Insight in the role of lipids and other systemic factors in hand and knee osteoarthritis: lessons from clinical studies

Loef, M.

Insight in the role of lipids and other systemic factors in hand and knee osteoarthritis

Lessons from clinical studies

Marieke Loef

ISBN: 978-94-6458-476-9

Cover photo: Camelina Saliva, Rothamsted Research
Printing: Ridderprint | www.ridderprint.nl

Printing of this thesis was financially supported by Alrijne Ziekenhuis and Chipsoft which is gratefully acknowledged.

Insight in the role of lipids and other systemic factors in hand and knee osteoarthritis

Lessons from clinical studies

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op dinsdag 15 november 2022
klokke 13.45 uur

door

Marieke Loef
geboren te Jakarta
in 1991

Promotiecommissie

Promotores	Prof. dr. M. Kloppenburg	
	Prof. dr. F.R. Rosendaal	
Leden promotiecommissie	Prof. dr. T.W.J. Huizinga	
	Prof. dr. J.G. van der Bom	
	Prof. dr. H.J. Lamb	
	Prof. dr. G.J.V.M. van Osch	(Erasmus MC)
	Dr. W.E. van Spil	(Dijklander Ziekenhuis)

“Isn’t it funny how day by day nothing changes, but when you look back everything is different?”

C.S. Lewis

Contents

1

General introduction

Osteoarthritis

"OA is a serious disease with no known cure and no interventions currently available to stop the progression or therapies to manage the pain and loss of mobility with an acceptable benefit:risk profile." – Osteoarthritis: A Serious Disease, OARSI White paper, 2016[1].

Rheumatic musculoskeletal disorders (RMDs) are among the leading causes of disability in middle-aged adults. One of the most prevalent RMDs is osteoarthritis (OA). OA may develop in any joint, but occurs most commonly in hands, knees and hips. Patients with OA suffer from pain, stiffness and an inability to perform day-to-day tasks, and these symptoms can have a tremendous impact on the patients' health-related quality of life (HRQoL)[2,3]. The prevalence and burden of OA has surged in the past decades, affecting approximately 300 million people globally in 2017. This rise in prevalence is accompanied by an increase in years lived with disability of more than 30% since 2007[4]. Most important risk factors for OA are sex, age and obesity[5]. Consequently, OA prevalence is expected to increase even further in the coming years due to ageing of the population and increasing prevalence of obesity. In the Netherlands, the year prevalence of OA is expected to increase with 21% from 2017 to 2030[6]. OA is a heterogeneous disorder, not solely because of the manifestation in multiple joints, but also in its pathophysiology. OA may be classified into different phenotypes, such as post-traumatic, ageing-related and metabolic OA[5,7,8], of which the latter phenotype is the main interest of the research described in this thesis.

Classification

OA can be classified in several ways, which is commonly based on either clinical symptoms alone, or in combination with structural features visualised on imaging. The American College of Rheumatology (ACR) has developed criteria for the classification of hand and knee OA based on clinical and radiographic features[9,10], as listed in **table 1**. The key feature in these classification criteria is the presence of joint pain, co-occurring with typical symptoms and findings during joint examination. For the fulfilment of the radiographic classification criteria, the additional presence of osteophytes is required. Differences in classification methods are important to keep in mind when interpreting study results, especially since clinical symptoms and radiographic findings may not correlate well. Of patients with symptomatic OA, nearly half of patients lack radiological features and vice versa[11,12].

Disease burden

In 2015, osteoarthritis was listed in the top ten of conditions with greatest disease burden in the Netherlands. The prevalence of RMDs is expected to increase based on the predicted demographic developments, which is anticipated to be accompanied by a 17% increase in health expenditure[6]. OA is a chronic disease, and a cure or disease-modifying OA drugs (DMOADs) are currently not available. This limits the adequate treatment of symptoms such as pain and disability, resulting in a further increase in societal and individual burden.

The patients' health status and disease burden can be measured in a standardised way using patient reported outcome measures (PROMs). PROMs may aid shared-decision making and facilitate patient-centred care, as well as structure the monitoring of disease progression or treatment effects[13–15]. Many different outcome measures are being used in OA practise and research, which may be aimed to measure a specific domain, such as pain or function, or give an overall view of the disease, such as quality of life.

Table 1. American College of Rheumatology criteria for hand and knee osteoarthritis

Hand osteoarthritis *Clinical*	**Knee osteoarthritis** *Clinical*	**Knee osteoarthritis** *Clinical and radiographic*
Pain, aching or stiffness +	Pain +	Pain +
≥ 3 of the following:	≥ 3 of the following:	≥ 1 of the following:
- Bony swelling of ≥2 of the 10 selected joints*	- Age >50 years	- Age >50 years
- Bony swelling of ≥2 DIP joints	- Stiffness <30 minutes	- Stiffness <30 minutes
- <3 swollen MCP joints	- Crepitus	- Crepitus +
- Deformity of ≥1 of the 10 selected joints*	- Bony tenderness	Osteophytes
	- Bony enlargement	
	- No palpable warmth	

*Selected joints: bilateral DIP 2 and 3, PIP 2 and 3, bilateral CMC-1 joints

While some PROMs have been specifically developed to evaluate knee or hand OA disease burden, generic PROMs are also frequently used. However, to make optimal use of PROMs data, and interpret them correctly, benchmarks and tools to visually present the results are imperative. Moreover, the clinimetric properties of the instrument need to be kept in mind when selecting the most appropriate measure to use. To improve outcome measurement and facilitate instrument selection, the OMERACT (Outcome Measures in Rheumatology) developed the "OMERACT Filter", which consists of three pillars: truth, discrimination, and feasibility[16]. Selection of an instrument should start by defining the target domain and an evaluation of the applicability of the instrument to the present case (Truth). Also, it should be assessed if the instrument is practical to use, or if is burdensome to the patient (Feasibility). Furthermore, the instrument should be able to discriminate between groups of interest (Discrimination), and needs to produce consistent findings and be able to detect meaningful change over time[17,18].

