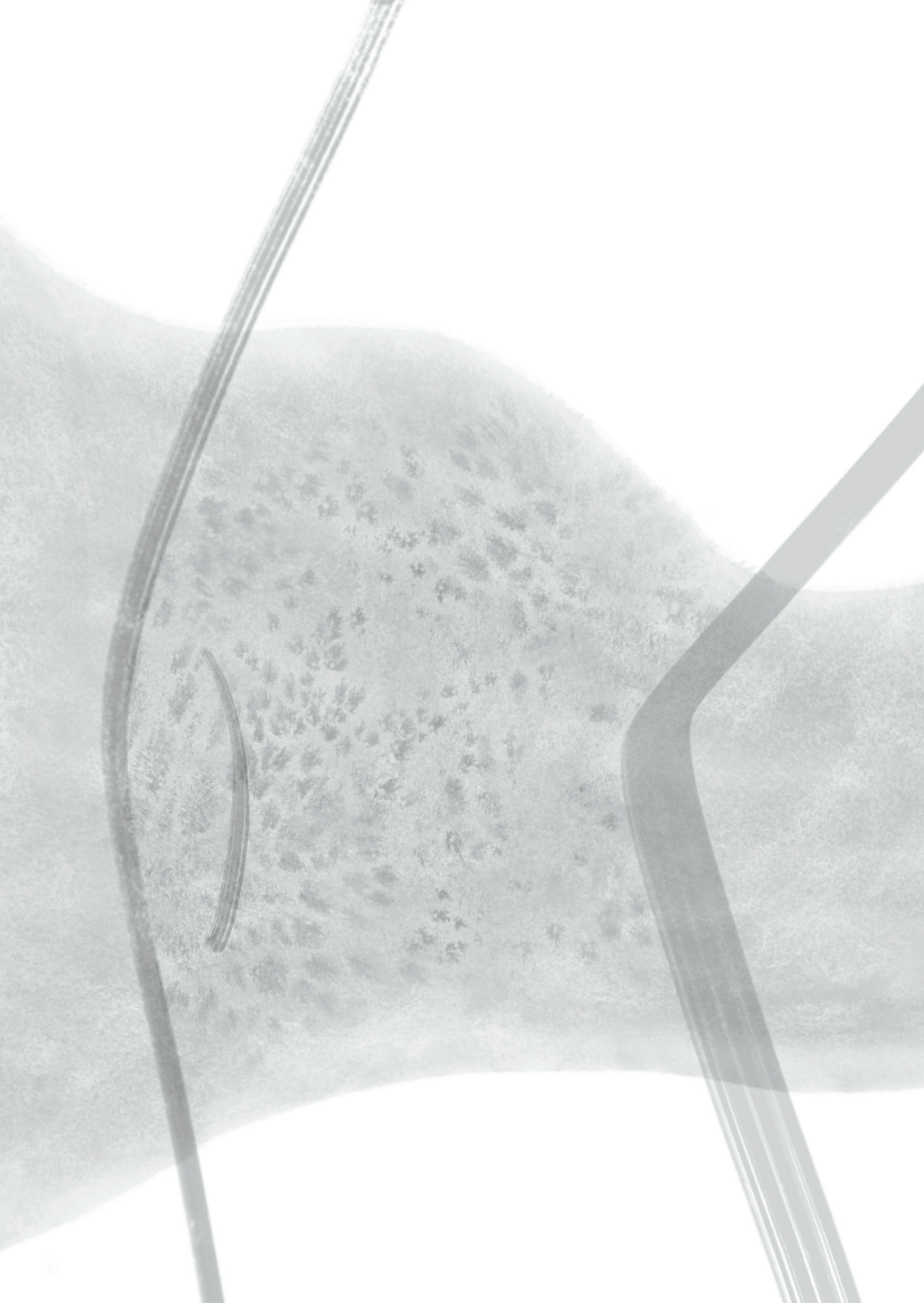

Chapter 8

Can we use patient-reported symptoms instead of joint inflammation on MRI to predict incident knee osteoarthritis?

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ABSTRACT

Objective

To compare the predictive performance of inflammation on magnetic resonance imaging (MRI), patient-reported swelling and patient-reported morning stiffness for incident radiographic (KLG≥2) and clinical (ACR) knee OA (KOA) after 2.5 and 6.5 years.

Methods

Data of the PROOF study were used, consisting of 407 overweight and obese (BMI≥27kg/m^2) middle-aged women without ACR and KLG≥2 knee OA at baseline. To determine the relation of inflammation on MRI (effusion and synovitis) and patient-reported symptoms with incident radiographic and clinical KOA at follow-up times, sensitivity, specificity, likelihood ratios (LR), pre- and post-test probabilities were calculated.

Results

Radiographic KOA incidence was 4.7% after 2.5 years and 15.5% after 6.5 years. Clinical KOA incidence was 7.0% after 2.5 years and 11.7% after 6.5 years. Patient-reported morning stiffness yielded the highest estimated positive predictive value (PPV) for clinical KOA at 6.5 years (24.5 [95% CI 15.3, 36.7]) and the highest sensitivity and LR+ at 2.5 years (32.6 [95% CI 19.5, 48.0] and 2.9 [95% CI 1.8, 4.6] respectively). For radiographic KOA, inflammation on MRI had the highest PPV (36.1 [95% CI 26.4, 47.0] at 6.5 years) and LR+ (2.9 [95% CI 1.8, 4.6] at 2.5 years). Both patient-reported symptoms had smaller increases in PPV and smaller LR+ at both time-points.

Conclusion:

For the prediction of clinical KOA, patient-reported morning stiffness had better predictive performance than inflammation on MRI and patient-reported swelling. It has the potential to be helpful for the selection of high-risk individuals and to enrich preventive trials in clinical KOA.

INTRODUCTION

Knee osteoarthritis (OA) is one of the most common causes of disability in the ageing population[1]. Worldwide, symptomatic knee OA affects up to 10% of men and 18% of women aged 60 years or older[2]. Due to the ageing population and the global increase in obesity prevalence, the number of people affected with knee OA is likely to increase rapidly, with associated burden for society[1]. Up to now there is no curative treatment available that can reverse the disease[3]. In this light, emphasis on primary prevention or early treatment of the disease is of utmost importance. Therefore, it is crucial to be able to detect those at high risk of developing the disease, since OA will develop faster and more frequently within this group[4].

While traditionally considered as a disease of hyaline cartilage, there is growing evidence that synovial inflammation plays a key role in OA pathophysiology, with effects on articular cartilage and pain[5-7]. With the increased availability of magnetic resonance imaging (MRI), the evaluation of multiple joint features, including synovitis, has become widespread in OA research, as it provides more direct insight in early joint changes compared to traditional radiography[8, 9]. Contrast-enhanced (CE) MRI allows direct visualization of synovitis[10]. In larger epidemiologic studies, non-CE MRIs are usually used due to lower costs and less risk of adverse events[11]. Several studies have shown that inflammation, assessed as effusion-synovitis and Hoffa-synovitis on non-CE MRI[10, 11], is an independent risk factor for incident radiographic knee OA[12-14], for radiographic and symptomatic progression[15], and plays a role in the development of centralized pain[16]. Although there have been a large number of studies published on the association between synovitis and the risk of knee OA, this is not the case for the predictive value of synovitis; its usefulness as a clinical predictive tool for future development of clinical knee OA in a high-risk population is currently unknown. Further, patient-reported symptoms such as pain, morning stiffness and swelling have been associated with signs of inflammation on non-CE MRI[5, 7, 17-19]. If patient-reported symptoms are able to predict incident knee OA in a vulnerable population, it could help clinicians to stratify their patients at risk and help to enrich clinical trials in early stage knee OA[20], without the need of using expensive imaging techniques.

Therefore, the objective of the present study was to evaluate and compare the predictive value of inflammation on MRI, patient-reported swelling and patient-reported morning stiffness for the incidence of radiographic and clinical knee OA at 2.5 and 6.5 years in a high-risk cohort of middle-aged overweight and obese women without established knee OA at baseline.

PATIENTS AND METHODS

Study design and population

Data of the Prevention of Knee Osteoarthritis in Overweight Females (PROOF) study were used. A description of the design and results of the PROOF study (ISRCTN 42823086) has been published previously[21]. The PROOF study was approved by the Medical Ethics Committee of Erasmus MC University Medical Center in 2005. Informed consent of all eligible women willing to participate was obtained according to the declaration of Helsinki. The study was performed among a high-risk group of middle-aged women (50-60 years) with a body mass index (BMI) ≥27kg/m^2. In principal, all women with ACR (American College of Rheumatology[22]) defined clinical and radiographic (combined) knee OA or with Kellgren and Lawrence (KL) grade ≥2 knee OA in both knees were excluded. However, some participants had (one of) these features in one of the knees and only participated in the study with the knee in which this was absent at baseline. We selected 685 knees from 345 women with complete follow-up data at 2.5 years. Additionally, from those completed at 2.5 years, 452 knees from 227 individuals with complete follow-up at 6.5 years were available. Complete follow-up data was defined as availability of MRI at baseline and a completed questionnaire and availability of radiographs at 2.5 years and 6.5 years follow-up. In total, 5 knees from 5 individuals were excluded from the analyses due to a recent severe knee trauma (n = 1) or the inability or unwillingness to continue MRI scanning of the second knee (n = 4).

Questionnaires

All subjects filled out a questionnaire that included questions on knee complaints, number of days with knee pain, presence of frequent knee pain ("Did you experience knee pain on most days of the last month?"), history of knee injury ("Did you ever injure your knee badly enough to visit a doctor or other health care professional?") and the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire[23]. Swelling was assessed with KOOS subscale on 'Symptoms' ("Do you have swelling in your knee during the last week?") and dichotomized into any swelling ('rarely/sometimes/often/ always') versus 'never'. Morning stiffness was assessed with KOOS (symptoms) subscale on 'Stiffness' ("How severe is your knee joint stiffness after first wakening in the morning?") and dichotomized into 'moderate/severe/extreme' stiffness versus 'none/mild'. The cut-off points of both KOOS variables were based on the distribution of the variables, with 10% as lower limit. All measurements were repeated after 2.5 and 6.5 years of follow-up. Additionally, baseline menopausal status was assessed.

Physical examination

At baseline, 2.5 and 6.5 years follow-up, body weight and height were measured at the research center. Crepitation at active knee motion was determined by a trained research assistant.

Radiography

A standardized semi-flexed posterior-anterior radiograph of both knees was taken according to the metatarsophalangeal protocol[24]. Radiography of the patellofemoral (PF) joint was no common practice at the inception of the original study and therefore not available for current analyses[21]. KL grading[25] and medial knee alignment angle[26] were assessed on all knee radiographs (paired and sequence known) by trained researchers blinded for clinical outcomes and treatment assignment (MR and JR). Normal alignment was defined as angles between 182° and 184°, valgus and varus alignment were defined as angles >184° and <182° respectively[27]. The reproducibility of KL grading (kappa = 0.6) and knee alignment (kappa = 0.7) was assessed through independent scoring of a random subset of 20% of the radiographs by the researchers.

MRI acquisition and assessments

An MRI of both knees was made at baseline on a 1.5 Tesla scanner (Philips or Siemens). The MRI protocols included coronal and sagittal non-fat suppressed proton density weighted sequences, a coronal T2 weighted sequence, an axial dual spin-echo sequence, and a sagittal 3D water selective (WATS) sequence with fat saturation. The protocol of both scanners is shown in the Supplementary data. All MRIs were scored by two blinded researchers (JR and PvdP) using the semi-quantitative MRI Osteoarthritis Knee Score (MOAKS)[11]. For the purpose of adequate implementation of MOAKS, an extensive training for the two researchers was organized under supervision of an experienced musculoskeletal radiologist (EO: >12 years of experience with musculoskeletal MRI in clinical and research settings)[28,29]. Synovitis, cartilage defects, bone marrow lesions (BMLs), osteophytes, meniscal abnormalities and meniscal extrusions were scored in all MRIs. Effusion-synovitis was scored 0-3 according to the distension of the joint capsule as 1 = small, 2 = moderate and 3 = large. Hoffa-synovitis was scored 0-3 according to the amount of hyperintensity signal in Hoffa's fat pad on the fat-saturated images as 1 = mild, 2 = moderate, 3 = severe. The presence and absence of effusion-synovitis and Hoffa-synovitis was dichotomized by 0 vs ≥1. The inter-rater reliability was determined with prevalence-adjusted bias-adjusted kappa (PABAK) statistics[30]. The PABAK value for both readers was 0.600 ('substantial') for effusion-synovitis and 0.642 ('substantial') for Hoffa-synovitis. To create a sum of the amount of inflammation, we summed the scores from effusion-synovitis and Hoffa-synovitis, creating a 0-6 score. The presence of any inflammation (≥1) was compared with absence of inflammation. This cut-off was chosen since the study population consisted of relatively 'healthy' knees, e.g. without

established knee OA. Similarly, the scores of the other MOAKS structural features were summed over the different subregions and dichotomized into presence vs. absence of that particular feature. The reliability for reading of the other MOAKS features has been reported in the paper of Runhaar et al.[28]

Outcome assessment

For each knee, incident radiographic knee OA was defined as KL<2 at baseline and KL≥2 or knee prosthesis at 2.5 years or 6.5 years follow-up. Incidence of ACR knee OA was defined as absence of the combined clinical and radiographic ACR criteria at baseline and presence of these combined criteria at 2.5 years or 6.5 years follow-up. Definition of the combined clinical and radiographic ACR criteria is as follows[22]: Frequent knee pain and a definite tibiofemoral (TF) osteophyte and one of the following: age >50 years, morning stiffness <30 minutes, crepitus on active knee motion. In the further text, 'clinical knee OA' is used instead of 'ACR knee OA'.

Statistical analyses

The analyses were performed on knee level. Descriptive data were presented as mean ± standard deviation (SD), as median (interquartile range (IQR)) for a non-normal distribution, or as frequencies/proportions for discrete variables. The predictive performance of inflammation on MRI and the two patient-reported symptoms, morning stiffness and swelling, was evaluated and compared by calculating the sensitivity, specificity, pre-test probability, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LR), using incident radiographic or clinical knee OA at 2.5 years and at 6.5 years as outcome measures. The LR summarizes how many times more likely the risk on disease is with a positive test result (positive LR) and how many times less likely the risk on disease is with a negative test result (negative LR)[31]. In this way, it summarizes the predictive value of the test in one single number. Overall, positive LRs above 10 and negative below 0.1 are considered to provide strong evidence to rule in or rule out diseases respectively[31]. Regression analyses were performed to assess the cross-sectional association between inflammation on MRI and the patient-reported symptoms. These results will be presented in the Supplementary data, since they contribute insignificantly to our research question, but might give an indication whether the patient-reported symptoms could be used as a proxy for inflammation on MRI. In addition, the longitudinal association between inflammation on MRI, morning stiffness and swelling as independent variables and incident radiographic or incident clinical knee OA at 2.5 years and 6.5 years as dependent variables was assessed. The regression analyses were performed with Generalized Estimating Equations (GEE) for a binomial outcome, which takes into account the correlation of measurement between two knees within one subject. Adjustments were made for age, BMI, medial knee alignment, previous knee injury, postmenopausal status, and baseline presence of cartilage defects, BMLs, osteophytes, menis-

cal abnormalities and meniscal extrusions. Since study participants were part of a randomized trial, analyses were additionally run with adjustments for the original randomization groups[21]. For all independent variables, preliminary analyses were conducted to ensure no violation of the multicollinearity assumption. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated for all regression analyses. All tests were two-sided and P values less than 0.05 were considered statistically significant. Analyses were performed with SPSS version 24.0 (Chicago, IL).

RESULTS

Population characteristics

The whole cohort consisted of 407 women. From these women, 345 (85%), of whom the data was complete at baseline and 2.5 years, were included. After 6.5 years, complete data was available for 227 (56%) women. Loss to follow-up was not related to the presence of inflammation on MRI or to self-reported swelling or morning stiffness at baseline (data not shown). The cohort with complete data at 2.5 years, presented in table 1, was used as the reference. Baseline characteristics for the total study population and for the cohort with complete data at 6.5 years are approximately the same and are presented in Supplementary table 1. Significant different baseline characteristics of the included participants with participants with incomplete data are marked. The reference cohort consisted of middle-aged [median age = 55.2 (IQR 5.5) years], obese women [median BMI = 31.1 (5.2)kg/m^2], of which nearly 70% was post-menopausal. In total, presence of inflammation on MRI was found in 103 knees (15.0%) at baseline. Effusion-synovitis was present in 87 (12.7%) of the knees and Hoffa-synovitis in 19 (2.8%) of the knees. Patient-reported symptoms of swelling and morning stiffness were present at baseline in 82 (12.0%) and 98 (14.3%) of the knees, respectively. 3.2% of 685 knees had both swollen knee and morning stiffness. The pre-test probability (incidence) of radiographic knee OA was 4.7% after 2.5 years and 15.5% after 6.5 years. For clinical knee OA this was 7.0% after 2.5 years and 11.7% after 6.5 years.

The predictive performance for radiographic knee OA

Overall, sensitivity values were low for both inflammation on MRI and the patient-reported symptoms (table 2). The highest sensitivity was for inflammation on MRI (40.0% [95% CI: 22.7 – 59.4] at 2.5 years and 32.8% [95% CI: 21.8 – 45.4] at 6.5 years). Specificity values were higher. The patient-reported symptom of swollen knee had the highest specificity (90.1% [95% CI: 87.4 – 92.3]) at 2.5 years and 90.7% [95% CI: 87.2 – 93.5] at 6.5 years), but CI with the other two tests were largely overlapping. Inflammation on MRI yielded a higher probability of radiographic knee OA (PPV 14.1 [95% CI: 9.2 – 21.1] at 2.5 years and PPV 36.1 [95% CI: 26.4 – 47.0] at 6.5 years) than the other two tests, but the PPVs had overlapping CI, especially at 6.5 years. Altogether, inflammation on MRI yielded the highest

Table 1. Baseline patient (and knee) characteristics.