The Knee injury and Osteoarthritis Outcome Score (KOOS) is an example of a disease-specific PROM that evaluates several aspects of disease burden in patients with knee complaints. The KOOS is a self-administered questionnaire and consists of five subscales, aimed to measure pain, symptoms, function in activities of daily living, function in sport and recreation activities, and quality of life[19]. The KOOS is an extension of the Western Ontario McMaster Universities (WOMAC) questionnaire, which consists of three dimensions: pain, stiffness and limitations in everyday activities. The WOMAC has been extensively evaluated for its clinimetric properties, and is found to be a valid, reliable and responsive measure. The WOMAC is copyrighted, however a request for use may be placed via the website[20]. In contrast to the WOMAC, the KOOS is free of use, and also considered valid, reliable and responsive to change[21].

In patients with hand OA, the Australian/Canadian Hand Osteoarthritis Index (AUSCAN) is frequently applied. The AUSCAN is disease-specific PROM developed using the WOMAC as a template, and similarly measures the domains pain, stiffness and function[22]. In addition, the Functional Index for Hand OsteoArthritis (FIHOA) is commonly used for the measurement of hand function in daily activities in patients with hand OA.

Another important domain of disease impact is the health-related quality of life (HRQoL), which is often assessed with the Short Form (SF)-36. The SF-36 is a generic, self-administered, quality of life measure. The SF-36 consists of 36 questions, providing scores for eight health domains, which may be summarised in the physical component summary (PCS) and the mental component summary (MSC) scores. Moreover, the use of norm-based scoring allows

comparison of HRQoL between different conditions and study populations[23].
Specific domains may also be measured with a visual analogue scale (VAS), which is typically a 0-100mm scale. The VAS is an often used measure for the quantification of pain, as well as to get a global assessment of the disease by the patient or physician.

Imaging
Structural OA can be measured with a variety of imaging modalities, of which radiography is the most frequently used in clinical practise and research. The Kellgren-Lawrence (KL) grading system is an often used scoring method, that allows for the assessment of OA severity with a single score from 0 to 4 for each joint. Grade 0 represents no OA, grade 1 doubtful OA, grade 2 definite, but minimal OA, grade 3 moderate OA, and grade 4 represents severe OA[24]. Example radiographs of the distal interphalangeal joint and the knee joint, representing the different grades of OA severity according to the KL system, are depicted in figure 1. In addition, the Osteoarthritis Research Society international (OARSI) developed an atlas to guide the evaluation of individual OA features[25], which has been updated by Altman and Gold[26]. The radiographic OA features included in the atlas differ slightly per joint, but include amongst others: osteophytes, joint space narrowing, malalignment, erosions, subchondral sclerosis and subchondral cysts. Following this atlas, osteophytes and joint space narrowing are scored from 0 to 3, while all other features are scored as absent or present[26].

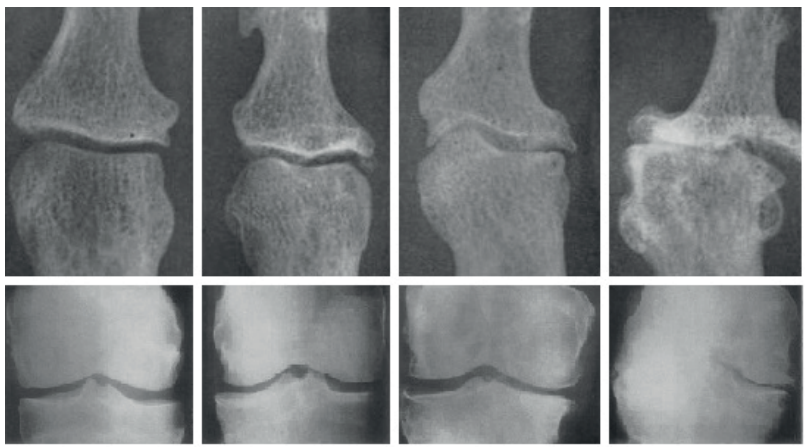

Figure 1. X-ray images of the distal interphalangeal joints (top row) and knee joint (bottom row), graded according to the Kellgren-Lawrence scoring system. *Figure by Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis and Cartilage 2007; 15: A1–56.*

Radiography is commonly used because of the ample experience with this imaging modality, the ease of use, accessibility, and relatively low costs. However, radiography is limited by the visualization of bony structures only. Therefore, magnetic resonance imaging (MRI) is increasingly used in research settings, which has the advantage of assessment of all affected joint tissues including soft tissues. To standardize the scoring of structural OA features on MRI, several scoring systems have been developed. For the assessment of knee OA, the Knee Osteoarthritis Scoring system (KOSS) may be used, which includes in addition to osteophytes and cartilaginous lesions many other OA-related features such as meniscal abnormalities, subchondral cysts, bone marrow lesions (BMLs), effusion and synovitis[27]. Using these features,

the criteria developed by Hunter et al.[28] can be used to determine the presence of structural knee OA. The Oslo Hand Osteoarthritis MRI score was the first scoring system developed to score hand OA features[29]. More recently, the OMERACT Hand OA MRI Scoring System (HOAMRIS) has been developed for the assessment of the distal and proximal interphalangeal joints[30]. Additionally, the OMERACT Thumb Base Osteoarthritis MRI Scoring System (TOMS) was developed, which can be used in a complementary fashion with the HOAMRIS[31].

Metabolic OA: the role of obesity

Obesity has been recognised many years ago as one of the major risk factors for the development of OA[32,33]. However, for long this association was solely ascribed to an increase in mechanical loading. In line with the hypotheses at that time, this association was explained by a cartilage-driven wear-and-tear process, with subsequent degradation of the cartilage[34]. Although mechanical stress due to increased body weight indeed plays a large role in OA risk[35], this does not fully explain the association between obesity and OA, which is apparent from the association between obesity and non-weightbearing joints such as the hand[36,37]. Moreover, OA is a disease of the entire joint. The osteoarthritic process is not only characterised by cartilage degradation, but also by subchondral bone remodelling, formation of osteophytes, degradation of ligaments and synovial inflammation (figure 2)[5,38]. Furthermore, detrimental changes to the periarticular muscles, nerves, bursa and local fat pads may also contribute to OA[38].