	Study sample with 2.5-years data
N-subjects	**345**
Age (years, IQR)	55.2 (5.5)
BMI (kg/m^2, IQR)	31.1 (5.2)
Postmenopausal status (%)	234 (67.8)
N-knees	**685**
Previous knee injury (%)	95 (13.9)[a]
Varus malalignment (%)	267 (39.0)
Morning stiffness < 30 minutes[b] (%)	98 (14.3)
None	436 (63.6)
Mild	151 (22.0)
Moderate	74 (10.8)
Severe	20 (2.9)
Extreme	4 (0.6)
Swollen knee[c] (%), any	82 (12.0)
Never	603 (88.0)
Rarely	62 (9.1)
Sometimes	14 (2.0)
Often	2 (0.3)
always	4 (0.6)
KLG 0 (%)	339 (49.5)
KLG 1 (%)	305 (44.5)
KLG ≥ 2 (%)	41 (6.0)
ACR (clinical and radiographic)(%)	29 (4.2)
MRI structural features	
Inflammation (effusion/Hoffa)[d] (%)	103 (15.0)
Effusion-synovitis (%)	87 (12.7)
Hoffa-synovitis (%)	19 (2.8)
Cartilage defects (%)	479 (69.9)[a]
Bone marrow lesions (%)	436 (63.6)[a]
Osteophytes (%)	165 (24.1)
Meniscal pathologies (medial and/or lateral) (%)	452 (66.0)[a]
Meniscal extrusions (medial and/or lateral) (%)	359 (52.4)

KLG = Kellgren and Lawrence grade; ACR = the American College of Rheumatology.

[a]Included participants differed significantly with participants with incomplete follow-up at 2.5 years ($p < 0.05$).

[b]Morning stiffness, evaluated with KOOS subscale on 'Stiffness', was present when the women had moderate, much or very much knee joint stiffness after sleeping.

[c]Swollen knee, evaluated with KOOS subscale on 'Symptoms', was present when the women had any swelling of the knee during the last week. [d]Inflammation = presence of any effusion-synovitis (small, medium or large) and/or any Hoffa-synovitis (mild, moderate or severe), dichotomized in presence vs. absence.

Table 2. Predictive value of inflammation on MRI, patient-reported morning stiffness and patient-reported swelling of the knee at baseline for radiographic (KLG≥2) and clinical (ACR clinical and radiographic) knee OA at follow-up.

	Time point (year)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR+ (95% CI)	LR- (95% CI)	Pre-test probability (%) (95% CI)	PPV/post-test probability+ (%) (95% CI)	NPV/post-test probability- (%) (95% CI)
Outcome radiographic knee OA								
Inflammation on MRI (Effusion/Hoffa)*	2.5[a]	40.0 (22.7 – 59.4)	88.1 (85.3- 90.6)	3.4 (2.1 – 5.5)	0.7 (0.5 – 0.9)	4.7 (3.2 – 6.7)	14.1 (9.2 – 21.1)	96.8 (95.7 – 97.6)
	6.5[b]	32.8 (21.8 – 45.4)	89.3 (85.7 – 92.3)	3.1 (1.9 – 4.8)	0.75 (0.6 – 0.9)	15.5 (12.3 - 19.4)	36.1 (26.4 - 47.0)	87.9 (85.9 – 89.6)
Patient-reported morning stiffness**	2.5[e]	13.3 (3.8 – 30.7)	86.2 (83.2 – 88.8)	1.0 (0.4 – 2.5)	1.0 (0.9 – 1.2)	4.7 (3.2 – 6.7)	4.5 (1.8 – 10.7)	95.3 (94.6 – 96.0)
	6.5[f]	20.9 (11.9 – 32.6)	89.3 (85.7 – 92.3)	2.0 (1.1 – 3.4)	0.9 (0.8 – 1.0)	15.5 (12.3 - 19.4)	26.4 (17.1– 38.4)	86.0 (84.0 – 87.5)
Patient-reported swelling ***	2.5[e]	23.3 (9.9 – 42.3)	90.1 (87.4 – 92.3)	2.4 (1.2 – 4.7)	0.9 (0.7 – 1.0)	4.7 (3.2 – 6.7)	10.3 (5.4 – 18.6)	96.0 (95.2 – 96.7)
	6.5[f]	19.4 (10.8 – 30.9)	90.7 (87.2 – 93.5)	2.1 (1.2 – 3.7)	0.9 (0.8 – 1.0)	15.5 (12.3 - 19.4)	27.7 (17.6 -40.7)	86.0 (84.4 – 87.4)
Outcome clinical knee OA								
Inflammation on MRI (Effusion/Hoffa)	2.5[c]	17.4 (7.8 – 31.4)	86.0 (83.0 – 88.7)	1.2 (0.6 – 2.4)	1.0 (0.8 – 1.1)	7.0 (5.2 -9.3)	8.6 (4.6 – 15.4)	93.2 (92.3 – 94.0)
	6.5[d]	19.6 (9.8 – 33.1)	85.4 (81.5 – 88.8)	1.3 (0.7 – 2.5)	0.9 (0.8 – 1.1)	11.7 (10.3 – 15.2)	15.2 (7.9 – 26.6)	88.9 (85.1 – 91.8)
Patient-reported morning stiffness	2.5[g]	32.6 (19.5 – 48.0)	88.7 (86.0 – 91.1)	2.9 (1.8 – 4.6)	0.8 (0.6 – 0.9)	7.0 (5.2 -9.3)	17.9 (12.0– 25.8)	94.6 (93.4 – 95.5)
	6.5[h]	23.5 (12.8 – 37.5)	90.4 (87.0 – 93.1)	2.4 (1.4 – 4.4)	0.9 (0.7 – 1.0)	11.7 (10.3 – 15.2)	24.5 (15.3– 36.7)	89.9 (88.4 – 91.2)
Patient-reported swelling	2.5[g]	17.4 (7.8 – 31.4)	89.5 (86.8 – 91.8)	1.7 (0.9 – 3.2)	0.9 (0.8 -1.1)	7.0 (5.2 -9.3)	11.1 (6.0 – 19.7)	93.5 (92.6 – 94.3)
	6.5[h]	9.8 (3.3 – 21.4)	88.8 (85.2 – 91.8)	0.9 (0.4 – 2.1)	1.0 (0.9 – 1.1)	11.7 (10.3 – 15.2)	10.4 (4.6 – 21.9)	88.1 (87.1 – 89.1)

LR+ = positive likelihood ratio; LR- = negative likelihood ratio; NPV = negative predictive value (=post-test probability of no disease of a negative test); OA = osteoarthritis; Pre-test probability = prevalence; PPV = positive predictive value (=post-test probability of a positive test);

* inflammation = presence of any effusion-synovitis (small, medium or large) and/or any Hoffa-synovitis (mild, moderate or severe), dichotomized in any vs none.

** Patient-reported morning stiffness, evaluated with KOOS subscale on 'Stiffness', was present when the women had moderate, much or very much knee joint stiffness after sleeping.

*** Patient-reported swelling of knee is evaluated with KOOS subscale on 'Symptoms' and dichotomized into any vs. none.

[a]number of included knees is n = 643, [b]n = 655, [c]n = 604, [d]n = 616, [e]n = 432, [f]n = 435, [g]n = 406, [h]n = 408, [i]n = 644, [j]n = 656, [k]n = 613, [l]n = 624, [m]n = 432, [n]n = 435, [o]n = 411, [p]n = 413

LR+ for incident radiographic knee OA (LR+ 3.4 [95% CI 2.1 – 5.5] at 2.5 years and LR+ 3.1 [95% CI 1.9 – 4.8] at 6.5 years), although CIs were mostly overlapping with self-reported swelling and morning stiffness. The estimated LR- were all close to one, with the lowest LR- (0.70 [95% CI 0.50 – 0.90]) for inflammation on MRI.

The predictive performance for clinical knee OA

Similar to radiographic knee OA, sensitivity values for clinical knee OA were low for both inflammation on MRI and for the patient-reported symptoms (table 2). Nevertheless, patient-reported morning stiffness had a higher sensitivity than inflammation on MRI and patient-reported swelling at 2.5 years (32.6% [95% CI: 19.5 – 48.0] vs. 17.4% [95% CI: 7.8 – 31.4] respectively). Morning stiffness yielded a higher specificity than inflammation on MRI at 6.5 years (90.4% [95% CI: 87.0 – 93.1] vs. 85.4% [95% CI: 81.5 – 88.8] respectively). Overall, the presence of morning stiffness yielded the highest probability of developing clinical knee OA (PPV 17.9% [95% CI: 12.0 – 25.8]) at 2.5 years and PPV 24.5% [95% CI 15.3 – 36.7] at 6.5 years). All estimated LR- were close to one, with the lowest LR- (0.80 [95% CI 0.60 – 0.90) for morning stiffness.

Regression analyses

The association between inflammation on MRI and patient-reported morning stiffness was statistically significant (OR 2.89, 95% CI 1.66 - 5.03; adjusted(a)OR 2.31, 95% 1.27 – 4.20). There was no statistically significant association between inflammation on MRI and patient-reported swelling (OR 1.71, 95% CI 0.90 – 3.26; aOR 1.24, 95% CI 0.60 -2.57). The association between the presence of inflammation on MRI at baseline and incident radiographic knee OA at 2.5 (aOR = 4.03, 95% CI: 1.89 – 8.58) and 6.5 years (aOR = 3.23, 95% CI: 1.56 – 6.68) was statistically significant (Supplementary table 2). For patient-reported morning stiffness, the association with incident clinical knee OA was significant. For patient-reported swelling, the association with incident radiographic knee OA at 6.5 years was significant (aOR 2.50, 95% CI 1.08 – 5.77).

DISCUSSION

Summary

Among overweight and obese middle-aged women without clinical knee OA, predictive performance of inflammation on MRI and of patient-reported knee swelling and morning stiffness were evaluated for the prediction of incident radiographic knee OA and incident clinical knee OA after 2.5 and 6.5 years. Overall sensitivity of the three different tests was low, but specificity values were higher. For the prediction of radiographic knee OA, inflammation on MRI yielded the highest PPV, although differences with the other two tests seemed small. Compared to 15.5% incidence of radiographic knee OA after 6.5 years in the entire

population (±1 in 7), the incidence rate was ±1 in 3 (36.1%) among those with inflammation on MRI. Among those with self-reported morning stiffness or swelling of the knee, the incidence rate was ±1 in 4 (26.4% and 27.7% respectively). For the prediction of clinical knee OA, patient-reported morning stiffness seemed to have slightly better predictive performance than inflammation on MRI and patient-reported knee swelling. Compared to 11.7% incidence of clinical knee OA after 6.5 years in the entire population (±1 in 9), the incidence rate was ±1 in 4 (24.5%) among those with self-reported morning stiffness. Among those with inflammation on MRI or knee swelling, the incidence rate did not change significantly (15.2% and 10.4% respectively), so they were not predictive for this outcome.

Context and comparison with existing literature

Recently, one other study assessed the predictive value of effusion for the development of radiographic knee OA after 5 years[14]. Predictive performance of the presence of effusion was comparable with our findings, with a PPV of 39%. Sensitivity was slightly higher (51%) and specificity slightly lower (69%) in comparison with our results. The cohort consisted of subjects with symptoms of knee pain. Hoffa synovitis was not scored. This might explain the differences with our results. The self-reported symptoms of knee swelling and morning stiffness in the present study, have been evaluated previously in a case-control study with data of the Osteoarthritis Initiative[32]. This study found self-reported morning stiffness and self-reported knee swelling important prodromal symptoms 2-3 years prior to the time of incident radiographic knee OA[32]. These symptoms might indicate the presence of early (pre-radiographic) knee OA. This is according to the consensus paper of Luyten et al., in which it is proposed that also KOOS stiffness and other symptoms (including swelling) should be thought of as classification criteria for an early knee OA definition[33]. In the current study, the pretest probability of having clinical knee OA at least doubled with the presence of self-reported morning stiffness, from 7.0% to a positive predictive value of 17.9% after 2.5 years and from 11.7% to 24.5% after 6.5 years. Although showing some predictive potential for clinical knee OA, it yields not enough discriminative power to be useful as predictor for knee OA in a clinical setting. However, morning stiffness might be a useful factor for the selection of individuals at high risk for future clinical knee OA. In that way, it might have potential to enrich preventive trials in knee OA and consequently improve their feasibility.

The regression analyses presented in the supplementary data showed a statistically significant association between joint inflammation on MRI and incident radiographic knee OA, confirming previous literature[13, 34]. Further, the association in the present study between inflammation on MRI and self-reported morning stiffness has been described previously in a cohort of persons with symptomatic knee OA, in which a large joint effusion was strongly associated with stiffness of the knee[7]. Our results suggest that this association holds on in an at risk population as well.

Strengths and limitations

The strength of our study is that the study population consists of high-risk overweight and obese women largely without established knee OA. 94% of the knees was free of radiographic knee OA and >96% was free of clincal knee OA. As far as we know, we are the first to propose the predictive value of inflammation on MRI, patient-reported morning stiffness and patient-reported swelling on both radiographic and clinical knee OA in a high-risk study population.

This study has also some limitations that should be noted. After 6.5 years, 44.2% of the initial study population was lost to follow-up. As reported previously, due to the nature of the disease and therefore duration of the study, this loss was to be expected[35]. This loss seems even less than the average dropout rate in obesity trials, as this is estimated at approximately 40% in already the first twelve months[36, 37]. Although there were some significant differences in baseline characteristics between participants with complete data versus those lost to follow-up, the percentages of these characteristics did not differ substantially. Therefore, no effect on the association between predictors and outcome measures is expected. Further, the present study used non-CE MRIs, while the gold standard for detecting synovitis is CE MRI. However, synovitis can be assessed indirectly with non-CE MRI using the surrogate marker Hoffa synovitis[38], with the only disadvantage that signal changes in Hoffa's fat pad could also be attributed to post-arthroscopic changes, cysts or ganglion[11, 39]. In addition, to overcome the difficulty of distinguishing inflamed synovium from joint fluid, the imaging measure 'effusion-synovitis' is used on non-CE MRI. Overall, because of possible side-effects, ethical considerations and increased expense of CE MRI, non-CE MRI is generally the most commonly used modality to assess synovitis in large epidemiological OA studies, especially in populations of pre-symptomatic subjects as in our study[33, 38].

Conclusion and implications

In a population of overweight and obese middle-aged women at high risk for knee OA, inflammation on MRI had the best predictive performance for incident radiographic knee OA, compared to patient-reported morning stiffness and patient-reported swelling. For the prediction of clinical knee OA, patient-reported morning stiffness had slightly better predictive performance than inflammation on MRI and patient-reported swelling. Although the discriminative power of morning stiffness on its own was not high enough to enable the prediction of knee OA in a clinical setting, it has the potential to be a useful factor for the selection of individuals at high risk for future clinical knee OA and enrich preventive trials in knee OA. Further research is warranted to explore the value of clinical questionnaires versus MR imaging in early knee OA. Therefore, exploring other easy obtainable factors that can further enhance prediction of knee OA is needed.

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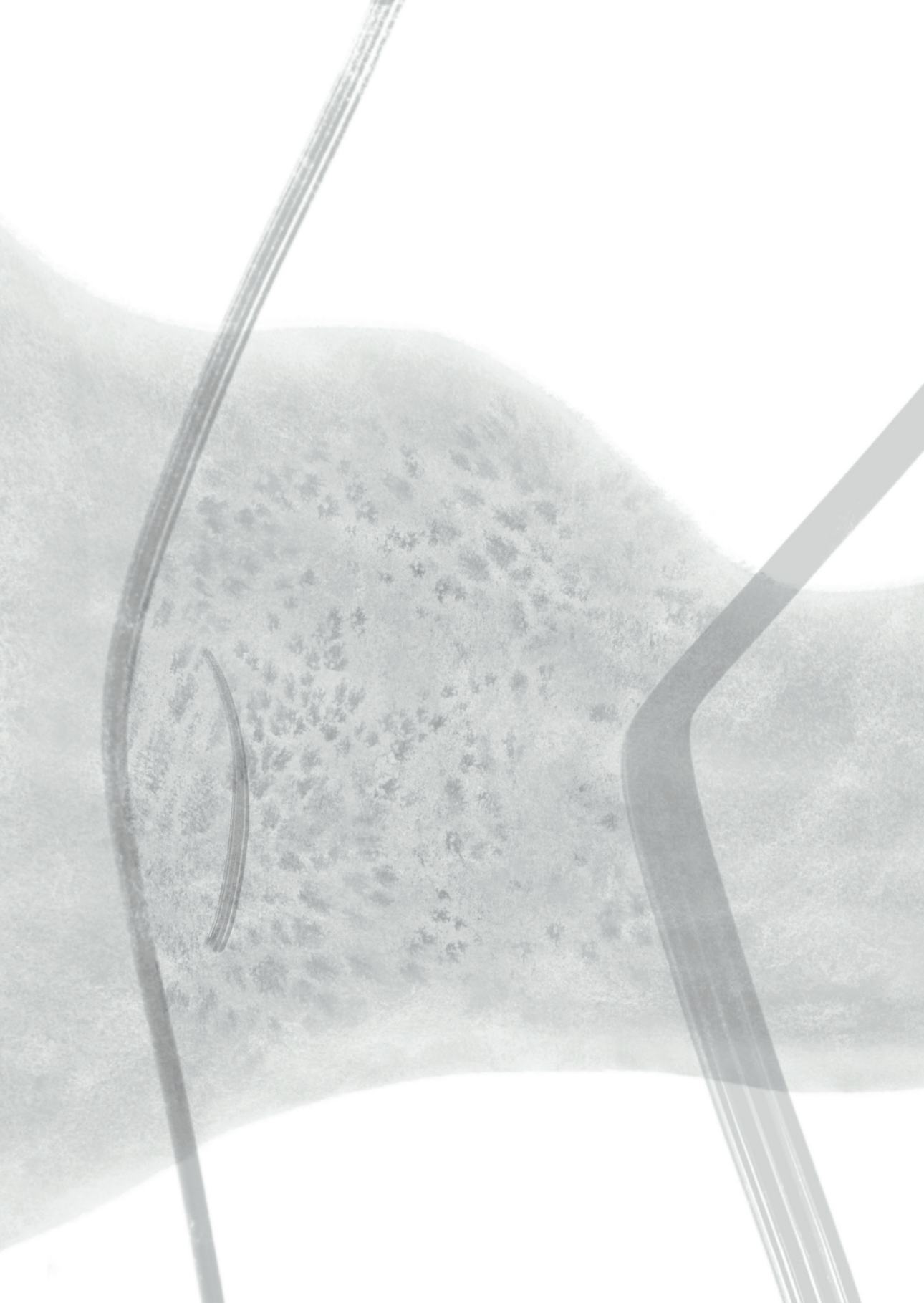

Chapter 9

General discussion

Based on the Trend Scenario of the Dutch Public Health Foresight Study, the Dutch population prevalence of doctor-diagnosed osteoarthritis (hip, knee, hand, or feet OA) is estimated to increase from 7% in 2015 to almost 13% in 2040[1]. As a result, OA will become the number one most common disease in 2040. Worldwide, knee OA accounts for approximately 85% of the disease burden of OA[2]. Mainly due to ageing population and the obesity epidemic, knee OA will be responsible for one of the largest increases in years lived with disability in 2040[1]. The disease has a substantial impact on the health of the individuals affected, on the health-care system and on socio-economic costs[3]. Moreover, knee OA is also associated with an increased risk in important comorbidities, especially cardiovascular diseases[4]. To date, there is no curative treatment and the therapeutic options of the accompanying pain and functional limitation are scarce and symptoms are often unsatisfactorily controlled[5]. Recent literature has described the urgent need to increase the focus on the primary prevention of knee OA[3, 5-7]. Also, more attention should be shifted to the identification of factors that increase the risk of OA in vulnerable populations, i.e. the risk prediction of knee OA, in order to be able to give recommendations to prevent the onset of knee OA[8].