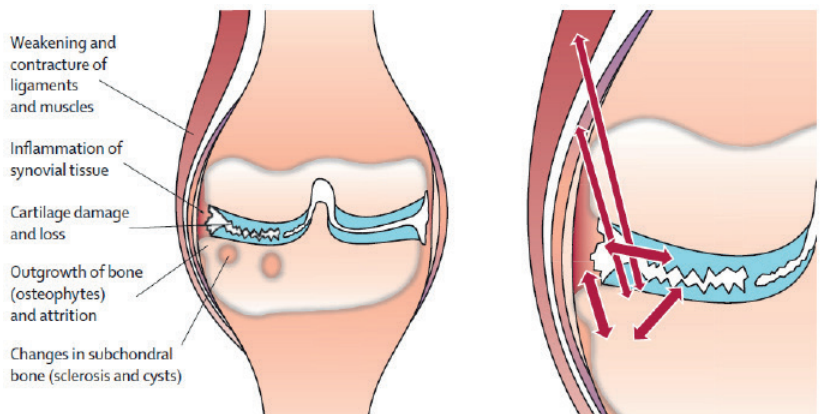

Figure 2. Schematic drawing of the osteoarthritic joint.
Osteoarthritis is a disease of the whole joint (left), and the osteoarthritis processes in the different joint tissues are tightly connected (right). *Figure by Bijlsma, Berenbaum and Lafeber. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011 Jun 18;377(9783):2115-26.*

Obesity is associated with a broad spectrum of systemic effects, which likely are involved in OA, especially in non-weightbearing joints. Obesity is associated with a chronic low-grade inflammation, sometimes also referred to as “metaflammation”, what may lead to metabolic dysregulation and the metabolic syndrome[39]. Components of the metabolic syndrome, such as hypertension, dyslipidaemia and hyperglycaemia, have been previously studied as possible players in OA (figure 3)[40]. However, conflicting results have been reported[41–44]. Furthermore, soluble mediators released by adipose tissue, such as adipokines[45,46] and lipids, could mediate the systemic effects of obesity.

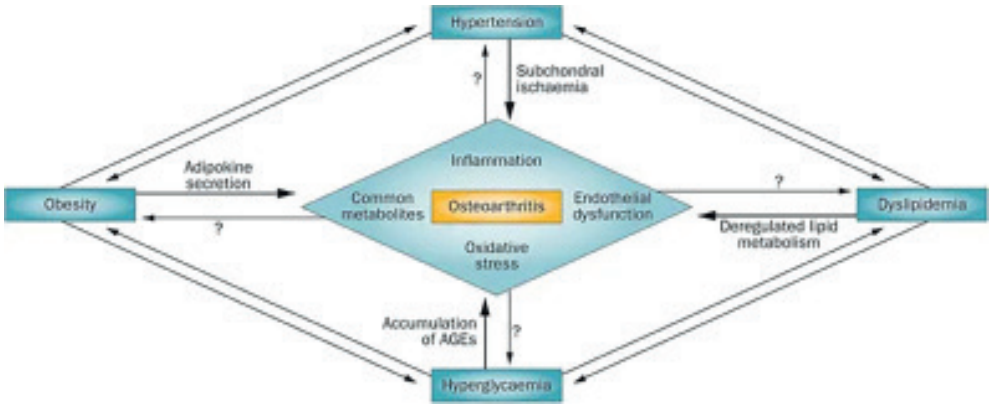

Figure 3. The metabolic syndrome and its components may be associated with osteoarthritis.
AGEs = advanced glycation end-products. *Figure by Zhuo, Yang, Chen and Wang. Metabolic syndrome meets osteoarthritis. Nat Rev Rheumatol. 2012 Dec;8(12):729-3*

Dysregulation of lipid metabolism

Obesity and excessive nutrient intake may exceed the adipose tissues' lipid storage capacity. Subsequently, circulating fatty acid levels will increase, accompanied by ectopic lipid accumulation. The resulting systemic lipotoxicity may be a key driver of the obesity-related metaflammation[47]. Not just the quantity of fat intake plays a role, but also the type of fat may be important[48]. The term lipids comprises many different molecules, such as higher order lipids including, but not limited to, sterols, sphingomyelins and phospholipids, as well as free fatty acids and downstream lipid metabolites. Fatty acids can be categorised depending on their length and degree of saturation into the commonly used subclasses of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids.

The different fatty acids and lipid metabolites likely have distinct effects on inflammation. For example, while saturated fatty acids and omega-6 polyunsaturated fatty acids have a pro-inflammatory profile, omega-3 polyunsaturated fatty acids have anti-inflammatory effects *in vitro*[49]. These opposing effects have also been observed in preclinical OA research. Saturated fatty acids and omega-6 polyunsaturated fatty acids increased prostaglandin release and upregulate gene expression related to apoptosis and cartilage degradation[50,51], while omega-3 polyunsaturated fatty acids reduced cartilage proteinase mRNA levels and inflammatory cytokines in chondrocyte cultures[52]. However, human epidemiological studies on the association of fatty acids and other lipids on OA are few, inconclusive, and limited to structural knee and hip OA[53–55].

Inflammation

Despite that not always all of the classical clinical features of inflammation, *rubor, calor, dolor* and *tumour*, can be observed in OA, a chronic, low-grade local and systemic inflammation is present. The role of inflammation in the development of OA has only been given full consideration since the last decades, but is now accepted as having a pivotal role in OA pathogenesis. This paradigm shift occurred following advances in molecular biology, showing the involvement of soluble inflammatory mediators.

Serum levels of various proinflammatory cytokines are increased in OA patients, which is believed to be produced by inflamed synovium and activated chondrocytes. Breakdown products of the extracellular matrix may act as damage-associated molecular patterns (DAMPs). DAMPs, as well as intracellular proteins, or "alarmins" , may activate the innate immune system, resulting in upregulation of proinflammatory cytokines and catabolic mediators such as metalloproteinases (MMPs) and disintegrin and metalloproteinase with thrombospondin-1 domains (ADAMTS)[56–58]. In particular, cytokines from the interleukin (IL) family, as well as tumour necrosis factor alpha (TNFα) have been extensively investigated[59]. In addition to stimulating MMPs and ADAMTS proteinases, these cytokines stimulate the expression of COX-2, increasing the synthesis of prostaglandins, and upregulate the production of nitric oxide. Moreover, IL-1β and TNFα may induce other proinflammatory cytokines[58], further enhancing the inflammatory response.

Additionally, the involvement of inflammation is overtly apparent from imaging studies. Inflammatory signs such as gray-scale synovitis and power Doppler signals on ultrasonography[60,61], as well as synovitis and BMLs on MRI scans[62], are often present in patients with OA. Importantly, structural inflammatory features are not merely a reflection of damage but likely contribute to the OA process. Inflammatory features detected by ultrasound, were associated with the development of joint erosions in patients with hand OA[63]. In addition, in patients with knee OA synovitis and BMLs have been associated with cartilage deterioration[64,65]. Moreover, synovial inflammation and BMLs were associated with commonly reported symptoms such as pain in both hand and knee OA[60–62,66].