The aim of this thesis was therefore to explore the primary prevention and the prediction of knee OA in a high-risk population. For this purpose, data from the PROOF study, the first randomized controlled trial in the prevention of clinical and radiographic knee OA, were used[9]. The participants were all overweight or obese middle-aged women, but did not have clinical signs of knee OA at the start of the study. The previous chapters described the methods and results of the studies that were conducted. This chapter will discuss how to interpret these results in the context of existing literature. Further, I will address important methodological issues, implications for clinical practice, and future research.

MAIN FINDINGS AND CLINICAL RELEVANCE

The work in this thesis is based on the PROOF study. In this first ever preventive trial in knee OA, the preventive effects of a diet and exercise program (DEP) and of oral crystalline glucosamine sulphate were evaluated in overweight and obese middle-aged women without clinical knee OA at inclusion based on X-ray and symptoms[9]. The 2.5-years effects of both interventions on different structural features of knee OA on MRI were evaluated in **Chapter 2**. At baseline, the prevalence of the different MRI OA features, assessed with the MRI Osteoarthritis Knee Score (MOAKS), was already high, ranging from a prevalence of 24% for osteophytes to 70% for cartilage defects in the patellofemoral (PF) and/or tibiofemoral (TF) joint. The DEP intervention resulted in significantly less progression of meniscal extrusion (13%) compared to the DEP control group (21%). The other MRI OA features that were evaluated were cartilage defects, bone marrow lesions (BMLs), osteophytes and other meniscal abnormalities. Progression of these features was not significantly influenced by the DEP intervention, glucosamine sulphate or their combination. **Chapter 3** evaluated the

2.5-years effects of three weight change subgroups, obtained by latent class growth analyses (LCGA), on the progression of MRI OA features. In addition to the OA features described in **Chapter 2**, the progression of MOAKS synovitis and/or effusion was also evaluated. The most important finding was the 2.5-times increase in progression of synovitis/effusion in the weight gain subgroup (18%) compared to the stable weight subgroup (7%). The high baseline prevalence of the OA MRI features described in **Chapter 2 and 3** indicates that the development of these features must have started before the age of 50 - 60 years. In a concise report, presented in **Chapter 4**, the effects of differences in body weight between the age of 40 years and the age at enrolment in the PROOF study were explored. The outcome was the baseline prevalence of MRI knee OA based on a Delphi consensus definition[10]. At 40 years, 20% of the women had normal weight, 55% of them had overweight and 25% had obesity. A 16% increase in MRI OA in women who changed from normal weight at age 40 to overweight at enrolment of the study was found while women who were obese at both time points demonstrated an increase of 44%. Suggestions were made to target weight loss at a younger age to prevent the development of (MRI) OA. Since all women in the PROOF study were overweight and obese and there was no 'normal weight group' at baseline, it was not possible to determine an optimal time period for weight loss. In theory, the greatest influence on the risk of knee OA might not be weight loss, but rather a life-long prevention of becoming overweight or obese, as suggested previously in literature[11]. **Chapter 5** evaluated the long-term effects of the PROOF interventions after 6.5 years on incident clinical knee OA. It was hypothesized that prolongation of the follow-up time after 2.5 years could possibly result in greater effects. To note, the 2.5-years follow-up results on the primary outcome measure, incidence of clinical and radiographic knee OA, showed no significant preventive effects of the interventions[9]. Also, after 6.5 years, no significant preventive effects of either the DEP intervention or the glucosamine sulphate intervention were found, although per protocol effects were stronger than intention-to-treat effects for the DEP intervention. This trend was not seen for the glucosamine sulphate intervention. As a proof-of-concept, the effect of moderate weight loss in 1 year on the incidence of clinical knee OA was evaluated. This demonstrated that losing ≥ 5 kg or ≥ 5% of baseline weight in the first 12 months resulted in a 3.0 times reduction in incident clinical knee OA (7% vs. 21% incidence) and a 2.5 times reduction in radiographic knee OA development (6% vs. 16% incidence) after 6.5 years.

To implement primary prevention, it is necessary to identify the increased risk on knee OA in an early, pre-clinical and pre-radiographic phase. In **Chapter 6** the potency of the biochemical (inflammatory) marker Coll2-1NO_2 in detecting disease activity in an early phase, was explored. The outcome measure 'incident knee OA' was defined as incidence of either Kellgren & Lawrence[12] (KL) ≥ 2, clinical knee OA (clinical and radiographic ACR criteria[13]) or joint space narrowing of ≥ 1.0mm in the medial or lateral compartment. This is in accordance with the primary outcome measure of the PROOF study[9]. Lower baseline urinary Coll2-1NO_2 levels were significantly associated with an increased risk of incidence

of knee OA after 2.5 years. This was a counterintuitive outcome, as it was hypothesized that not lower but higher concentrations would have been associated with increased risk of incident knee OA. This emphasizes further exploration of this biomarker. In **Chapter 7**, the pre-clinical and pre-radiographic phase of knee OA was further explored. A prediction model with easy-obtainable variables was developed for the outcomes incident frequent knee pain (FKP) and incident symptomatic knee OA after 2.5 and/or 6.5 years. The prediction models included the variables age, body mass index (BMI), mild knee symptoms, knee problems while climbing stairs, morning stiffness, postmenopausal status and heavy work. The discriminative ability of the prediction models was only moderate. Therefore, prediction seems not yet clinically applicable, but results could be of important value to improve the feasibility of preventive trials in OA research, e.g. by enriching the study sample. In **Chapter 8** the predictive value of two easy-obtainable variables, self-reported knee swelling and morning stiffness was explored and compared with MRI. Swelling and stiffness may be seen as a manifestation of knee inflammation[14, 15]. As known from previous studies, inflammation on MRI is an independent risk factor for the development of radiographic knee OA[16, 17]. Therefore, the predictive value of the inflammation marker synovitis/effusion on MRI was compared with the predictive value of self-reported swelling and self-reported morning stiffness. The outcome was the incidence of radiographic and clinical knee OA after 2.5 or 6.5 years. Inflammation on MRI had the best predictive performance for incident radiographic knee OA. For the prediction of clinical knee OA, patient-reported morning stiffness had slightly better predictive performance than inflammation on MRI and patient-reported swelling. None of the patient-reported variables had enough predictive power to be used for the prediction of knee OA in daily clinical practice, but morning stiffness in particular might be a helpful variable for the selection of the study sample for preventive trials and external validation of these findings is therefore necessary.

PREVENTION OF KNEE OA

Weight loss

Overweight and obesity are the most important modifiable risk factors for knee OA[7]. At the same time, reducing weight and maintaining weight loss is challenging. Especially in primary prevention of knee OA, when there is not yet any disease activity, the willingness to undergo lifestyle changes and to maintain those changes is often even more difficult[7]. It is widely acknowledged that weight loss results in significant improvements in pain and function among patients with established osteoarthritis[18]. The question is whether new cases of knee OA can be prevented with weight loss. Studies that evaluated the preventive effect of weight loss on intermediate outcomes, such as cartilage thickness and volume, suggested a beneficial effect[19-21]. This is also the conclusion of a recent systematic review that evaluated the effect of weight loss on imaging outcomes in patients with or at risk for knee OA. The authors

concluded that there are indications that pathophysiological manifestations like cartilage compositional measures are positively influenced during weight loss in early knee OA[22].

In the PROOF study, 28% of the 203 overweight and obese women randomized to the DEP intervention were compliant, which means that they attained at least ≥ 6 dietary consultations and ≥ 7 exercise classes[9]. After 2.5 years, those women had a mean weight reduction of 1.4 ± 5.2kg from their baseline body weight of 89.2 ± 12.9kg[9]. Weight loss of ≥ 5kg or ≥ 5%, associated with several cardiovascular health benefits[23, 24], was reached by 19% after 12 months and by 15% after 2.5 years in the DEP intervention group[25]. The exploratory analysis in **Chapter 5** showed a considerable effect of losing ≥ 5kg or ≥ 5% of baseline body weight in the first year of the study on the incidence of clinical and radiographic knee OA after ± 6.5 years. To note, this was independent of the intervention group. This effect of weight loss is in line with the results from the Framingham study, that observed that a reduction of BMI with 2 or more units decreased the development of knee OA significantly (OR 0.46; 95% CI, 0.24 - 0.86)[26]. Due to a large amount of missing data during the 6.5 years follow-up period of PROOF, the finding should be interpreted with caution. However, it might give indications that weight loss could be a successful strategy in preventing knee OA in an overweight and obese population.

The cut-off of 5kg or 5% does not distinguish those with steady weight over time from those with large fluctuations in weight, which can be seen as a disadvantage. The use of three different weight change subgroups (steady, weight gain, weight loss) in **Chapter 3**, obtained by LCGA, may be a more reliable method to analyse effects of weight loss on knee OA[27, 28]. No significant differences were found between the stable weight and weight loss subgroup on the change in MRI OA features over 2.5 years. However, when we explored the effect with the weight gain subgroup as reference (instead of the stable weight subgroup), there was a 52% decrease in the odds of progression of patellofemoral (PF) cartilage defects in the weight loss group compared to the weight gain group (aOR 0.48, 95% CI 0.24 – 0.97). This finding might give some support to the 6.5 years results in the ≥ 5kg or ≥ 5% weight loss group as described in **Chapter 5**. However, for the outcome of incident knee OA after 6.5, only radiographs of the TF joint were available and not of the PF joint, since PF joint radiographs were no common practice at the time of the inception of the original study[9]. This prohibits us to draw definitive conclusions on the relation between the preventive effects of weight loss on the cartilage in the PF joint after 2.5 years and the lower incidence of symptomatic knee OA (based on radiographs of the TF joint) after 6.5 years. In literature, there are only two studies that reported on the natural sequence of development of OA in the PF and TF joint. Both of them found evidence that PF joint OA increases the risk of TF joint OA incidence and progression[29, 30]. Given the above, if weight loss decreases PF joint cartilage damage, this might reduce the development of PF and TJ joint OA over time.

In conclusion, weight loss does probably have preventive effects on knee articular cartilage. This suggests an important role in prevention of early knee OA. In addition, a substantial amount of weight loss in 1 year among overweight and obese middle-aged women seems indeed preventive for incident clinical knee OA several years later.

Intervention effects of the PROOF trial

Diet and exercise program

The results of the DEP intervention on the progression of different MRI OA features (**Chapter 2**), showed that the women in the DEP intervention group had less progression of meniscal extrusion after 2.5 years compared to the control group (13% vs. 21%). This was largely based on medial meniscal extrusion, since progression of lateral meniscal extrusion was seen in only 3% of the knees. The role of the meniscus is to stabilize the knee joint, to distribute loads and to reduce shock and friction during movement[31]. Several papers have shown that a disturbed function of the meniscus, due to damage or extrusion of the meniscus, is a strong risk factor for the development and progression of knee OA[17, 32-34]. Also in the PROOF study, meniscal extrusion was associated with a significantly higher incidence of radiographic knee OA and medial joint space narrowing, as shown by van derVoet et al. [35]. In the DEP intervention group, a significant larger amount of total weight loss and weight loss of ≥ 5 kg or ≥ 5% was found during the first year (although this weight loss did not continue during the next 1.5 years of the follow-up)[25, 36]. Based on other studies, weight loss or even preventing weight gain seems to have protective effects on the progression of meniscal degeneration in subjects at risk for or with early knee OA[37, 38]. Physical activity levels, defined with SQUASH[39], were also significantly higher in the DEP intervention group throughout the total follow-up period, although these results had to be interpreted with caution as the effect sizes based on Cohen's d were varying from 0.16 – 0.19 at the different follow-up measurements over 2.5 years[25, 36]. Exercise programs are intended to increase upper leg muscle strength and improve knee stability[40, 41], which might have induced protective effects on meniscal extrusion in the DEP intervention group of the PROOF study. Additional mediation analyses to evaluate this, were done by our research group (data not shown in this thesis). Remarkably, they did not find an effect of either the physical activity or the weight loss on the progression of meniscal extrusion in the PROOF study[42]. It is well possible that power issues or inaccuracy of reporting physical activity have prohibited to detect effects. Another possible mechanism is that not the biomechanical load on the meniscus, but systemic anti-inflammatory pathways explain the effect of diet and exercise on the progression of meniscus extrusion [43, 44]. How this prevents progression of meniscal extrusion needs further investigation.

There was no effect of the DEP intervention on cartilage defects after 2.5 years. This might be due to the poor adherence rate to the DEP intervention (28% of 203 women) and the relatively low amount of weight loss in the intervention group over 2.5 years (-1.4 ± 5.2 kg

vs 0.0 ± 6.7 kg in the control group). In addition, the impact of the exercise program in terms of mechanical stimuli to the cartilage (e.g. Nordic walking, volleyball, modern dance) might be too low to promote beneficial cartilage effects, as suggested by a recent systematic review[45]. The only other study available that evaluates the effect of therapeutic exercise on cartilage in patients at risk for knee OA, was performed in (mostly) younger men, having had meniscectomy[46]. This RCT reported a positive effect of adequate loading therapeutic exercise on cartilage glycosaminoglycan content, suggesting that there are treatment effects on articular cartilage, especially when the weight-bearing impact of the exercise treatment is adequate[46]. Nevertheless, with only two studies available, more high-quality studies are needed to investigate the preventive impact of exercise on articular cartilage in patients at increased risk of knee OA. Probably, more supervised exercise, performed among younger subjects and with both structural and compositional changes of the cartilage as outcome measures might increase the possibility of finding preventive effects. Up to now, such studies have not been performed[46].

Although we showed reduced progression of meniscal extrusion in the DEP intervention group after 2.5 years, knee OA development was not reduced after 6.5 years (**Chapter 5**). A large amount of lost-to-follow-up after 6.5 years and low compliance rates in the DEP intervention group are factors that might have contributed to an absence of long-term effects. Furthermore, no significant difference in weight loss was found at the end of long-term follow-up between the intervention and control group[36]. With the tailor-made intervention and the motivational interviewing by trained dieticians, it was expected that compliance rates and actual weight loss would have been higher after 2.5 and 6.5 years. The fact that they were disappointing, emphasizes the difficulty of lifestyle interventions on sustained effects in the long-term. An even more personalized approach and long-term support might be necessary to achieve and sustain weight-loss and adequate physical activity. A combined lifestyle intervention, that focuses on diet, adequate weight-loading exercise and moreover on psychological aspects, is thought to have the best results on the long-term, especially when long-term personal coaching is applied[47].

Effect of Glucosamine sulphate on prevention of knee OA

For established knee OA patients, the use of glucosamine is controversial. Many different studies are performed, showing varying effects on outcomes such as pain and stiffness; the studies with the highest quality showed no or only little effect[48]. A recent systematic review and individual patient data subgroup meta-analysis from the OA Trial Bank found that glucosamine was no better than placebo for pain and function and concluded that there was therefore no good evidence to support the use of glucosamine in established OA patients[49]. As literature suggested larger effects of glucosamine over placebo when used in a very early phase of the disease[50], glucosamine sulphate was part of the preventive intervention of the PROOF study. Both **Chapter 2 and 5** showed no preventive effects of oral glucosamine

sulphate on the progression of the different OA MRI features after 2.5 years or on the incidence of radiographic and clinical knee OA after 6.5 years. Although previous results from the PROOF population showed that glucosamine sulphate diminished the 2.5 years progression of medial joint space narrowing compared to placebo (5.9% vs 12.8%, OR 0.41; 95% CI 0.20 – 0.85)[51], this finding could not be established after 6.5 years, when the radiographic and clinical definition of knee OA was used as outcome measure (**Chapter 5**). Moreover, the reported reduction in joint space narrowing could not be translated into changes in OA MRI structures, as glucosamine sulphate did not show preventive effects on progression of meniscal extrusion or cartilage damage after 2.5 years (**Chapter 2**). Based on all the above results from PROOF, a preventive effect cannot be assigned to glucosamine sulphate for the follow-up time of either 2.5 or 6.5 years. Since no other preventive trials with glucosamine sulphate have been performed yet, this is up to now, the best information we have.

PREDICTION OF KNEE OA

Biochemical markers (biomarkers)

Knee radiography has a relatively large precision error and low sensitivity in detecting early knee OA[52]. Moreover, once structural changes are found, these are irreversible[5]. Therefore, there is an increasing interest to assess the role of alternatives. Since metabolic changes of joint tissues start long before the onset of structural changes, biochemical markers might help in detecting early signs of OA[53]. The urine nitrated form of peptide Coll2-1 (Coll2-1NO_2), a biochemical marker of inflammation-related cartilage degradation, was assessed in **Chapter 6** of this thesis. Coll2-1NO_2 was found to be negatively associated with the combined outcome definition of incident knee OA after 2.5 years. Mechanisms such as lower NO production in the preclinical phase or an overall lower cartilage volume in people developing knee OA are possible suggestions to explain this counterintuitive outcome[54-56]. However, a clear understanding is lacking and it seems that the

uColl2-1NO_2 biomarker is not able to play a useful role in the prediction or early detection of knee OA. Overall, only few biochemical markers have been identified that are associated with incident knee OA[53]. Two of them, urinary C-terminal telopeptide of collagen type II (uCTX-II) and serum cartilage oligomeric protein (COMP) (both markers of cartilage and bone metabolism) might be promising candidates for prognostic risk assessment of OA[53]. However, although they seem to have prognostic abilities, their potential usefulness in individual OA prediction is far from clinical practice. More understanding about reference intervals, level changes over time, joint specificity of the biomarker and clear knowledge about sensitivity and specificity of these prognostic biomarkers is necessary[57, 58].

Prediction models for incident knee OA

In **Chapter 7** we presented a prediction model for incident frequent knee pain and for incident knee OA in overweight middle-aged women with an area under the curve (AUC) of 0.71 and with an explained variance of 21%. Age, BMI, mild knee symptoms, knee problems while climbing stairs, morning stiffness, postmenopausal status and physically demanding work were the selected model variables. One other study, that used subject demographics, clinical factors and other risk factors without using imaging variables[59], found a similar discriminative power. Although our prediction model can be generally characterized as a model with 'fair' performance (our external validation showed similar results), we are aware that this is not enough to be clinically applicable in daily practice. Variables such as mild knee symptoms, morning stiffness and pain while climbing stairs are proposed classification criteria for a definition of early knee OA[60, 61]. In this light, the models do not predict incidence of new disease but rather predict progression of early OA to established OA when radiographic and clinical criteria according to the ACR are present[13]. So, the variables derived from this prediction model might be more helpful for the identification of subjects in the earliest stage of disease instead of subjects without disease at high risk to develop it. In that light they might have a role in the optimization of the study population of secondary prevention trials, in which treatment effects from the earliest symptoms are evaluated.

Only conventional predictors were included in the models, although there are many others, including imaging markers from radiography and MRI. Recently, prediction models have been built that evaluated the added value of these imaging factors[62-64]. They showed that adding minor X-ray damage or MRI tissue lesions (in knees without X-ray damage) to conventional risk factors, improved the prediction of knee OA with AUCs reaching ≥ 0.80. However, for general practice, MRIs are not implemented in daily work and not easily applicable. In addition to this, the predictive performance of inflammation on MRI (synovitis and/or effusion) was compared with self-reported symptoms in **Chapter 8**. The results showed that patient-reported morning stiffness was associated with inflammation on MRI, but that patient-reported swelling of the knee was not, although in literature they are both linked with inflammation[65]. The low prevalence of self-reported swelling in the PROOF population may have hampered this association: 88% never experienced swelling and 9% only rarely experienced swelling of the knee in the last week. Remarkably, both synovitis/effusion and patient-reported swelling showed an increase in positive predictive value for radiographic knee OA after 6.5 years from 15.5% to 36.1% and 27.2% respectively. So, although patient-reported swelling could not be defined as a 'proxy' for inflammation on MRI in our study, it showed some predictive value for incident radiographic knee OA after 6.5 years. For the prediction of clinical knee OA, patient-reported swelling was not helpful. Therefore, it seems not (yet) useful to implement this question as a screening tool in a high-risk population. Patient-reported morning stiffness performed slightly better in identifying

patients at risk for clinical knee OA. This may be the consequence of the outcome definition used: frequent knee pain and a definite tibiofemoral (TF) osteophyte and one of the following: age > 50 years, morning stiffness < 30 minutes, crepitus on active knee motion[13]. As can be seen, morning stiffness is part of the outcome definition in some cases, which may have overfitted the model. When patient-reported morning stiffness was present at baseline, 1 in 4 women (instead of 1 in 8) developed clinical knee OA after 6.5 years. Still, 3 in 4 overweight/obese middle-aged women with morning stiffness, would not develop clinical knee OA, which hinders the use of this self-reported question for the prediction of knee OA in daily clinical practice. Morning stiffness was more prevalent than swelling of the knee: 14% reported moderate to severe and 22% had mild morning stiffness during the last week. Previous reviews have already mentioned that the clinical presentation of morning stiffness is likely to reflect synovial inflammation[65, 66]. As it is thought that synovial inflammation occurs even in early OA[67], this also links morning stiffness as a symptom of early OA. Previously, we showed that there was more progression of synovitis/effusion in the weight gain subgroup compared to the stable weight subgroup (**Chapter 3**). Within PROOF, we did not find significant effects for weight loss on reduction of effusion/synovitis. This is something that could be a topic for future (secondary) preventive studies. It would be interesting to know whether weight loss reduces the symptoms of morning stiffness in high-risk groups and whether there is an association with reduction in (progression of) synovitis/effusion.

METHODOLOGIC CONSIDERATIONS

In the previous paragraphs, some methodological aspects of the PROOF study are already described and are not further discussed here (e.g. poor adherence rate to the DEP intervention, low amount of weight loss in the intervention group, inadequate loading of the exercise intervention, 'shortages' in the prediction models). However, there are some aspects left that deserve attention.

As previously stated, MRI is able to visualize structural joint abnormalities, even in the earliest phases of disease, in which conventional radiographs are normal[68-70]. Thus, being a very important source in addition to radiographs. In the PROOF study, the MOAKS system was used to evaluate MRI data, which is a semi-quantitative scoring system that was originally developed for the cross-sectional evaluation of OA features to define disease status[71]. MOAKS is seen as one of the best tools for semi-quantitative analyses of knee OA. However, a definition of the longitudinal change of the individual OA MRI features was not described in the original paper. Therefore, the outcome measures in **Chapter 2 and 3** were based on the proposed definitions recently developed by Runhaar et al[72]. In accordance with this definition, the longitudinal change of each OA feature was defined per subregion and subsequently, the change scores were summed to obtain the frequencies of change. In **Chapter 2 and 3**, the summed scores were further dichotomized into progression

vs no progression (change score ≥ 1 = progression, change score < 1 = no progression) for the tibiofemoral and patellofemoral joint and their combination. As a disadvantage, detailed information about the number of affected regions or the degree of (within-grade) change per subregion is not available anymore. Moreover, sum scores are difficult to interpret, as they can represent widespread low grade damage or a focal severe lesion as well. Recently, a new definition of change over time for different MRI features has been published[73]. In this definition, also the number of subregions, the maximum change score per subregion and the maximum change score across all subregions are taken into account, as well as within-grade change. This approach helps to visualize the degree of change, which is lost in the summative approach we used. However, the aim of the present study was to evaluate whether there was any effect at all of the DEP intervention and of weight change on OA MRI features. A next step would be to evaluate degrees of change and evaluating specific subregions of the knee that are most at risk for progression (or improvement) of OA features. In future research, it would be recommendable to compare the definition we used with the one more recently developed by Roemer et al, e.g. by using both definitions as outcome measures in future (preventive or therapeutic) studies.

Another topic that deserves attention, is the amount of missing data during the 2.5 to 6.5 years follow-up period. The trial duration was defined for 2.5 years and after the interventions were ended, observation of the participants continued for 4 more years. It is known that particularly for long-term preventive studies, in which participants do not directly perceive benefit, adherence is a large problem. Moreover, during the recruitment phase of the study it was not yet implemented to extend the observation time with 4 more years after the trial interventions were ended. Due to ethical reasons, only the women that were still in the trial after 2.5 years (85%), could have been asked for informed consent for the next 4 years. After 6.5 years almost 40% of the women was lost to follow-up. It might have been that in the age range of the participants, other illnesses or life-events that deserved more attention on the short time, affected the adherence to the clinical trial. Generally known, low adherence rates lower the power of a study. At the same time, for the analyses of the different MRI features, a relatively large number of analyses had to be performed, increasing the possibility of type-1-errors. Therefore, it is important that when new preventive trials are planned and executed, the above considerations are taken into account.

IMPLICATIONS FOR CLINICAL PRACTICE

With this thesis, we aimed to study the primary prevention of knee OA in high-risk individuals. For other chronic conditions, such as cardiovascular disease and diabetes mellitus, prevention is a generally accepted intervention in primary care[6]. This is not yet the case for knee OA: too often general practitioners (GPs) and other health-care providers assume that it is an inevitable process with ageing. The findings in this thesis suggest that there

are effects of weight loss in overweight middle-aged women that might be important in the prevention of knee OA in this group. Weight loss at a younger age seems to be more effective, as was described in **Chapter 5.** It seems that exposure-time is the main way in which BMI influences the risk on knee OA, supporting the importance of weight control throughout life as primary prevention[11]. As stated in the 'Toekomstvisie huisartsenzorg 2022' (which will be updated soon), prevention should be one of the core tasks of a GP[74]. With the increased workload for GPs (due to transferral of tasks from secondary to primary care, the increase in responsibilities for (severe) ill (elderly) patients, rising public expectations, new development and treatment possibilities etc.), there is discussion about the role of the GP in primary prevention and whether it should be a more public/government task. However, living in an era of chronic diseases, mostly due to an unhealthy lifestyle, it would be counter-intuitive and also counter-effective for GPs (not time-saving) to await the disease instead of preventing it. Moreover, people with OA have an increased risk of important comorbidities such as cardiovascular diseases, diabetes, depression and also of all-cause mortality[4, 75-77]. The reason for this co-existence with many comorbidities is probably the disability and functional limitations resulting from OA. People affected by pain due to OA are less likely to achieve physical activity recommendations[78], necessary to keep a healthy weight. At the same time, walking disability in people with OA has been shown to be a strong risk factor for cardiovascular disease[4] and diabetes complications[79]. Therefore, the prevention of OA related disability will also help in the prevention of these comorbidities that are related to physical inactivity. This will lead to an increase in the overall health of the individual patient, one of the core values in general practice. The importance of weight control and physical activity throughout life requires a proactive attitude of the GP. He/she should put all effort to start the conversation about weight(loss) and raise awareness on the increased risk of knee OA and related comorbidities, also in the light of the current COVID-19 pandemic, in which obesity and diabetes are important risk factors for severe disease. With knowledge about healthy diet and physical exercise recommendations, GPs are very well able to give personalized advices about these topics. Recently, the Dutch government and secretary of state have sent a clear signal about the importance of prevention in healthcare. They presented the 'National Prevention Agreement'. This agreement focuses on reducing smoking, alcohol consumption and overweight prevalence[80]. The three topics to reduce overweight focus on A) promoting healthy eating, B) making it more appealing to do more sports, and engage in more physical activities, in a better way and C) creating a healthier environment and providing accessible and appropriate support and care for those who need it[80]. As part of the last topic, since 1 January 2019, the 'Combined Lifestyle intervention' (diet, physical activity and psychological counselling) is reimbursable under the Healthcare Insurance Act for adults with obesity (BMI $\geq$ 30kg/m^2), those with overweight (25kg/m^2 $\leq$ BMI $<$ 30kg/m^2) and related (risk on) comorbidity (e.g. risk factors for vascular and heart diseases, diabetes mellitus, osteoarthritis or sleep apnoea) and those with overweight and increased abdominal

circumference. This is a 2-years personalized program, including dietary and physical activity guidance and psychological counselling, that aims to adopt a sustained healthy lifestyle for the participants on the long-term[81]. That it is covered by the health care insurance offers the GP, who makes the referral to the local program, an important treatment possibility for those with overweight and/or obesity. It is a first step to stimulate patients to really start with the lifestyle intervention. In the coming years, the results of the combined lifestyle intervention will be closely monitored for effectiveness and cost-effectiveness[80].

Another topic to be discussed in the context of implementation, is the use of ultrasound in primary healthcare for patients with early knee symptoms (without fulfilling the established clinical diagnosis of knee OA). Radiographic changes are relatively late findings in knee OA and MRI, although seen as the gold standard for assessing the knee joint, is not widely available for routine clinical practice, due to practical reasons and expense[82]. A recent review showed that ultrasound can visualize osteophytes and degeneration of cartilage at least as well as radiography and that it can provide relevant additional information about pathologic changes in soft tissues, e.g. synovitis, meniscal extrusion and Baker's cyst[82]. Diagnostic ultrasound is increasingly available in physiotherapy and is less expensive than MRI. For early knee complaints, ultrasound may be a useful imaging technique. For instance, it can be helpful to detect early synovitis, when there are not yet radiographic changes. This may stimulate individuals to undertake action (weight loss, exercise therapy) to prevent further harm. Maybe, ultrasound can have the potential to play an additional role in the prediction of future knee OA in high-risk individuals. The use of ultrasound in early OA may also have opportunities for the development and evaluation of pharmacological interventions in early OA to prevent or slow down the disease process. In established painful knee OA, current best analgesic therapies are NSAIDs and intra-articular (IA) steroid injection; both work by anti-inflammatory mechanisms[83]. Up to now, it is yet unknown whether subjects with early knee complaints and concomitant inflammatory lesions on ultrasound could benefit long-lasting effects on articular structures and symptoms from anti-inflammatory pharmacological therapies. Improvements in appropriate 'inflammatory subgroup' stratification, in which ultrasound might play a role, and a better understanding of the inflammation pathway might provide more insight in (novel) therapeutic opportunities[84].

RECOMMENDATIONS FOR FUTURE RESEARCH

Trials addressing primary prevention of knee OA face the difficulty of a long trial duration, because of the insidious onset and overall slow progression of knee OA, as described neatly by Jordan et al[85]. The need for a (very) long trial duration can be prevented by the use of proxy outcome measures. In the PROOF trial, we used conventional non contrast enhanced MRI, to evaluate morphologic OA features (semi)quantitatively. Newer techniques, for instance compositional MRI, may be modalities of choice in future preventive research.

These techniques enable the evaluation of the biochemical composition of joint tissues, especially cartilage, but also meniscus and ligaments[86]. Potentially, they can supplement the current MRI sequences, as they are able to assess tissue changes at an earlier stage than is possible with the morphological MRI sequences that are used today[86-88]. In recent years, studies have been performed to evaluate whether pre-morphological changes in cartilage can predict later morphological changes and clinical symptoms of knee OA[89, 90]. These studies show promising results for compositional MRI techniques to serve as a very early prognostic imaging biomarker of knee OA. However, there are further studies needed to establish their potential value before these techniques can be actually implemented in clinical trials, e.g. the reproducibility and validity of these MRI techniques should be established in different populations[88].

Enrichment of the study sample by selection of individuals at highest-risk is another solution to prevent long trial duration. Study population selection within PROOF was based on age (50 - 60 years), gender (female) and weight (BMI ≥ 27kg/m^2). Including persons with additional risk factors will probably shorten the time for effect evaluation, as a combination of more factors will identify the individuals with the highest risk to develop knee OA on the short-time. Based on the results of **Chapter 7 and 8**, patient-reported problems with climbing stairs, morning stiffness and swelling of the knee are probably variables that are useful to enrich trial populations. Although we have to be aware that the presence of those symptoms may indicate an early state of knee OA, as discussed previously, and therefore prohibits a true primary preventive trial. However, at least for trials that face the early treatment (secondary prevention), this selection of subjects may be of additional value.

Further, the diet-and-exercise program of PROOF resulted in less meniscal extrusion, but did not find the underlying pathway in this. Since meniscal extrusion seems to play an important role in the development of knee OA, future research should focus on the elucidation of the pathway that prevents meniscal extrusion in overweight and obese individuals.

FINAL REMARKS

The overall aim of this thesis was to gain better understanding in prevention and prediction of knee OA in a high-risk population. As the papers in this thesis have shown, this is far from easy and has still a long way to go. Possible preventive effects of a lifestyle intervention and of weight change were found on the progression of meniscal extrusion and synovitis. But their role in the prevention of the clinical disease needs further exploration. The prediction model in this thesis, although not useful for daily clinical practice, might help to enrich future (secondary) preventive trials and help to shorten the time for effect evaluation. More work has to be done to make the prevention of knee OA just as generally accepted as it is for other chronic conditions, such as cardiovascular disease and diabetes mellitus in primary care.

With the studies in this thesis, we have tried to contribute in the process towards prevention of knee OA and in the improvement of prediction of this disease.