Treatment

There is a high unmet need for disease modifying drugs for the treatment of OA. Current guidelines recommend, in addition to non-pharmacological interventions such as (supervised) exercise and self-management programs, the use of oral or topical nonsteroidal anti-inflammatory drugs[67]. In addition, intra-articular injections with glucocorticoids may be considered[67,68].

At the moment, treatment options are limited to approaches aimed to reduce symptoms. The role of inflammation in OA and its association with structural progression and clinical outcomes has piqued an interest for targeting inflammation in therapeutic research. However, research investigating the use of biologics have been disappointing so far[69]. Possibly, potential treatment effects have been obscured by too short follow-up in the studies, small cohort sizes[70], or the inclusion of a heterogeneous study population. Conceivably, treatment effects may be observed in studies with a sufficiently long study duration, as OA often progresses slowly. Alternatively, study designs allowing the selection of patients with a high likelihood of fast progression or evident inflammatory disease may show different outcomes. In addition, the majority of available findings results from studies in patients with knee OA, and few studies have investigate soluble biomarkers in hand OA[70]. Another explanation may be that previous studies have targeted systemic effects with a relatively small role in OA pathogenesis. Increasing our understanding of OA pathogenesis may reveal potential new systemic treatment targets.

Aim of thesis

This thesis aimed to:

1. Increase our understanding of the role of inflammation-related systemic factors in the aetiology of OA, by focusing on the association of lipids with hand and knee OA.
2. Assess the disease burden associated with hand and knee OA in the general population as well as in secondary care patient populations, with a specific focus on improvement of the interpretation of PROMs.

Study populations

The research described in this thesis has been performed with data of several study populations, which enabled us to study OA in various stages of the disease, in differing settings, as well as multiple OA phenotypes. The main characteristics of the included study populations are briefly introduced below.

NEO study

The Netherlands Epidemiology of Obesity (NEO) study is a prospective population-based cohort study, which was designed to investigate pathways that lead to obesity-related diseases and conditions. In this thesis, we performed cross-sectional analyses of baseline measurements, for which individuals between 45 and 65 years of age were recruited between 2008 and 2012. Prior to the study visit, participants completed questionnaires on demographic and clinical information. The HRQoL was measured with the SF-36. In addition, participants completed the KOOS[19] and the AUSCAN[71], giving insight in OA specific disease burden of the knees and hands, respectively. At the study centre, blood samples were obtained after an overnight fast. Subsequently, a standardised liquid mixed-meal was consumed, and after 150 minutes postprandial blood samples were drawn. These blood samples were analysed for the quantification of lipid and metabolite measures. Furthermore, an extensive physical examination was performed, including measurement of body weight and total body fat by bioelectrical impedance analysis. In addition, a standardised examination of the hands and knees allowed us to define a clinical hand and knee phenotype according to the ACR classification criteria[9,10]. A subset of the population underwent an MRI of the right knee, on which OA features were scored using the KOSS[27], and a structural knee phenotype was defined according to modified criteria by Hunter et al.[28]. We investigated the association of several obesity-related factors with clinical hand and knee OA, as well as structural knee OA, in the general middle-aged Dutch population. In addition, we developed percentile curves for the KOOS questionnaire, to provide benchmarks to aid interpretation of KOOS subscale scores, and investigated differences in HRQoL of participants with and without hand OA.

HOSTAS study

The Hand OSTeoArthritis in Secondary care (HOSTAS) is an observational cohort study aimed to investigate determinants of disease outcome and the utility of clinimetric instruments in patients with primary hand OA. HOSTAS included consecutive patients from the LUMC rheumatology outpatient clinic between 2009 and 2015, based on the rheumatologist's diagnosis of primary hand OA and absence of other rheumatic diseases or secondary OA. The LUMC serves both as a secondary and tertiary referral centre for rheumatic diseases, enabling

inclusion of patients with primary hand OA in all disease stages. In the HOSTAS study, we assessed HRQoL in patients with primary hand OA, and compared differences with patients with concurrent knee OA. Moreover, we made an indirect comparison with individuals with hand OA in the general population (NEO study).

HOPE study

The Hand Osteoarthritis Prednisolone Efficacy (HOPE) study is a double-blind, randomised placebo-controlled trial, designed to investigate the efficacy and safety of short-term prednisolone treatment in patients with symptomatic, inflammatory hand OA. Patients were randomly assigned (1:1) to receive 10mg prednisolone or placebo daily for six weeks, followed by a two-week tapering schedule and six-week follow-up without study medication. Detailed description of the HOPE study, including the primary endpoint results, can be read elsewhere[72]. Briefly, patients with symptomatic hand OA, fulfilling the ACR criteria[9], and signs of inflammation on ultrasound were included. At baseline and week 6, patients filled in a visual analogue scale (VAS) for finger pain and VAS global assessment on a 0-100mm scale, and the AUSCAN pain and function subscales. At week 6, fulfilment of the OMERACT-OARSI responder criteria was assessed[73]. Blood samples were obtained non-fasted at baseline, 6 and 14 weeks, for the measurement of a large array of higher order lipids and fatty acids with the exploratory lipidomics platform Lipidyzer™, and measurement of oxylipins with an in-house developed LC-MS/MS platform. We developed a standardised procedure for the pre-processing of Lipidyzer™ data, and compared the reproducibility of Lipidyzer™ measurements over 6 weeks' time in commonly used plasma samples, with potentially more stable erythrocyte samples. In addition, we investigated the predictive value of baseline lipid levels for the response to prednisolone treatment after 6 weeks. Furthermore, we assessed if treatment with prednisolone alters lipid concentrations.

APPROACH study

The Applied Public-Private Research enabling OsteoArthritis Clinical Headway (APPROACH) is an exploratory, 2-year, prospective multi-centre cohort study, which started in 2018. The APPROACH study included patients with knee OA fulfilling the clinical ACR criteria[10] from five hospitals in four European countries: The Netherlands (University Medical Centre Utrecht, Leiden University Medical Center), Norway (Diakonhjemmet Hospital, Oslo), France (Assistance Public Hôpitaux de Paris) and Spain (Servizo Galego de Saúde, A Coruña). Patients were selected using machine learning models, developed on data from a previous OA cohort, to display a high likelihood of structural and/or pain progression. At baseline, participants completed an extensive booklet of questionnaires, among which the KOOS and the FIHOA[74,75] for assessment of knee and hand OA-specific disease burden, respectively. Radiographic knee and hand OA was measured according to the KL grading system[24]. Blood samples were drawn fasted, and plasma samples were used for measurements with the Lipidyzer™ platform, and measurement of oxylipin metabolites with an in-house developed LC-MS/MS platform. The research described in this thesis used baseline measurements to investigate the association of higher order lipid levels and downstream lipid metabolites, with radiographic knee and hand OA severity, as well as knee and hand pain and function.

LOAS study

The Longitudinal Leiden Orthopeadics Outcomes of Osteo-Arthritis study (LOAS) started in 2012, and is an ongoing, multi-centre, longitudinal prospective cohort study designed to

determine long-term outcomes of total knee arthroplasty (TKA) and total hip arthroplasty. Patients scheduled for primary or revision arthroplasty were recruited from seven hospitals in the Netherlands, as described previously[76]. For the present analyses, we aimed to validate the KOOS percentile curves developed in the general population (NEO study). Therefore, we included patients undergoing primary TKA between 45 and 65 years of age. KOOS subscale scores were obtained preoperatively and 6, 12 and 24 months after TKA. Preoperative knee radiographs were assessed according to the KL grading system[24] in a subset of patients (37%). We plotted the median population-level KOOS scores on the population-based KOOS percentile curves.

Outline of thesis

Part 1 – Lipids and other metabolic factors in osteoarthritis
In part one we aimed to increase our insight in OA pathogenesis, by investigating the association between inflammation-related systemic factors and OA, with a specific focus on the association of lipids with hand and knee OA.

Firstly, **chapter 2** summarizes the available preclinical and clinical evidence on the association between fatty acids and OA, showing clear gaps in our knowledge regarding fatty acids in OA. Observational human study results are few, and limited to structural OA. To advance this knowledge, we investigated the association between plasma fatty acids and clinical hand and knee OA, as well as structural knee OA, in the NEO study in **chapter 3**. In the human body, the majority of fatty acids is bound to carrier molecules to form higher order lipids species. In addition, fatty acids are metabolised to bioactive oxylipins. Lipidomics comprises the measurement of lipid species and their metabolites, and may be valuable in understanding the pathogenesis of diseases, as well as to predict treatment responses. However, biomarker discovery starts by being able to obtain reliable and reproducible measurements. Therefore, in **chapter 4** we investigated the reproducibility of lipid measurements in different sample types obtained by the standardised and commercially available Lipidyzer™ platform. Furthermore, we present a standardised method to pre-process the Lipidyzer™ data, to guide future biomarker research. In **chapter 5** we described the association of lipidomics with the severity of radiographic hand and knee OA, as well as hand and knee pain, using data from the APPROACH study. In addition, we explored the use of lipidomics for the prediction of prednisolone treatment response in **chapter 6**, and the influence of prednisolone treatment on lipid levels in **chapter 7**, in patients with inflammatory hand OA.

Lipid dysregulation also affects other conditions, such as cardiovascular disease. Moreover, associations between cardiovascular disease and OA have previously been reported. However, to which extent cardiovascular disease markers may explain the well-known association between obesity and OA, has not been investigated. Therefore, in **chapter 8** we assessed if the previously established association between obesity and OA is mediated by blood pressure, arterial stiffness and subclinical atherosclerosis.

Part 2 – Osteoarthritis disease burden

In part two we focussed on several PROMs, with a specific interest for epidemiological and methodological aspects. Disease burden can be measured in many ways, among which are quality of life, pain and function.

In **chapter 9** we investigated the association between OA and HRQoL in different study populations with hand OA. Firstly, we assessed the impact of hand OA in a population-based cohort, the NEO study. Furthermore, we examined this question in a secondary and tertiary care cohort with primary hand OA patients, the HOSTAS study.

OA symptoms, such as pain and functional impairment, may influence a patients' physical activity levels. Since physical activity is known to positivity influence disease burden, it presents a potential target for intervention. Therefore, it is essential to have insight in the relation between OA and physical activity. In **chapter** 10, we investigated the association of physical activity levels with clinical and structural knee OA, as well as the association between physical activity and several PROMs.

As a lack of benchmarks hinders adequate interpretation of PROMs, we explored in **chapter 11** factors that may influence KOOS scores, and developed percentile curves in the general Dutch population. Subsequently, we investigated the applicability of these curves by applying them on real-world clinical data of patients undergoing TKA included in the LOAS study, which is described in **chapter 12**.

To conclude, a summary and general discussion of the findings of this thesis, along with a research agenda for future investigations is provided in **chapter 13**. **Chapter 14** summarizes the main points raised in this thesis in Dutch.

References

1 OARSI White Paper- OA as a Serious Disease. Osteoarthritis Research Society International. 2018; published online April 16. https://www.oarsi.org/education/oarsi-resources/oarsi-white-paper-oa-serious-disease (accessed April 9, 2020).

2 Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. *Nat Rev Rheumatol* 2014; 10: 437–41.

3 Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet* 2019; 393: 1745–59.

4 GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; 392: 1789–858.

5 Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. *Lancet* 2011; 377: 2115–26.

6 Eysink PED, Poos M, Gijsen R, Kommer GJ, van Gool CH. Epidemiologische data van Ziekten van het botspierstelsel en bindweefsel: Achtergrondrapport voor Programma Zinnige Zorg. *Epidemiological data of disorders of the musculoskeletal system and connective tissue : Background report from 'Zinnige Zorg' Programme* 2019; published online Oct 3. DOI:10.21945/RIVM-2019-0180.

7 Dell'Isola A, Allan R, Smith SL, Marreiros SSP, Steultjens M. Identification of clinical phenotypes in knee osteoarthritis: a systematic review of the literature. *BMC Musculoskelet Disord* 2016; 17: 425.

8 Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013; 80: 568–73.

9 Altman R, Alarcón G, Appelrouth D, *et al.* The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. *Arthritis Rheum* 1990; 33: 1601–10.

10 Altman R, Asch E, Bloch D, *et al.* Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986; 29: 1039–49.

11 Altman RD. Criteria for classification of clinical osteoarthritis. *J Rheumatol Suppl* 1991; 27: 10–2.

12 Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. *J Rheumatol* 2000; 27: 1513–7.