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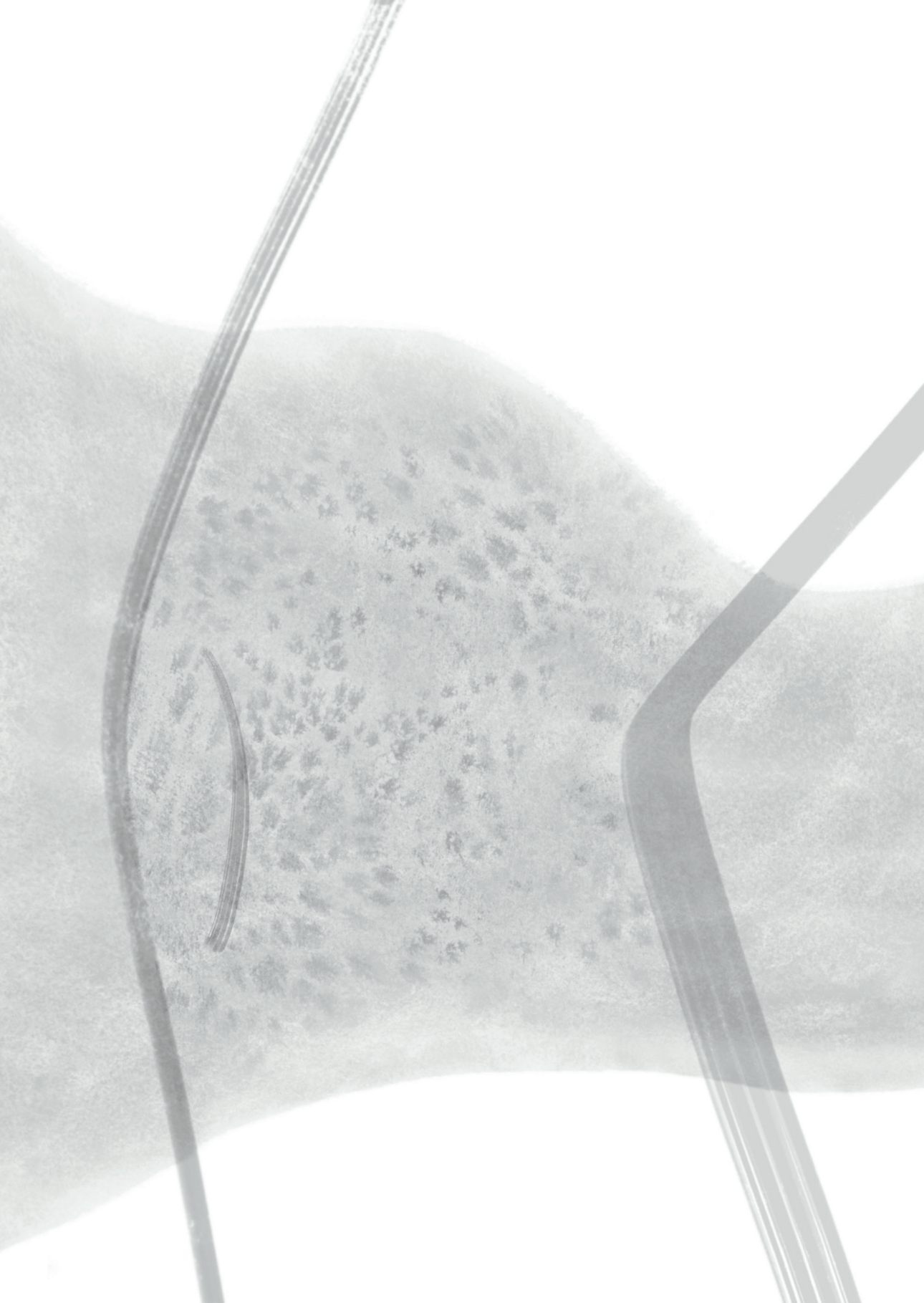

Chapter 10

Appendices

APPENDIX TO CHAPTER 2, 3 AND 4

Table 1. Definitions of progression, improvement and unchanged status of main MOAKS features(1).

Features described in MOAKS	Progression	Improvement	No change
BMLs without cyst at baseline	- Incidence of one or more cysts *or* - increase in the size of the BML *or* - an increase in the number of BMLs when there is no change in the size of the BML	No cyst at follow-up and: - a decrease in the size of the BML *or* - a decrease in the number of BMLs when there is no change in the size of the BML	- No cyst at follow-up *and* - no change in size of the BML *and* - no change in number of BMLs
BMLs with cyst at baseline	One or more cysts at follow-up and: - an increase in the size of the BML *or* - an increase in the percentage of the lesion that is BML when there is no change in the size of the BML *or* - an increase in the number of BMLs when there is no change in the size of the BML or percentage of the lesion that is BML	- No cysts at follow-up *or* - one or more cysts at follow-up and: - a decrease in the size of the BML *or* - an decrease in the percentage of the lesion that is BML when there is no change in the size of the BML *or* - a decrease in the number of BML when there is no change in the size of the BML or the percentage of the lesion that is BML	- One or more cysts at follow-up *and* - no change in size of the BML *and* - no change in percentage of the lesion that is BML *and* - no change in the number of BMLs
Cartilage defects	- an increase in the percentage of full-thickness cartilage loss *or* - an increase in the size of any cartilage loss when there was no change in the percentage of full-thickness cartilage loss.	- a decrease in the percentage of full-thickness cartilage loss *or* - an decrease in the size of any cartilage loss when there was no change in the percentage of full-thickness cartilage loss	- No change in the percentage of full-thickness cartilage loss *and* – no change in the size of any cartilage loss
Osteophytes	- an increase in score for an osteophyte scored ≥ 2 at baseline *or* - a score ≥ 2 at follow-up for an osteophyte with a score < 2 at baseline	- an decrease in score for an osteophyte scored ≥ 2 at baseline	- a score <2 at baseline and follow-up *or* - no change in score for osteophytes scored ≥ 2 at baseline
Meniscal pathologies	- an increase in score of hypertrophy, cysts, partial maceration, complete maceration, progressive maceration, vertical tear, horizontal tear, complex tear, or root tear *or* - an increase in the score of signal when there is no improvement in any of the hypertrophy, cyst, maceration or tear scores*.	- a decrease in the score of hypertrophy when there is no increase in the cysts, maceration and tears scores *or* - a decrease in the score of cyst when there is no increase in the hypertrophy, maceration and tears scores *or* - a decrease in one of the maceration scores when there is no increase in hypertrophy, cyst, the other macerations and tear scores *or* - a decrease in one of the tear scores when there is no increase in hypertrophy, cyst, maceration and other tear scores *or* - a decrease in signal when there is no increase in hypertrophy, cyst, maceration and tear scores.	- No change in score of hypertrophy, cysts, partial maceration, complete maceration, progressive maceration, vertical tear, horizontal tear, complex tear, root tear, or signal
Meniscal extrusion	- an increase in extrusion score	- a decrease in extrusion score	- No change in extrusion score

*Since meniscal signal would then be regarded as a sequelae of the healing process. BML: bone marrow lesion.

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APPENDIX TO CHAPTER 3 AND 8

Appendix 1.

Philips Intera (1.5 T).

Slice direction	Sequence type	Weighting	Fat saturation	Slice thickness (mm)	Slice gap (mm)	TR	TE	NEX	ETL	Flip angle (degrees)	Matrix	Field of view (FOV) (cm)
Sagittal	TSE	Proton density	No	3.0	0.3	1460	24	2	4	90	512x512	16.0
Coronal	TSE	Proton density	No	3.0	0.3	1520	24	2	4	90	512x512	16.0
Coronal (SPIR)	TSE	T2	Yes	5.0	0.5	2790	70	4	9	90	512x512	18.0
Axial	Dual echo SE	Proton density and T2	No	4.5	0.8	2622	12.5/100	1	10	90	256x256	18.0
Sagittal	3D GE (WATS)	T1	Yes	3.0	0.0	22.5	11.3	2	0	25	512x512	16.0

Siemens Magnetom Essenza (1.5 T)

Slice direction	Sequence type	Weighting	Fat saturation	Slice thickness (mm)	Slice gap (mm)	TR	TE	NEX	ETL	Flip angle (degrees)	Matrix	Field of view (FOV) (cm)
Sagittal	TSE	Proton density	No	3.0	0.6	2700	27	1	6	150	320x320	16.0
Coronal	TSE	Proton density	No	3.0	0.6	2700	27	1	6	150	320x320	16.0
Coronal	TSE	T2	Yes	3.0	0.6	5030	71	2	12	150	256x192	16.0
Axial	Dual echo SE	Proton density and T2	No	3.0	0.6	3500	25/74	1	5	150	256x256	16.0
Sagittal	3D GE (WATS)	T1	Yes	1.5	0.0	21.4	8.0	1	1	25	320x320	16.0

SE = spin echo; TSE = turbo spin echo; GE = gradient echo; TR = repetition time; TE = echo time; NEX = number of excitations; ETL = echo train length; SPIR = Spectral Presaturation by Inversion Recovery.

APPENDIX TO CHAPTER 4

Appendix 2. Accepted propositions for definition of OA on MRI after Delphi voting completion[1]

Definitions

1. Tibiofemoral MRI OA: The presence of both group [A] features or one group [A] feature and two or more group [B] features.

* Group [A] after exclusion of joint trauma within the last 6 months (by history) and exclusion of inflammatory arthritis (by radiographs, history and laboratory parameters):
  - i) Definite osteophyte formation
  - ii) Full thickness cartilage loss

* Group [B]:
  - i) Subchondral bone marrow lesion or cyst not associated with meniscal or ligamentous attachments
  - ii) Meniscal subluxation, maceration or degenerative (horizontal) tear
  - iii) Partial thickness cartilage loss (where full thickness loss is not present)
  - iv) Bone attrition

2. Patellofemoral MRI OA (involving the patella and/or anterior femur):
  - i) A definite osteophyte
  - ii) Partial or full thickness cartilage loss

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APPENDIX TO CHAPTER 7

Appendix table 1. Multivariable models in prediction of incident symptomatic knee OA and internal validation (calibration and discrimination) of the risk prediction models.

	Basic model		**Backward model**	
Selected predictors*	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**
Age (years)	1.07 (0.98 - 1.17)	0.15	**1.14 (1.02 – 1.28)**	**0.02**
BMI (kg/m²)	**1.12 (1.04 - 1.20)**	**<0.01**	**1.16 (1.06 – 1.26)**	**0.001**
Mild knee symptoms[5]			1.73 (0.85 – 3.49)	0.13
Knee problems while climbing stairs[7]			**2.08 (1.03 – 4.23)**	**0.04**
Morning stiffness[8]			2.46 (0.97 – 6.25)	0.06
Postmenopausal status			0.54 (0.26 – 1.12)	0.10
Physically demanding work[4]			2.38 (0.83 – 6.83)	0.11
AUC of the model	0.63 (0.54 - 0.71)		0.72 (0.64 – 0.80)	
Calibration: Hosmer-Lemeshow p Value	0.96		0.20	
Variance explained (Nagelkerke)(%)	6.5		21.9	

OR = odds ratio; BMI = Body Mass Index; AUC = Area under the curve of the receiver operating characteristic. Bold indicates $p < 0.05$. *see Table 1 main document for variable definitions.

APPENDIX TABLE 2.

External validation for the prediction of incident symptomatic knee OA in Rotterdam Study-III-1.

	Study population (n=264)	
Selected predictors*	**OR (95% CI)**	**P Value**
Age (years)	1.13 (0.93 – 1.37)	0.22
BMI (kg/m^2)	1.09 (0.97 – 1.22)	0.15
Mild knee symptoms[5]	1.53 (0.49 – 5.08)	0.44
Knee problems while climbing stairs[7]	**4.47 (1.31 – 15.23)**	**0.02**
Morning stiffness[8]	2.14 (0.68 – 6.73)	0.19
Postmenopausal status	1.33 (0.22 – 7.98)	0.76
Physically demanding work**	1.50 (0.50 – 4.53)	0.48
AUC of the model	0.81 (0.71 – 0.90)	
Calibration: Hosmer-Lemeshow p Value	0.17	
Variance explained (Nagelkerke)(%)	21.9	

OR = odds ratio; BMI = Body Mass Index; AUC = Area under the curve of the receiver operating characteristic. Bold indicates $p < 0.05$. *see Table 1 main document for variable definitions. **Defined as doing intense work (regularly lifting heavy objects at work).

APPENDIX TO CHAPTER 8

Table 1.
Baseline characteristics for the included study participants 6.5 years.

	Overall	Complete data at 6.5 years
N-subjects	**407**	**227**
Age (years, IQR)	55.2 (5.5)	55.2 (5.4)
BMI (kg/m^2, IQR)	31.2 (5.3)	30.9 (4.9)
Postmenopausal status (%)	275 (67.6)	154 (67.8)
N-knees	**814**	**452**
Previous knee injury (%)	101 (12.4)	57 (12.6)
Varus malalignment (%)	323 (39.7)	182 (40.3)
Morning stiffness < 30 minutes[b] (%)	116 (14.3)	55 (12.2)
None	526 (64.6)	282 (62.4)
Mild	170 (20.9)	115 (25.4)
Moderate	90 (11.1)	47 (10.4)
Severe	22 (2.7)	8 (1.8)
Extreme	4 (0.5)	0
Swollen knee[c] (%), any	92 (11.3)	52 (11.5)
Never	720 (88.5)	400 (88.5)
Rarely	70 (8.6)	44 (9.7)
Sometimes	16 (2.0)	6 (1.3)
Often	2 (0.2)	2 (0.4)
always	4 (0.5)	0
KLG 0 (%)	410 (50.4)	219 (48.5)
KLG 1 (%)	349 (42.9)	213 (47.1)[d]
KLG ≥ 2 (%)	51 (6.3)	20 (4.4)[d]
ACR (clinical and radiographic)(%)	32 (3.9)	17 (3.8)
MRI structural features		
Inflammation (effusion/Hoffa)[c] (%)	113 (13.9)	70 (15.5)
Effusion-synovitis (%)	96 (11.8)	61 (13.5)
Hoffa-synovitis (%)	20 (2.5)	11 (2.4)
Cartilage defects (%)	537 (66.0)	316 (69.9)
Bone marrow lesions (%)	488 (60.0)	295 (65.3)[d]
Osteophytes (%)	186 (22.9)	100 (22.1)
Meniscal pathologies (medial and/or lateral) (%)	507 (62.3)	283 (62.6)
Meniscal extrusions (medial and/or lateral) (%)	413 (50.7)	233 (51.5)

KLG = Kellgren and Lawrence grade; ACR = the American College of Rheumatology.

[b] Morning stiffness, evaluated with KOOS subscale on 'Stiffness', was present when the women had moderate, much or very much knee joint stiffness after sleeping.

[c] Swollen knee, evaluated with KOOS subscale on 'Symptoms', was present when the women had any swelling of the knee during the last week.

[d] Included participants differed significantly with participants with incomplete follow-up at 6.5 years ($p < 0.05$).
[e] inflammation = presence of any effusion-synovitis (small, medium or large) and/or any Hoffa-synovitis (mild, moderate or severe), dichotomized in presence vs. absence.

Table 2. Association between inflammation on MRI, patient-reported morning stiffness or patient-reported swelling of the knee at baseline and radiographic (KLG≥2) and ACR (clinical and radiographic) knee OA at follow-up.

		Outcome measures			
	Time point (year)	**Incident radiographic OA OR (95% CI)**	***p* Value**	**Incident ACR OA^ OR (95% CI)**	***p* Value**
MRI structural feature					
Inflammation (effusion- and/or Hoffa-synovitis)*	2.5 (unadj.)	**4.76 (2.20 – 10.30)[a]**	**< 0.001**	1.28 (0.58 – 2.83)[b]	0.54
	2.5 (adj.)	**4.03 (1.89 – 8.58)[c]**	**< 0.001**	0.93 (0.42 – 2.02)[d]	0.85
	6.5 (unadj.)	**3.27 (1.76 – 6.09)[e]**	**< 0.001**	1.74 (0.96 – 3.18)[f]	0.07
	6.5 (adj.)	**3.23 (1.56 – 6.68)[g]**	**0.002**	1.51 (0.79 – 2.90)[h]	0.22
KOOS question					
Morning stiffness**	2.5 (unadj.)	0.96 (0.33 – 2.82)[i]	0.95	**3.73 (1.72 – 8.10)[j]**	**0.001**
	2.5 (adj.)	0.84 (0.29 – 2.46)[k]	0.75	**3.62 (1.51 – 8.68)[l]**	**0.004**
	6.5 (unadj.)	**2.16 (1.01 -4.61)[m]**	**0.05**	**2.79 (1.09 -7.16)[n]**	**0.032**
	6.5 (adj.)	2.20 (0.98 – 4.95)[o]	0.06	**2.69 (1.01 – 7.12)[p]**	**0.047**
Swelling of knee***	2.5 (unadj.)	**2.85 (1.17 – 6.96)[a]**	**0.02**	1.77 (0.81 – 3.90)[b]	0.15
	2.5 (adj.)	2.80 (0.99 – 7.89)[c]	0.05	1.83 (0.80 – 4.20)[d]	0.15
	6.5 (unadj.)	**2.34 (1.05 – 5.21)[e]**	**0.04**	0.93 (0.30 – 2.90)[d]	0.90
	6.5 (adj.)	**2.50 (1.08 – 5.77)[g]**	**0.03**	0.96 (0.30 – 3.09)[h]	0.96

OA = osteoarthritis; OR = odds ratio; ^ ACR OA = the combined clinical and radiographic ACR criteria: frequent knee pain and a definite tibiofemoral (TF) osteophyte and one of the following: age > 50 years, morning stiffness < 30 minutes, crepitus on active knee motion

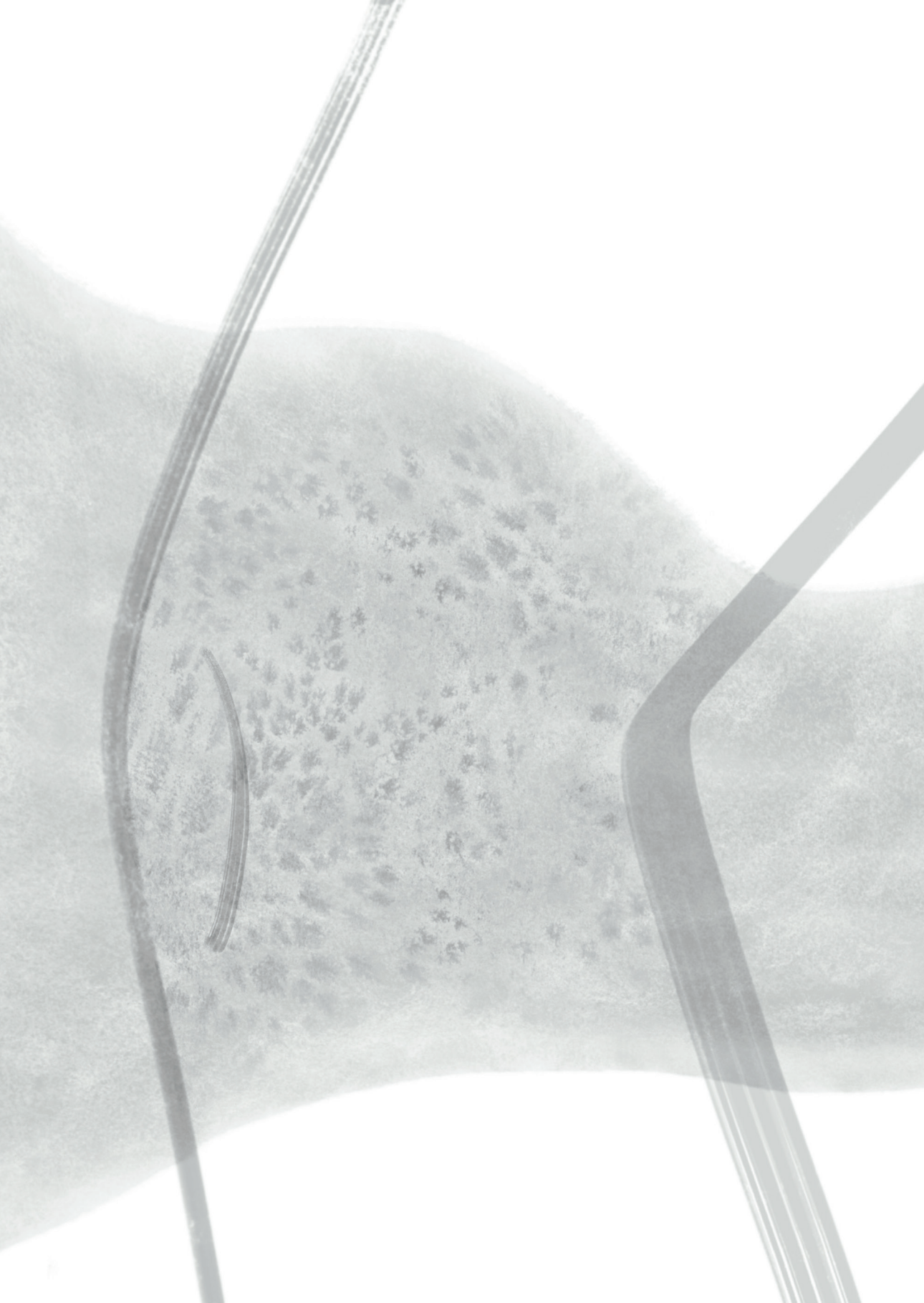
* Inflammation = presence of any effusion-synovitis (small, medium or large) and/or any Hoffa-synovitis (mild, moderate or severe), dichotomized in presence vs. absence.

** evaluated with KOOS subscale on 'Stiffness', was present when the women had moderate, much or very much knee joint stiffness after sleeping.

***evaluated with KOOS subscale on 'Symptoms', was present when the women had any swelling of the knee during the last week.

Unadj. = unadjusted; Adj. = Adjusted for age, body mass index (BMI), varus alignment, previous knee injury, postmenopausal status, presence of bone marrow lesions, cartilage defects, osteophytes, meniscal abnormalities, meniscal extrusion and randomization groups of the PROOF study.

[a]number of included knees is n = 643, [b]n = 655, [c]n = 604, [d]n = 616, [e]n = 432, [f]n = 435, [g]n = 406, [h]n = 408, [i]n = 644, [j]n = 656, [k]n = 613, [l]n = 624, [m]n = 432, [n]n = 435, [o]n = 411, [p]n = 413



Chapter 11

Summary

Worldwide, osteoarthritis (OA) is the most frequent form of arthritis, with more than 300 million cases reported in 2017. Its prevalence is higher in women and increases with age. It is one of the leading causes of disability among older adults. Its burden on healthcare use and costs is expected to rise due to the aging populations and the obesity epidemic. Clinically, knee OA is the most common form of OA, next to hand and hip OA. The disease is characterised by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function. This ultimately leads to joint pain, swelling and stiffness. Management is focused on reducing disability and controlling pain, but there is no curative treatment for knee OA. Given the estimated increase in knee OA prevalence, the primary prevention and the prediction of those at highest risk for knee OA are urgently needed. In the last years, magnetic resonance imaging (MRI) has become the most utilized and recommended imaging modality for knee OA in scientific research. It is able to detect early OA features in asymptomatic persons without radiographic knee OA. Therefore, this modality was also used as outcome measure in several studies of this thesis. The results described in this thesis were based on the PROOF study (PRevention of knee Osteoarthritis in Overweight Females) population. The PROOF study was, and still is, the first randomized controlled trial in the prevention of knee OA. The aim of the PROOF study was to study the preventive effects of a diet-and-exercise program and of oral crystalline glucosamine sulfate in women aged 50-60 years with a body mass index (BMI) ≥27kg/m^2. The 2.5-years follow-up results on the primary outcome measure, incidence of clinical and radiographic knee OA, have been described in a previous thesis by Jos Runhaar[1].

In the light of prevention and risk prediction, the main objectives of this thesis were as follows: 1) investigate the 2.5-years effects of the PROOF interventions and the 2.5-years effects of weight change on knee OA features on MRI; 2) assess the long-term effects of the PROOF interventions on incidence of clinical and radiographic knee OA; 3) evaluate the potency of the urinary Coll2-1NO_2 biochemical marker in detecting early knee OA; 4) investigate prediction of incident knee pain and incident knee OA using clinical signs and symptoms and MRI OA features as predictors.

Chapter 1 gives a general introduction to knee OA and the PROOF study and describes the main aims of this thesis. **Chapter 2** describes the 2.5-years follow-up effects of the PROOF interventions on different structural MRI OA features. We found that the baseline prevalence of the different MRI OA features, assessed with the MRI Osteoarthritis Knee Score (MOAKS), was high, ranging from 24% for osteophytes to 70% for cartilage defects. The diet-and-exercise program intervention resulted in significantly less progression of meniscal extrusion (13%) compared to the diet-and-exercise control group (21%). The other MRI OA features that were evaluated were cartilage defects, bone marrow lesions, osteophytes and other meniscal abnormalities. Progression of these features was not significantly influenced by the diet-and-exercise program intervention, glucosamine sulphate or their combination.

In **Chapter 3** we evaluated the 2.5-years follow-up effects of weight change on the progression of MRI OA features. Study participants were classified into a subgroup with steady weight, weight gain or weight loss with a technique called latent class growth analysis. This technique revealed the three subgroups with different weight change trajectories over time. A more than 2.5-fold increase in progression of synovitis, inflammation of the synovium, was found in women with steady weight gain (18%) compared to those with stable weight (7%) over time. Although not statistically significant, large effect sizes were also found for the difference in progression of patellofemoral bone marrow lesions and cartilage defects between the weight gain and stable weight subgroups. The high baseline prevalence of OA MRI features indicates that the development of these features must have started before the age of 50 - 60 years. This gives rise to the question what the impact is of weight status earlier in life. Therefore, in **Chapter 4**, we explored the effects of differences in body weight between the age of 40 years and the age at enrolment in the PROOF study on the prevalence of MRI knee OA (a Delphi consensus definition). At 40 years, 20% of the women had normal weight, 55% of them had overweight and 25% had obesity. The prevalence of MRI OA ranged from 16% in those with normal weight at 40 years and overweight at enrolment in the PROOF study, to 44% in those obese at both time points. So, those with a higher BMI at 40 years showed higher prevalence of MRI knee OA ±15 years later. When comparing the prevalence of MRI knee OA between the different weight status groups, suggestions were made for the optimal timing for a preventive weight loss strategy on the development of (MRI) OA. Since all women in the PROOF study were overweight and obese and there was no 'normal weight group' at baseline, it was not possible to precisely determine the optimal time period for weight loss. We suggested that the greatest influence on the risk of knee OA might not be weight loss, but rather a life-long prevention of becoming overweight or obese.

Chapter 5 presents the long-term effects (6.5 years) of the PROOF interventions on incident clinical knee OA. The diet-and-exercise intervention and the glucosamine sulphate intervention showed no significant effect on the long-term incidence of knee OA. For the diet-and-exercise intervention a trend was found that indicated a possibility of a significant preventive effect, if the compliance rate for the intervention would have been higher. This was not found for the glucosamine intervention. In addition, the effect of moderate weight loss in the first year of the study on the incidence of clinical knee OA after 6.5 years was evaluated. This demonstrated that losing ≥5 kg or ≥5% of baseline weight in the first 12 months resulted in a 3.0 times reduction in incident clinical knee OA (7% vs. 21% incidence) and a 2.5 times reduction in radiographic knee OA development (6% vs. 16% incidence) after 6.5 years. The conclusions should be interpreted with caution, since there was a lot of missing data and the compliance rates were low.

In **Chapter 6** we explored the potency of the biochemical marker Coll2-1NO_2, seen as a marker of inflammation, in detecting disease activity in an early phase. We found that

lower baseline urinary Coll2-1NO_2 levels were significantly associated with increased risk of incidence of knee OA after 2.5 years. This was a counterintuitive outcome, as we had hypothesized that not lower but higher concentrations would have been associated with increased risk of incident knee OA. The cross-sectional association between uColl2-1NO_2 at 2.5 years and prevalent knee OA at 2.5 years and the association between the change of uColl2-1NO_2 and incident knee OA at 2.5 years were not statistically significant. We emphasized the importance of early detection of knee OA, but showed that the role of uColl2-1NO_2 needs more validation.

Chapter 7 assessed a risk prediction model for general practitioners with easy-obtainable questionnaire and physical examination variables for incident frequent knee pain and symptomatic knee OA. A basic model, with only age and BMI had little discriminative power. With the variables age, BMI, mild knee symptoms, knee problems while climbing stairs, morning stiffness, postmenopausal status and physically demanding work, the area under the curve of the prediction model for incident frequent knee pain increased to 0.71 (0.63 to 0.78). Similar results were found for the prediction model of incident symptomatic knee OA. Since the discriminative ability of the prediction models was moderate, prediction seems not yet clinically applicable. We discuss that the results could be of important value to enrich the study populations of preventive trials for knee OA and improve their feasibility. In **Chapter 8** the predictive performance of inflammation on MRI and of patient-reported knee swelling and morning stiffness were compared for the prediction of incident radiographic and incident clinical knee OA after 2.5 and 6.5 years. Inflammation on MRI had the best predictive performance for incident radiographic knee OA. For the prediction of clinical knee OA, patient-reported morning stiffness had slightly better predictive performance than inflammation on MRI and patient-reported swelling. We suggested that patient-reported morning stiffness has the potential to be a helpful factor for the selection of high-risk individuals in order to increase the feasibility of preventive trials in knee OA.

Finally, **Chapter 9** provides an overview of the main findings of this thesis and a general interpretation of the results in the light of existing knowledge. In addition, it discusses the clinical implications of the results and provides recommendations for future research. With the studies in this thesis, we have tried to contribute in the process towards prevention of knee OA and in the improvement of prediction of this disease. Continued efforts remain necessary to make the prevention of knee OA just as generally accepted as it is for other chronic diseases.

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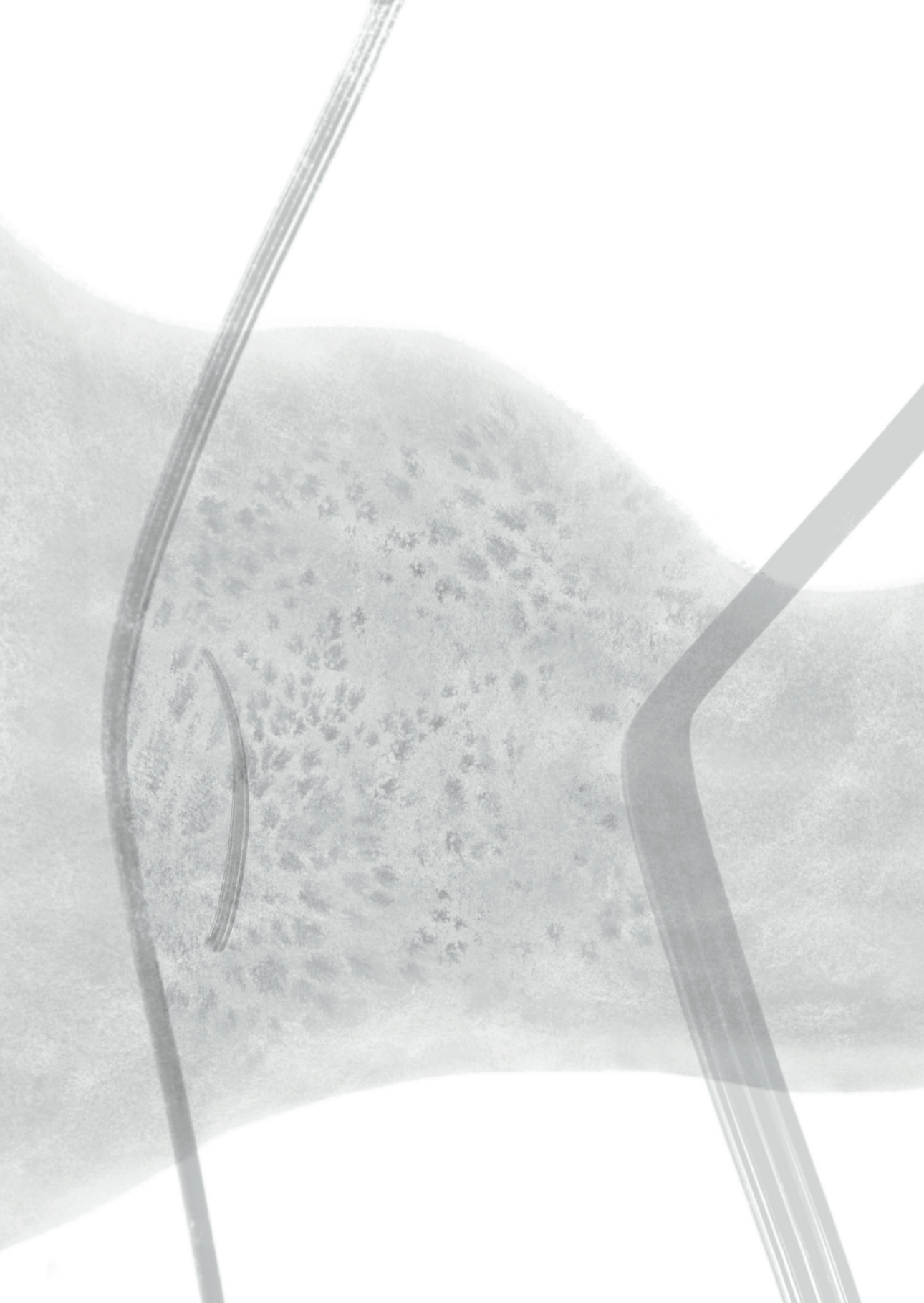

Samenvatting

Artrose is wereldwijd de meest voorkomende gewrichtsaandoening, met een geschatte prevalentie in 2017 van meer dan 300 miljoen. De prevalentie is hoger bij vrouwen en neemt toe met de leeftijd. Artrose is een van de belangrijkste oorzaken van functionele beperking onder ouderen. Als gevolg van de vergrijzing en de obesitas-epidemie, zullen zorgkosten ten gevolge van artrose fors toenemen. Knieartrose is naast hand- en heupartrose de meest voorkomende vorm van artrose. De ziekte wordt gekenmerkt door kraakbeenafbraak, subchondrale botomvorming en botcysten, osteofytvorming en ontsteking van het gewrichtsslijmvlies en leidt tot verlies van de normale gewrichtsfunctie. Dit leidt uiteindelijk tot gewrichtspijn, zwelling en stijfheid. De behandeling is gericht op het verminderen van functionele beperking, het onder controle houden van pijn en verbeteren van kwaliteit van leven, maar er is geen curatieve behandeling voor knieartrose. Gezien de voorspelde toename van de prevalentie van knieartrose zijn de preventie van knieartrose en het in kaart brengen van personen met het grootste risico op knieartrose, van groot belang. Sinds een aantal jaar is MRI (magnetic resonance imaging) de meest gebruikte en aanbevolen beeldvorming in het wetenschappelijk onderzoek naar knieartrose. MRI is in staat om vroege artrose kenmerken te detecteren bij asymptomatische personen zonder afwijking op de röntgenfoto. Daarom werd deze beeldvormingsmodaliteit ook toegepast als uitkomstmaat voor verschillende studies in dit proefschrift. De resultaten die in dit proefschrift worden beschreven komen voort uit de PROOF studie (PRevention of knee Osteoarthritis in Overweight Females). De PROOF studie was en is de eerste en enige gerandomiseerde, gecontroleerde trial naar preventie van knieartrose. Het doel van de PROOF studie was het bestuderen van de preventieve effecten van een dieet- en beweegprogramma en van glucosamine sulfaat bij vrouwen tussen de 50 en 60 jaar oud met een BMI ≥27kg/m^2. Het ontstaan van klinische en radiologische knieartrose na 2,5 jaar was de primaire uitkomstmaat van de PROOF studie. Deze resultaten zijn eerder beschreven in het proefschrift van Jos Runhaar[1].

In het kader van de preventie en het voorspellen van knieartrose, waren de doelstellingen van dit proefschrift als volgt: 1) het onderzoeken van de 2,5-jaars effecten van de PROOF interventies en van gewichtsverandering op het ontstaan van artrose kenmerken op MRI; 2) het onderzoeken van de lange-termijn effecten van de PROOF interventies op het ontstaan van klinische en radiologische knieartrose; 3) het evalueren van de rol van biomarker Coll2-1NO$_2$ bij het opsporen van vroege knieartrose; 4) het onderzoeken van de rol van demografische kenmerken, patiënt-gerapporteerde symptomen en van artrose kenmerken op MRI bij het voorspellen van het ontstaan van knieartrose.