13 Yang LY, Manhas DS, Howard AF, Olson RA. Patient-reported outcome use in oncology: a systematic review of the impact on patient-clinician communication. *Support Care Cancer* 2018; 26: 41–60.

14 Appleby J, Poteliakhoff E, Shah K, Devlin N. Using patient-reported outcome measures to estimate cost-effectiveness of hip replacements in English hospitals. *J R Soc Med* 2013; 106: 323–31.

15 Devlin NJ, Appleby J. Getting the most out of PROMs: putting health outcomes at the heart of NHS decision-making. London: King's Fund, 2010.

16 Boers M, Brooks P, Strand CV, Tugwell P. The OMERACT filter for Outcome Measures in Rheumatology. *J Rheumatol* 1998; 25: 198–9.

17 Kianifard F. Evaluation of Clinimetric Scales: Basic Principles and Methods. *Journal of the Royal Statistical Society Series D (The Statistician)* 1994; 43: 475–82.

18 de Vet HCW, Terwee CB, Bouter LM. Current challenges in clinimetrics. *J Clin Epidemiol* 2003; 56: 1137–41.

19 Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. *J Orthop Sports Phys Ther* 1998; 28: 88–96.

20 WOMAC Osteoarthritis Index- WOMAC 3.1 - Knee and Hip Osteoarthritis. http://www.womac.com/womac/index.htm (accessed April 30, 2020).

21 Knee injury and Osteoarthritis Outcome Score. http://www.koos.nu/ (accessed April 30, 2020).

22 Bellamy N, Campbell J, Haraoui B, *et al.* Dimensionality and clinical importance of pain and disability in hand osteoarthritis: Development of the Australian/Canadian (AUSCAN) Osteoarthritis Hand Index. *Osteoarthr Cartil* 2002; 10: 855–62.

23 Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; 30: 473–83.

24 Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. *Annals of the Rheumatic Diseases* 1957; 16: 494–502.

25 Altman RD, Hochberg M, Murphy WA, Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. *Osteoarthr Cartil* 1995; 3 Suppl A: 3–70.

26 Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. *Osteoarthritis and Cartilage* 2007; 15: A1–56.

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

28 Hunter DJ, Arden N, Conaghan PG, *et al.* Definition of osteoarthritis on MRI: results of a Delphi exercise. *Osteoarthritis Cartilage* 2011; 19: 963–9.

29 Haugen IK, Lillegraven S, Slatkowsky-Christensen B, *et al.* Hand osteoarthritis and MRI: development and first validation step of the proposed Oslo Hand Osteoarthritis MRI score. *Annals of the Rheumatic Diseases* 2011; 70: 1033–8.

30 Haugen IK, Ostergaard M, Eshed I, *et al.* Iterative development and reliability of the OMERACT hand osteoarthritis MRI scoring system. *J Rheumatol*

2014; 41: 386–91.
31 Kroon FPB, Conaghan PG, Foltz V, *et al.* Development and Reliability of the OMERACT Thumb Base Osteoarthritis Magnetic Resonance Imaging Scoring System. *J Rheumatol* 2017; 44: 1694–8.
32 Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis. The Framingham Study. *Ann Intern Med* 1988; 109: 18–24.
33 Davis MA, Ettinger WH, Neuhaus JM. Obesity and osteoarthritis of the knee: evidence from the National Health and Nutrition Examination Survey (NHANES I). *Semin Arthritis Rheum* 1990; 20: 34–41.
34 Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. *Lancet* 1972; 1: 519–22.
35 Visser AW, de Mutsert R, le Cessie S, *et al.* The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. *Ann Rheum Dis* 2015; 74: 1842–7.
36 Visser AW, Ioan-Facsinay A, de Mutsert R, *et al.* Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. *Arthritis Res Ther* 2014; 16: R19.
37 Yusuf E, Nelissen RG, Ioan-Facsinay A, *et al.* Association between weight or body mass index and hand osteoarthritis: a systematic review. *Ann Rheum Dis* 2010; 69: 761–5.
38 Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* 2012; 64: 1697–707.
39 Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017; 542: 177–85.
40 Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012; 8: 729–37.
41 Monira Hussain S, Wang Y, Cicuttini FM, *et al.* Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014; 43: 429–36.
42 Yoshimura N, Muraki S, Oka H, *et al.* Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthr Cartil* 2012; 20: 1217–26.
43 Dahaghin S, Bierma-Zeinstra SMA, Koes BW, Hazes JMW, Pols H a. P. Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. *Ann Rheum Dis* 2007; 66: 916–20.
44 Garessus EDG, de Mutsert R, Visser AW, Rosendaal FR, Kloppenburg M. No association between impaired glucose metabolism and osteoarthritis. *Osteoarthr Cartil* 2016; 24: 1541–7.
45 Richter M, Trzeciak T, Owecki M, Pucher A, Kaczmarczyk J. The role of adipocytokines in the pathogenesis of knee joint osteoarthritis. *Int Orthop* 2015; 39: 1211–7.
46 Kroon FPB, Veenbrink AI, de Mutsert R, *et al.* The role of leptin and adiponectin as mediators in the relationship between adiposity and hand and knee osteoarthritis. *Osteoarthr Cartil* 2019; 27: 1761–7.
47 Ertunc ME, Hotamisligil GS. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J Lipid Res* 2016; 57: 2099–114.
48 Cordain L, Eaton SB, Sebastian A, *et al.* Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 2005; 81: 341–54.
49 Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc Natl Acad Sci USA* 2003; 100: 1751–6.
50 Bastiaansen-Jenniskens YM, Siawash M, van de Lest CHA, *et al.* Monounsaturated and Saturated, but Not n-6 Polyunsaturated Fatty Acids Decrease Cartilage Destruction under Inflammatory Conditions: A Preliminary Study. *Cartilage* 2013; 4: 321–8.
51 Alvarez-Garcia O, Rogers NH, Smith RG, Lotz MK. Palmitate has proapoptotic and proinflammatory effects on articular cartilage and synergizes with interleukin-1. *Arthritis Rheumatol* 2014; 66: 1779–88.
52 Zainal Z, Longman AJ, Hurst S, *et al.* Relative efficacies of omega-3 polyunsaturated fatty acids in reducing expression of key proteins in a model system for studying osteoarthritis. *Osteoarthr Cartil* 2009; 17: 896–905.
53 Baker KR, Matthan NR, Lichtenstein AH, *et al.* Association of plasma n-6 and n-3 polyunsaturated fatty acids with synovitis in the knee: the MOST study. *Osteoarthr Cartil* 2012; 20: 382–7.
54 Hill CL, March LM, Aitken D, *et al.* Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. *Ann Rheum Dis* 2016; 75: 23–9.
55 Castro-Perez JM, Kamphorst J, DeGroot J, *et al.* Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. *J Proteome Res* 2010; 9: 2377–89.
56 Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013; 5: 77–94.
57 Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthr Cartil* 2013; 21: 16–21.
58 Goldring MB, Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol* 2011; 23: 471–8.
59 Rahmati M, Mobasheri A, Mozafari M. Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges. *Bone* 2016; 85: 81–90.
60 Kortekaas MC, Kwok W-Y, Reijnierse M, Watt I, Huizinga TWJ, Kloppenburg M. Pain in hand osteoarthritis is associated with inflammation: the