Hoofdstuk 1 geeft een algemene inleiding op knieartrose en de PROOF studie en beschrijft de belangrijkste doelstellingen van dit proefschrift**. Hoofdstuk 2** beschrijft de 2,5-jaars effecten van de PROOF interventies op het ontstaan van artrose kenmerken op MRI. De artrose kenmerken werden gescoord met de MRI Osteoarthritis Knee Score (MOAKS). MOAKS kenmerken die werden geëvalueerd waren kraakbeendefecten, beenmergafwijkin-

gen, osteofyten, uitpuiling van de meniscus en andere meniscusschade. We ontdekten dat de prevalentie van de MRI kenmerken bij aanvang van de studie al hoog was, variërend van 24% voor osteofyten tot 70% voor kraakbeendefecten. Het dieet- en beweegprogramma resulteerde in minder progressie van de meniscusuitpuiling (13%) in vergelijking met de dieet- en beweeg controlegroep (21%). De progressie van de andere kenmerken werd niet significant beïnvloed door de dieet- en beweeginterventie, glucosamine sulfaat of hun combinatie. In **hoofdstuk 3** evalueren we de 2,5-jaars effecten van gewichtsverandering op de progressie van artrose kenmerken op MRI. De deelneemsters aan de studie zijn hierbij ingedeeld in een subgroep met een relatief stabiel gewicht, een subgroep met gewichtstoename en een subgroep met gewichtsverlies gedurende 2,5 jaar. Dit is gedaan met een techniek genaamd 'Latent Class Growth Analysis', waarbij drie subgroepen werden geïdentificeerd met een duidelijk verschillend beloop van gewichtsverandering. In de groep met gewichtstoename werd meer dan 2,5 keer zoveel progressie van synovitis (ontsteking van het gewrichtsslijmvlies) gevonden (18%) dan in de groep vrouwen met een stabiel gewicht gedurende 2,5 jaar (7%). Er werden ook grote verschillen gezien in de progressie van beenmergafwijkingen en kraakbeendefecten in het patellofemorale gewricht, maar deze verschillen waren statistisch niet significant. De hoge prevalentie van de artrose kenmerken op MRI bij aanvang van de studie geeft aan dat de ontwikkeling van deze kenmerken al begonnen moet zijn voor de leeftijd van 50 – 60 jaar. Dit leidt tot de vraag wat het effect van lichaamsgewicht op jongere leeftijd is op het ontstaan van knieartrose. In **hoofdstuk 4** onderzoeken we daarom de effecten van lichaamsgewicht op 40-jarige leeftijd en bij aanvang van de PROOF studie op de baseline prevalentie van knieartrose op MRI. Op 40 jarige leeftijd had 20% van de vrouwen een normaal gewicht, 55% van hen had overgewicht en 25% had obesitas. De prevalentie van knieartrose op MRI varieerde van 16% bij vrouwen met een normaal gewicht op 40 jarige leeftijd en overgewicht bij aanvang van de PROOF studie, tot 44% bij vrouwen met obesitas op beide tijdstippen. Degenen met een hogere BMI op 40 jarige leeftijd toonden bij eenzelfde gewicht op middelbare leeftijd een hogere baseline prevalentie van MRI knieartrose. Aangezien de vrouwen in de PROOF studie bij aanvang allemaal overgewicht of obesitas hadden en er geen 'normale' BMI groep was, was het niet mogelijk om een optimaal tijdstip voor een preventieve gewicht-reducerende interventie te bepalen.

In **hoofdstuk 5** worden de lange termijn effecten (6,5 jaar) van de PROOF interventies op het ontstaan van knieartrose gepresenteerd. De dieet- en beweeginterventie en de glucosamine sulfaat interventie toonden geen significant effect. Voor de dieet- en beweeginterventie werd wel een trend gevonden, wat inhoudt dat er mogelijk wel een significant preventief effect gevonden zou kunnen worden als de compliantie voor de interventie hoger zou zijn geweest. Voor de glucosamine sulfaat interventie werd dit niet gevonden. Ook werd het effect van gewichtsverlies in 1 jaar op het ontstaan van knieartrose na 6,5 jaar geëvalueerd. Hieruit bleek dat gewichtsverlies van ≥5kg of ≥5% gedurende het eerste jaar resulteerde in een lagere kans op het ontwikkelen van klinische knieartrose (7% vs. 21%) en radiografische knieartrose

(6% vs. 16%) na 6,5 jaar. Deze bevinding toont aan dat het in principe mogelijk lijkt om door gewichtsverlies knieartrose te voorkomen, maar moet wel met voorzichtigheid worden geïnterpreteerd aangezien er veel gegevens ontbraken en de compliantie laag was.

In **hoofdstuk 6** onderzoeken we de rol van de biochemische marker Coll2-1NO_2 in urine bij het opsporen van ziekteactiviteit in de vroege fase van knieartrose onder de deelnemers van de PROOF studie. Coll2-1NO_2 wordt gezien als een ontstekingsmarker. We vonden dat juist lagere aanvangswaardes van Coll2-1NO_2 significant geassocieerd waren met een verhoogd risico op het ontstaan van knieartrose na 2,5 jaar. We hadden verwacht dat niet lagere maar hogere aanvangsconcentraties geassocieerd zouden zijn met een verhoogd risico op het ontstaan van knieartrose. De associatie tussen de concentratie Coll2-1NO_2 op 2,5 jaar en de aanwezigheid van knieartrose op 2,5 jaar was niet significant, evenals de associatie tussen de verandering van Coll2-1NO_2 concentraties gedurende 2,5 jaar en het ontstaan van knieartrose. Het belang van vroege detectie van knieartrose is groot, maar biomarker Coll2-1NO_2 heeft zijn rol hierin (nog) niet bewezen.

Hoofdstuk 7 onderzoekt een predictiemodel voor frequente kniepijn en voor klinische knieartrose met verschillende gegevens uit vragenlijsten en lichamelijk onderzoek bij de deelneemsters van de PROOF studie. Een basismodel met alleen de gegevens over leeftijd en BMI heeft nauwelijks onderscheidend vermogen. Een model met de gegevens over leeftijd, BMI, milde knieklachten, knieproblemen bij traplopen, ochtendstijfheid, postmenopauzale status en fysiek zwaar werk had het beste voorspellende vermogen met een 'area under the curve' (AUC) van 0,71 (95% CI: 0,63 – 0,78). Het voorspellende vermogen is echter niet hoog genoeg om dit model te kunnen toepassen in de klinische praktijk. Wel kunnen de resultaten van deze studie van belang zijn voor het optimaliseren van de studiepopulatie van toekomstige onderzoeken naar de preventie van knieartrose.

In **hoofdstuk 8** worden tekenen van inflammatie op de MRI vergeleken met patiënt-gerapporteerde klachten van kniezwelling en ochtendstijfheid om radiografische en klinische knieartrose te kunnen voorspellen. Inflammatie op de MRI had de beste voorspellende waarde op het ontstaan van radiologische knieartrose. Ochtendstijfheid had de beste voorspellende waarde op het ontstaan van klinische knieartrose. Geen van beide patiënt-gerapporteerde klachten had genoeg voorspellend vermogen om gebruikt te kunnen worden in de klinische praktijk. Ochtendstijfheid heeft mogelijk wel de potentie om gebruikt te worden voor de selectie van hoog-risico individuen bij preventieve trials.

Tot slot geeft **hoofdstuk 9** een overzicht van de belangrijkste bevindingen van dit proefschrift en een algemene interpretatie van de resultaten in het licht van bestaande kennis op het gebied van preventie en predictie van knieartrose. Daarnaast wordt de klinische implicatie van de resultaten besproken en worden aanbevelingen gedaan voor toekomstig onderzoek.

Met de studies in dit proefschrift hebben we getracht een bijdrage te leveren aan de verbetering van preventie en voorspelling van knieartrose. Continue inzet zal nodig blijven om de preventie van knieartrose meer op de kaart te zetten, net zoals dat het geval is voor andere chronische ziektes.

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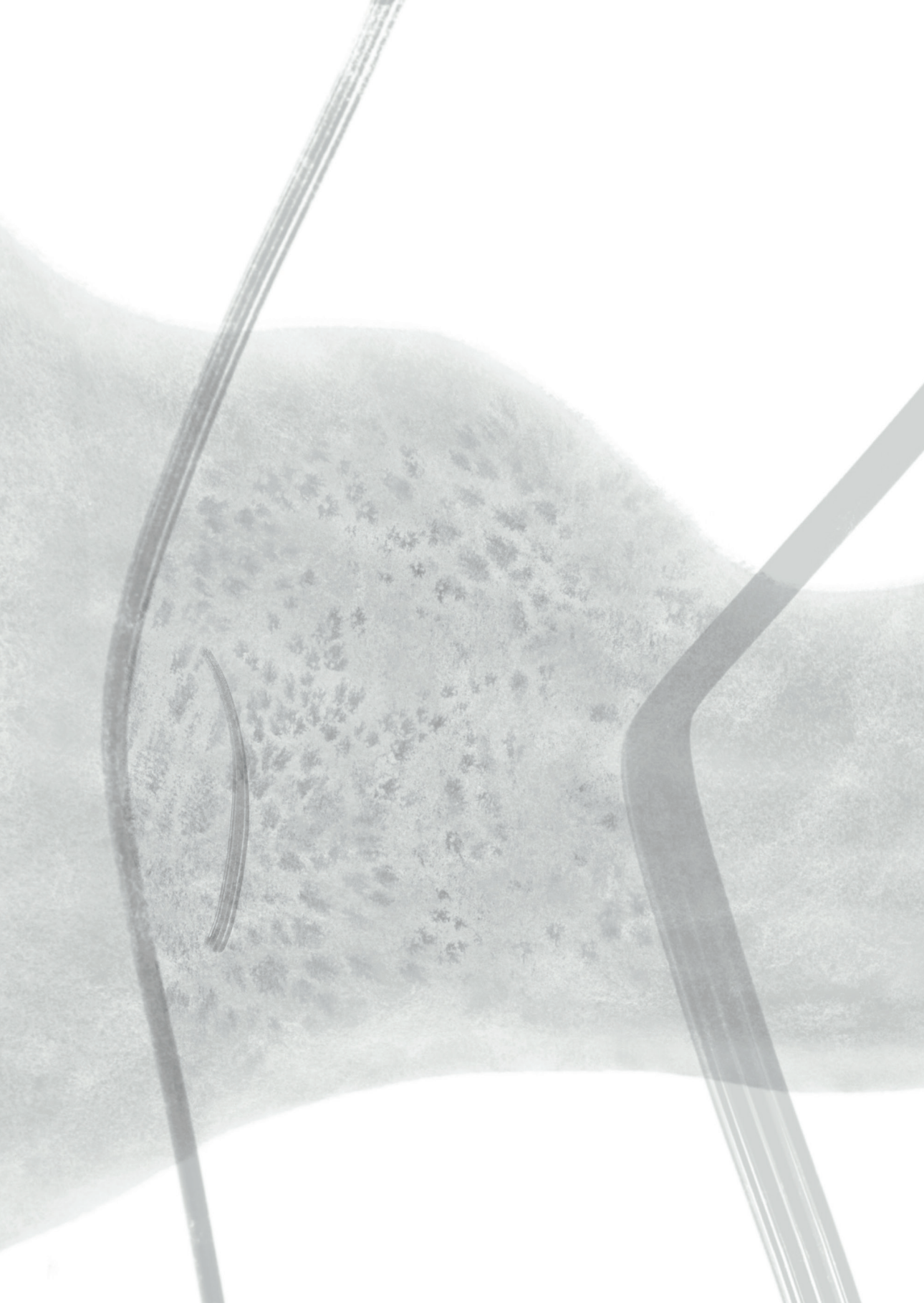
1. Runhaar, J., *Development and prevention of knee osteoarthritis: The load of obesity*. 2013, Erasmus University Rotterdam.



Addendum

Dankwoord

Begonnen in december 2012 als 'aiotho', schrijf ik nu in Corona tijd dit laatste stukje. Ik vond het een voorrecht dat ik de huisartsopleiding kon combineren met promotie onderzoek en daarbij ook de master klinische epidemiologie heb kunnen doen. Een aantal mensen wil ik heel graag bedanken voor hun bijdrage aan dit traject.

Ten eerste gaat mijn dank uit naar alle **deelneemsters, betrokken huisartsen, fysiotherapeuten en diëtisten** die hebben deelgenomen aan de PROOF studie. Jullie bereidwilligheid en inspanning heeft ertoe geleid dat deze studie kon worden uitgevoerd en dat de gegevens beschikbaar kwamen waarmee ik (en andere onderzoekers) het onderzoek kon uitvoeren. Daarmee zijn de eerste stappen gezet op het gebied van preventie van knieartrose! Speciale dank ook voor **Diana,** voor al haar werk als onderzoeksassistent van de PROOF studie. Zeer nauwkeurig heb jij alles bijgehouden en de deelneemsters in de studie weten te houden. Jouw bijdrage is daarmee enorm.

Mijn grote dank gaat uit naar mijn co-promotor **dr. Jos Runhaar**, de drijvende kracht achter de PROOF studie. **Jos**, wat heb ik een enorm geluk met jou als begeleiding. Bedankt voor het vertrouwen om met de door jou opgezette PROOF data te mogen werken. Je bent een onderzoeker in hart en nieren. Ik ben onder de indruk van je slimheid, snelheid en positiviteit. Je leerde me scherp formuleren, hoofd- en bijzaken scheiden en bood overzicht als ik dat even niet meer had. Als ik na een commentaar van reviewers dacht weer opnieuw te kunnen beginnen, liet jij me al snel het tegendeel inzien en kwam ik duizendmaal lichter uit ons overleg. Je bent en blijft altijd relaxt, dat is super fijn! Bedankt voor het her- en herlezen van stukken, het meedenken over tabellen, het schrappen en doorvragen. Door jou ben ik gegroeid als onderzoeker en heb ik dit proefschrift kunnen volbrengen. Zeer veel dank!

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Daarnaast gaat mijn dank uit naar mijn **co-auteurs**: Bart Koes, Bastiaan de Vos, Dammis Vroegindeweij, Dieuwke Schiphof, Edwin Oei, Gerjo van Osch, Marienke van Middelkoop, Max Reijman, Patrick Bindels, Peter van der Plas en Yves Henrotin. Veel dank voor de tijd die jullie gestoken hebben in het grondig lezen van mijn stukken, jullie input, stimulans en kritische feedback. Dit alles was van grote toegevoegde waarde. Bastiaan, mede PROOF

aiotho, samen hebben we geploeterd rondom de statistiek van de 'missing values'. Dank voor het schrijven van het stuk over de lange termijn resultaten van PROOF, dat ik heb opgenomen in dit proefschrift. Dieuwke, dank voor het uitzoeken en aanleveren van jouw data uit de Rotterdam Studie ter validatie van mijn predictiemodel. Patrick, bedankt voor je kritische vragen over de relevantie voor de huisartspraktijk. Hartelijke dank allemaal voor de fijne samenwerking!

Leden van de kleine commissie, prof.dr. Jacobijn Gussekloo, associate prof.dr. Max Reijman en prof.dr. Liesbeth van Rossum, veel dank voor het lezen en beoordelen van mijn proefschrift en jullie deelname in de oppositie.

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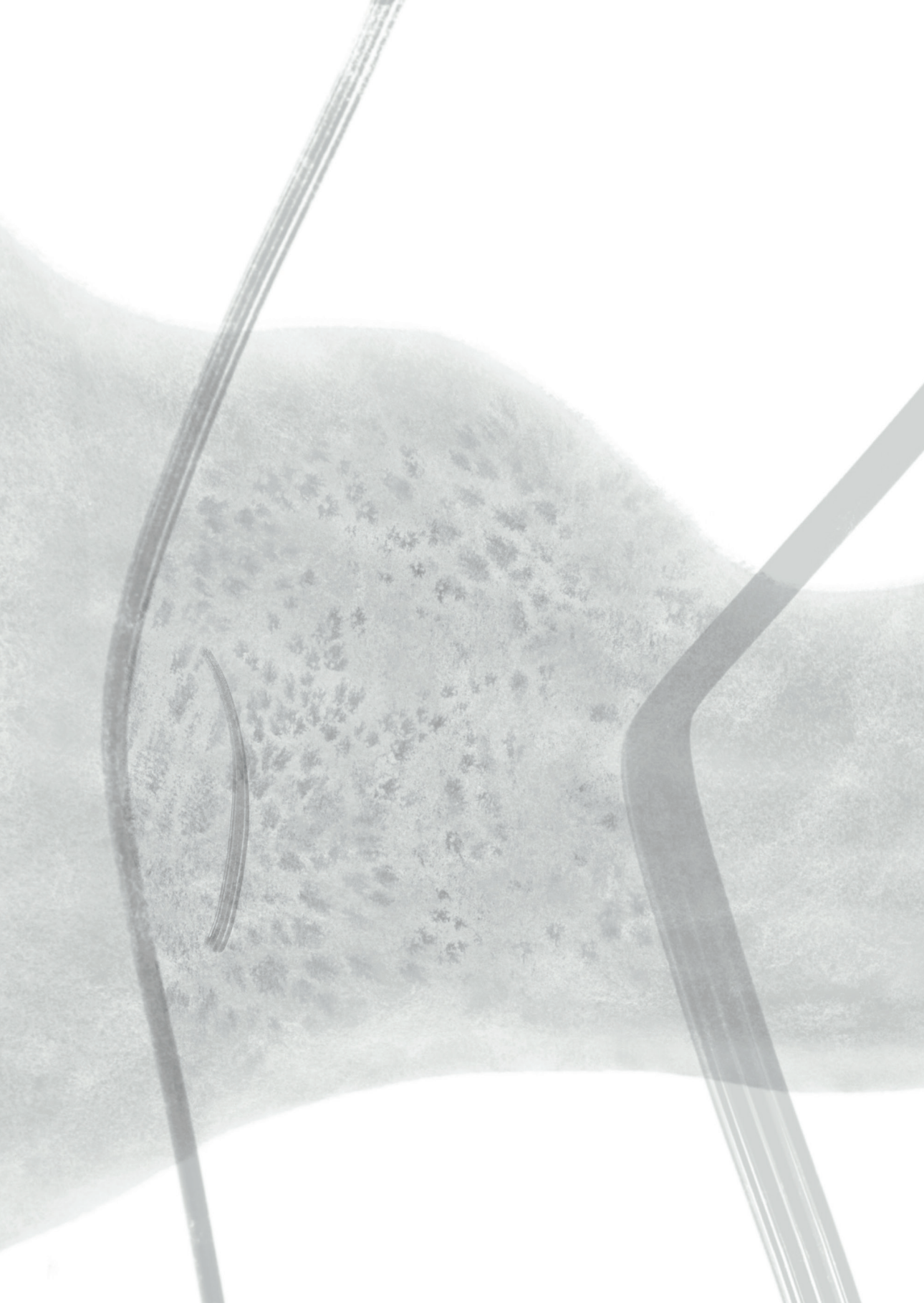