value of ultrasound. *Ann Rheum Dis* 2010; 69: 1367–9.
61 Keen HI, Wakefield RJ, Grainger AJ, Hensor EMA, Emery P, Conaghan PG. An ultrasonographic study of osteoarthritis of the hand: synovitis and its relationship to structural pathology and symptoms. *Arthritis Rheum* 2008; 59: 1756–63.
62 Haugen IK, Christensen BS, Boyesen P, Sesseng S, van der Heijde D, Kvien TK. Increasing synovitis and bone marrow lesions are associated with incident joint tenderness in hand osteoarthritis. *Annals of the Rheumatic Diseases* 2016; 75: 702–8.
63 Kortekaas MC, Kwok W-Y, Reijnierse M, Stijnen T, Kloppenburg M. Brief Report: Association of Inflammation With Development of Erosions in Patients With Hand Osteoarthritis: A Prospective Ultrasonography Study. *Arthritis & Rheumatology (Hoboken, NJ)* 2016; 68: 392–7.
64 de Lange-Brokaar BJ, Bijsterbosch J, Kornaat PR, *et al.* Radiographic progression of knee osteoarthritis is associated with MRI abnormalities in both the patellofemoral and tibiofemoral joint. *Osteoarthritis Cartilage* 2016; 24: 473–9.
65 Roemer FW, Guermazi A, Javaid MK, *et al.* Change in MRI-detected subchondral bone marrow lesions is associated with cartilage loss: the MOST Study. A longitudinal multicentre study of knee osteoarthritis. *Ann Rheum Dis* 2009; 68: 1461–5.
66 Torres L, Dunlop DD, Peterfy C, *et al.* The relationship between specific tissue lesions and pain severity in persons with knee osteoarthritis. *Osteoarthritis and Cartilage* 2006; 14: 1033–40.
67 Kolasinski SL, Neogi T, Hochberg MC, *et al.* 2019 American College of Rheumatology/ Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. *Arthritis Care Res (Hoboken)* 2020; 72: 149–62.
68 Kloppenburg M, Kroon FP, Blanco FJ, *et al.* 2018 update of the EULAR recommendations for the management of hand osteoarthritis. *Ann Rheum Dis* 2019; 78: 16–24.
69 Chevalier X, Eymard F, Richette P. Biologic agents in osteoarthritis: hopes and disappointments. *Nat Rev Rheumatol* 2013; 9: 400–10.
70 van Spil WE, Szilagyi IA. Osteoarthritis year in review 2019: biomarkers (biochemical markers). *Osteoarthr Cartil* 2020; 28: 296–315.
71 Bellamy N, Campbell J, Haraoui B, *et al.* Dimensionality and clinical importance of pain and disability in hand osteoarthritis: Development of the Australian/Canadian (AUSCAN) Osteoarthritis Hand Index. *Osteoarthr Cartil* 2002; 10: 855–62.
72 Kroon FPB, Kortekaas MC, Boonen A, *et al.* Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. *The Lancet* 2019; 394: 1993–2001.
73 Pham T, van der Heijde D, Altman RD, *et al.* OMERACT-OARSI initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthr Cartil* 2004; 12: 389–99.
74 Wittoek R, Vander Cruyssen B, Maheu E, Verbruggen G. Cross-cultural adaptation of the Dutch version of the Functional Index for Hand Osteoarthritis (FIHOA) and a study on its construct validity. *Osteoarthritis and Cartilage* 2009; 17: 607–12.
75 Dreiser RL, Maheu E, Guillou GB, Caspard H, Grouin JM. Validation of an algofunctional index for osteoarthritis of the hand. *Rev Rhum Engl Ed* 1995; 62: 43S-53S.
76 Leichtenberg CS, Meesters JJL, Kroon HM, *et al.* No associations between self-reported knee joint instability and radiographic features in knee osteoarthritis patients prior to Total Knee Arthroplasty: A cross-sectional analysis of the Longitudinal Leiden Orthopaedics Outcomes of Osteo-Arthritis study (LOAS) data. *The Knee* 2017; 24: 816–23.
77 Goekoop-Ruiterman YPM, de Vries-Bouwstra JK, Allaart CF, *et al.* Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial. *Ann Intern Med* 2007; 146: 406–15.

Part 1

Lipids, inflammation and other metabolic factors in osteoarthritis

2

Fatty acids and osteoarthritis: different types, different effects

Marieke Loef, Jan Schoones, Margreet Kloppenburg, Andreea Ioan-Facsinay

Joint Bone Spine 2019;86:451-8

Abstract

While the association between obesity and osteoarthritis used to be solely regarded as a result of increased mechanical loading, systemic factors also likely play a role in the pathophysiology of osteoarthritis. Nutrient excess leading to obesity may result in lipotoxicity, which might be involved in the development of osteoarthritis. The different fatty acid types have distinct effects on inflammation. This review focusses on the currently available studies, summarizing the effects of the different fatty acid types on osteoarthritis and involved joint tissues.

In animal studies omega-3 polyunsaturated fatty acids reduced the expression of inflammatory markers, cartilage degradation and oxidative stress in chondrocytes. In contrast, these markers were increased upon omega-6 polyunsaturated fatty acid and saturated fatty acid stimulation. Additionally, a decrease in pain and dysfunction was observed upon omega-3 supplementation in cats and dogs.