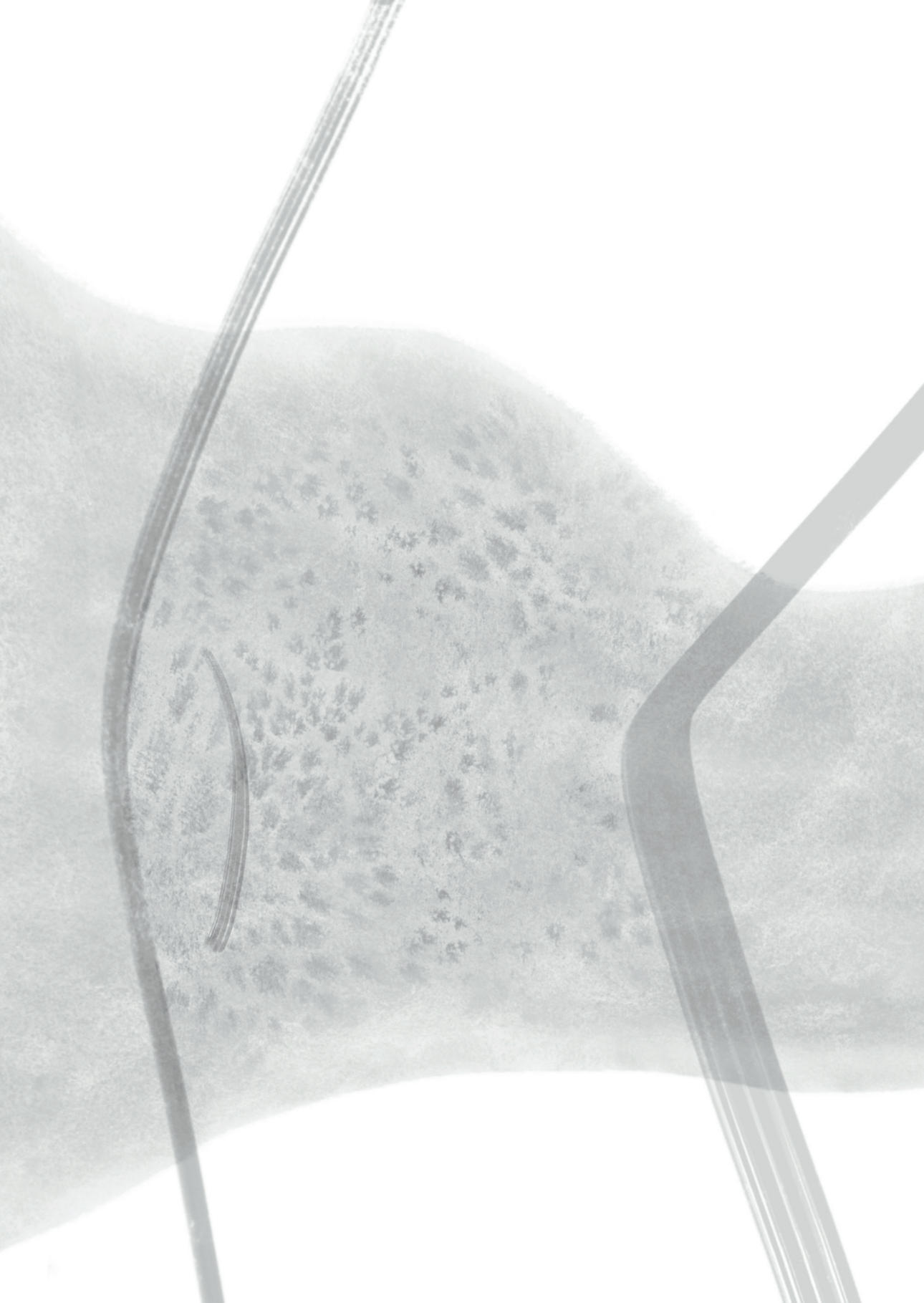
Portfolio

PhD Portfolio

Name PhD student: Marieke Landsmeer	**PhD period**: 2013 - 2021
Erasmus MC Department: General Practice	**Promotor**: S.M.A. Bierma-Zeinstra
Research School: NIHES	**Supervisor:** dr. J. Runhaar

	year	Workload (ECTS*)
PhD training		
General courses		
Master of Science in Clinical Epidemiology, NIHES, Rotterdam	2013 – 2016	70
BROK course ('Basiscursus Regelgeving en Organisatie voor Klinische onderzoekers'), Erasmus MC, Rotterdam	2015	1
Course on Scientific Integrity, Erasmus MC, Rotterdam	2014	0.3
Course on Scientific Writing, Maastricht University	2013	0.6
Professional education		
Vocational training for general practitioner, Erasmus MC, Rotterdam	2011 – 2017	
Presentations		
Oral		
NHG Wetenschapsdag, Rotterdam	2015	1
NHG Wetenschapsdag, Amsterdam	2016	1
Poster		
NAPCRG Annual Meeting, Ottawa, Canada	2013	1
Osteoarthritis Research Society International (OARSI) World Congress, Amsterdam	2016	1
OARSI World Congress, Liverpool	2018	1
NHG Wetenschapsdag, Amsterdam	2018	1
Seminars/workshops/conferences		
AIOTHO-conferences, the Netherlands	2013 – 2015	0.6
OARSI, Philadelphia	2013	1
WONCA, Praag	2013	1
NHG Wetenschapsdag, moderator poster session, Leiden	2013	1
Teaching		
Supervising practical meetings, tutoring		
EBM course for general practitioners	2015	0.2
Supervising writing and publication of letter to the editor by medical students	2013 – 2015	1
Moderator at mini-symposium of department	2018	0.3
Other		
Oral presentation at weekly work discussion meetings	2013 – 2016	0.6
Oral presentation at monthly interdisciplinary research group meetings	2014 – 2016	0.3
Oral presentation at department's mini-symposium	2018	0.3

1 ECTS (European Credit Transfer System) equals a workload of 28 hours.



List of publications

This thesis

Landsmeer ML, Runhaar J, Henrotin YE, Middelkoop van M, Oei EH, Vroegindeweij D, Reijman M, van Osch GJ, Koes BW, Bindels PJ, Bierma-Zeinstra SM. Association of urinary biomarker COLL2-1NO_2 with incident clinical and radiographic knee OA in overweight and obese women. Osteoarthritis Cartilage. 2015 Aug;23(8):1398-404.

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de Vos BC, Landsmeer MLA, van Middelkoop M, Oei EHG, Krul M, Bierma-Zeinstra SMA, Runhaar J. Long-term effects of a lifestyle intervention and oral glucosamine sulphate in primary care on incident knee OA in overweight women. Rheumatology (Oxford). 2017 Aug 1;56(8):1326-1334.

Landsmeer MLA, de Vos BC, van der Plas P, van Middelkoop M, Vroegindeweij D, Bindels PJE, Oei EHG, Bierma-Zeinstra SMA, Runhaar J. Effect of weight change on progression of knee OA structural features assessed by MRI in overweight and obese women. Osteoarthritis Cartilage. 2018 Dec;26(12):1666-1674.

Landsmeer MLA, Runhaar J, van Middelkoop M, Oei EHG, Schiphof D, Bindels PJE, Bierma-Zeinstra SMA. Predicting Knee Pain and Knee Osteoarthritis Among Overweight Women. J Am Board Fam Med. 2019 Jul-Aug;32(4):575-584.

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Nauta M, Landsmeer ML, Koren G. Codeine-acetaminophen versus nonsteroidal anti-inflammatory drugs in the treatment of post-abdominal surgery pain: a systematic review of randomized trials. Am J Surg. 2009 Aug;198(2):256-61.

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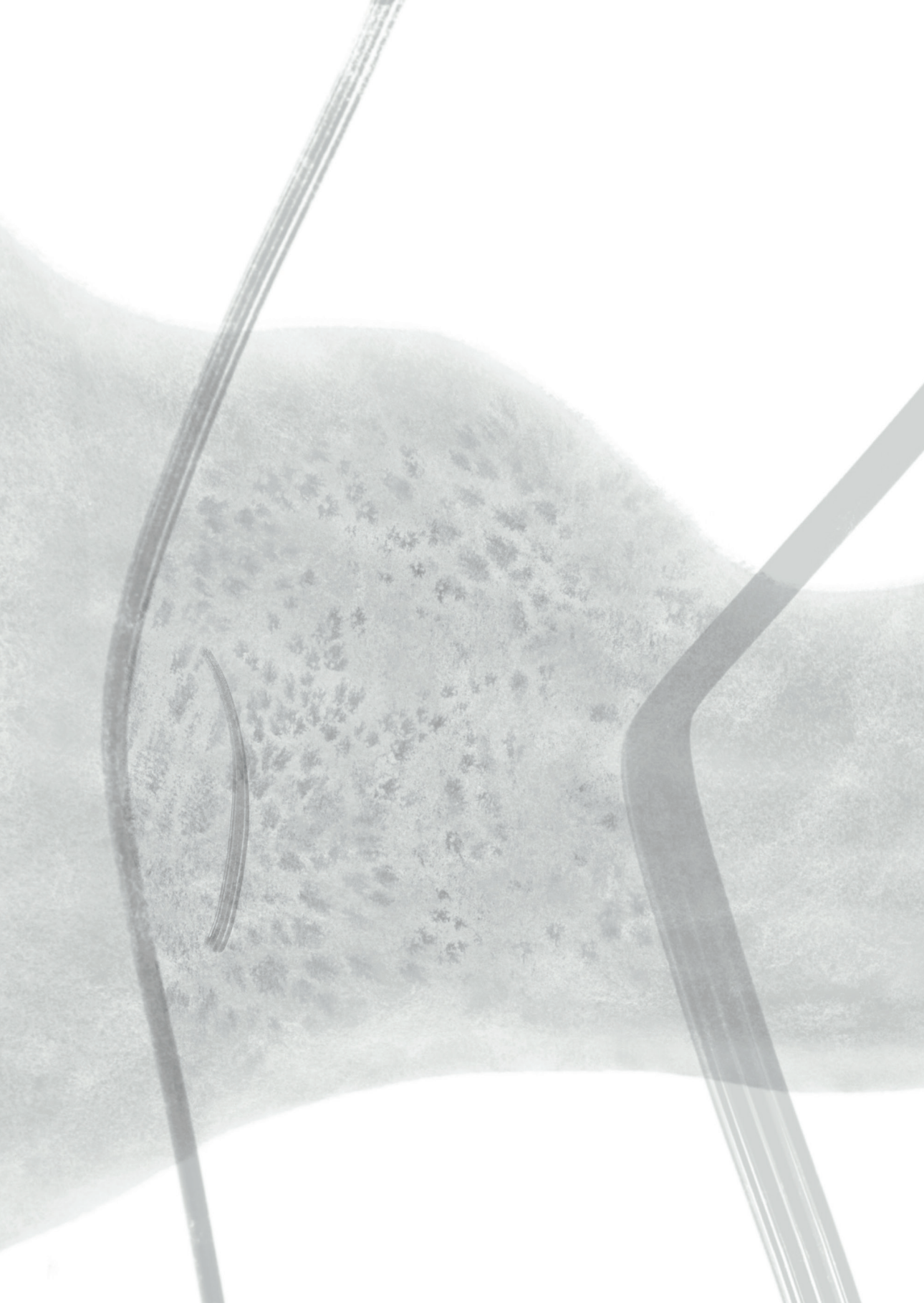
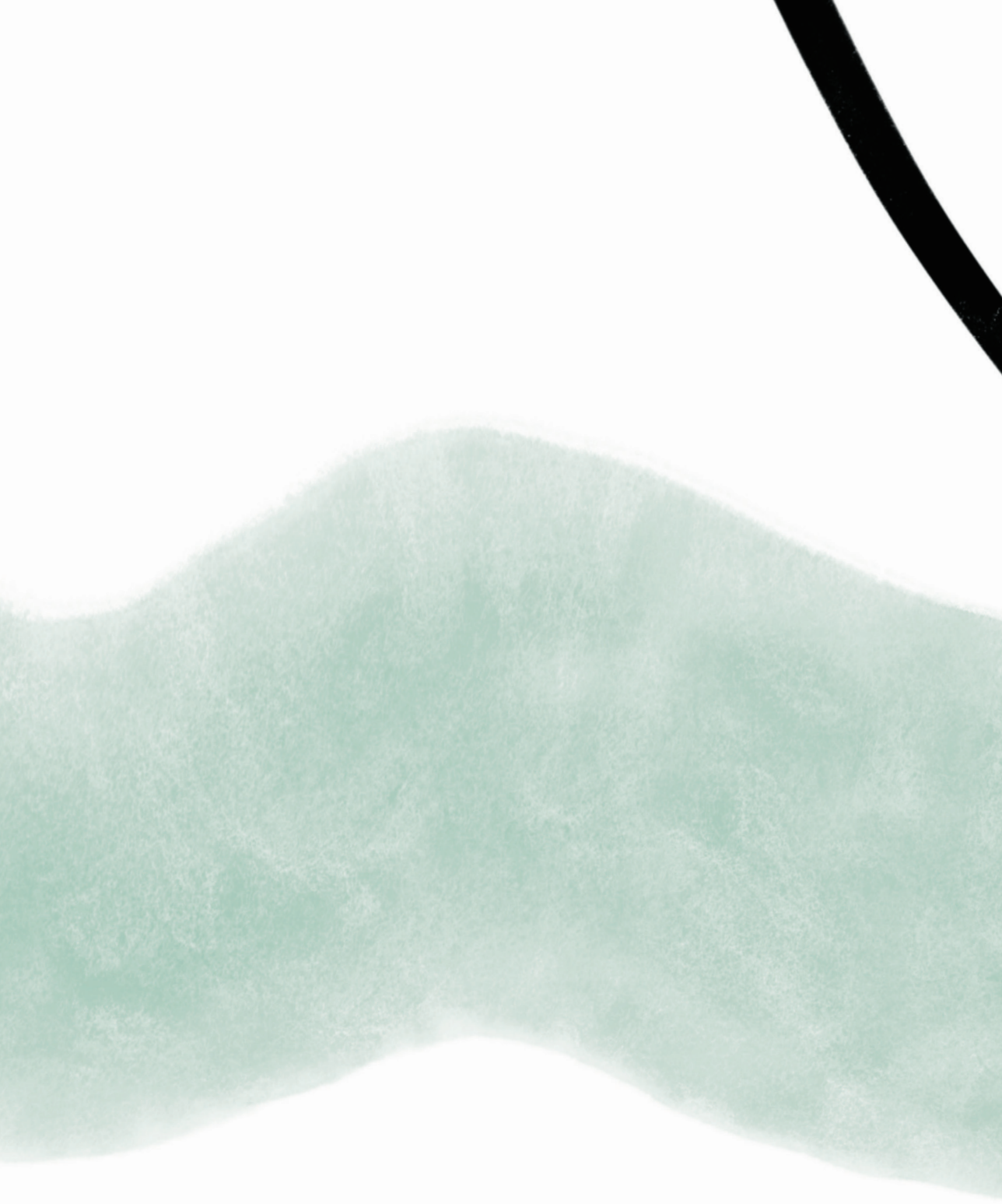
Sluimers D, Willemse NL, Landsmeer ML. Re: Magnesium Intake and Depression in Adults. J Am Board Fam Med. 2015 Sep-Oct;28(5):683.

Landsmeer, M. Weinig effect corticosteroïdinjecties bij knieartrose. Huisarts en wetenschap. 2016 May;59(5):233-233



About the author

Marieke Landsmeer was born on September 21st, 1983 in Leiderdorp, the Netherlands. In 2001, she graduated cum laude from secondary school 'Stedelijk Gymnasium' in Leiden, after which she followed the liberal arts year at the 'Vrije Hogeschool' in Driebergen. In 2002, she started Medical School at the University of Utrecht. During her study, she travelled to Paramaribo, Suriname, for her gynaecology internship and worked on a research project on 'pharmacotherapy during lactation' at the Hospital for Sick Children (SickKids) in Toronto, Canada. During her studentship, she worked as a nursing aid of the 'Medical Student Team' at different surgical departments of the UMC Utrecht. After completing Medical School in 2009, she worked as a resident in the internal medicine department at the Diakonessenhuis in Utrecht, followed by a sailing journey of several months in Greece and Turkey with her partner Stijn. In 2011 she began her specialty training for general practitioners (GP) at Erasmus MC in Rotterdam, after having worked as a resident at the surgical department of the 'Franciscus Gasthuis' in Rotterdam for 9 months. She combined her GP specialty training with the research project described in this thesis under supervision of dr. J. Runhaar and prof. dr. S.M.A. Bierma-Zeinstra and obtained a Master of Health Sciences in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES) in 2016. After completing her GP-training in 2017, she is now working as a GP in and around Delft. Besides her work, she loves the outdoors and enjoys life together with her partner Stijn and their three children Freek (2014), Siem (2017) and Anouk (2019).



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