In line, most human *in vitro* studies show pro-apoptotic and pro-inflammatory actions of saturated fatty acids. While all polyunsaturated fatty acids reduced markers of oxidative stress, omega-3 polyunsaturated fatty acids additionally decreased prostaglandin production. Human intervention studies with omega-3 polyunsaturated fatty acid supplementation may indicate a beneficial effect on pain and function and might be associated with less structural damage. In contrast, an adverse effect of saturated fatty acids on osteoarthritis has been observed. Monounsaturated fatty acids have been infrequently studied and findings are inconclusive.

Existing studies indicate a promising effect of especially omega-3 polyunsaturated fatty acids on osteoarthritis signs and symptoms. However, more human intervention studies are warranted to draw robust conclusions.

Introduction

Obesity and osteoarthritis, a twofold effect

In middle-aged adults, musculoskeletal disorders are among the leading causes of disability. Osteoarthritis (OA) is a prevalent disorder, affecting 237 million people globally. The prevalence and burden of OA has greatly increased in the past decade[1], and it is expected to increase even further with ageing of the population and increasing prevalence of obesity[2]. OA may develop in any joint, but occurs most commonly in spine, hands, knees and hips. Multiple tissues are involved in the osteoarthritic process, which is characterized by cartilage degradation, synovial inflammation and (subchondral) bone remodelling[2]. OA is a heterogeneous disorder that is starting to be recognized as a cluster of diseases that can be subdivided into different phenotypes, such as post-traumatic, ageing-related and metabolic OA[2–4].

In the last decades a profound change in lifestyle and diet has occurred. The Western diet and the increasingly sedentary lifestyle have led to an increase in overweight and obese individuals. The association of obesity with OA has been recognized many years ago[5], and has been supported by a great amount of evidence since then[6–10]. For long it was thought that obesity mainly resulted in an increased mechanical loading, leading to mechanical stress, increased wear-and-tear, and subsequently to cartilage degradation and OA[11]. However, a paradigm shift has occurred more recently, as obesity is also associated with OA in non-weight-bearing joints like the hands, in which systemic processes are likely more involved[7,8,12]. Therefore, systemic effects of obesity have emerged as possible players in OA. Among these, metabolic syndrome has been most intensively studied and conflicting results were reported[13–18]. Similarly, soluble mediators released by adipose tissue such as adipokines and fatty acids could mediate the systemic effects of obesity. Adipokines have been extensively investigated in relation to OA[19]. In the present review, we will focus on fatty acids and their possible involvement in OA development and progression.

Lipid triggered meta-inflammation

Excess nutrient intake may result in a lipid influx that exceeds the capacity of the adipose tissue to store lipids. Consequently, an excess of fatty acids is observed in the circulation, which is associated with the accumulation of lipids at ectopic sites such as the liver and skeletal muscle. This might lead to systemic lipotoxicity and could influence inflammatory responses. There is evidence indicating that chronic nutrient excess results in increased inflammation through cytokines such as tumour necrosis factor (TNF) and cellular receptors such as toll-like receptor (TLR)-4, and these might be have an important contribution to the pathogenesis of metabolic diseases[20]. However, although excess fat intake is widely accepted as unhealthy, it seems that not just the quantity of fat intake plays a role, but also that the type of fat is important[21].

Fatty acids can be categorized depending on their length and degree of saturation into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The latter group can be further divided into omega-6 (n-6) and omega-3 (n-3) PUFAs (figure 1), based on the location of the last double bond relative to the terminal methyl end of the molecule. The change towards our Western diet increased our intake in SFA, decreased our intake of PUFA and increased the ratio between n-6 and n-3 PUFAs from 1-4:1,

which is deemed optimal, to a ratio of 16-20:1[21]. The different fatty acid types are believed to have distinct effects on inflammation. SFA and n-6 PUFAs have a more pro-inflammatory effect, while n-3 PUFAs have anti-inflammatory effects[22]. Therefore, it is likely that they also play different roles in metabolic OA.

The relationship between fatty acids and OA pathophysiology needs further elucidation. In this review we outline the evidence for a role of fatty acids in the development and progression of OA from studies in animals and humans. We will focus on the association of different fatty acid types with OA and their distinct effects on different joint tissues.

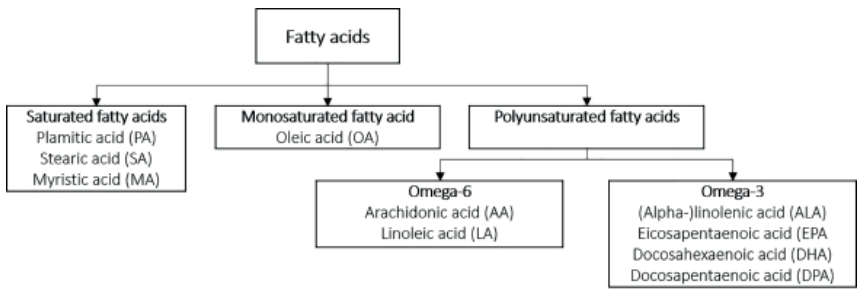

Figure 1. Fatty acid subtypes

Methods

Literature search details

We performed a PubMed literature search to obtain *in vitro*, animal and human studies describing the association of fatty acids with OA. Included search terms were; "fatty acids", "fatty acids, omega-6", "fatty acids, omega-3", "fatty acids, unsaturated", "fatty acids, monounsaturated", "dietary fats", "lipids", "osteoarthritis" and "degenerative arthritis". The search was restricted to the English language and excluded review articles.

Study selection

The above described search retrieved 256 potential studies, which were screened on title and abstract to determine eligibility by one review author (ML). Fifty-five full-text papers were retrieved and assessed for eligibility to determine inclusion, resulting in a total of 29 studies relevant to the subject of this review. Additionally, three relevant articles were identified by other sources. All studies are described in table 1 and 2.

Evidence from animal studies

Fatty acid treatment of chondrocytes

A number of *in vitro* studies investigated the effect of supplementation of different fatty acids to chondrocytes of animal origin[23–26]. Many of these studies have investigated the effect of fatty acids on the secretion or expression of inflammatory factors, such as interleukins (IL), TNF, matrix metalloproteinase (MMPs) and/or prostaglandins. Zainal et al. pre-incubated bovine chondrocytes with the n-3 PUFAs eicosapentaenoic acid (EPA), docosahexaenoic (DHA) and alpha-linolenic acid (ALA), followed by IL-1 stimulation. They observed delayed IL-

1α induced cell death in cultures pre-incubated with EPA. In addition, IL-1 induced expression of A Disintegrin And Metalloproteinase with ThromboSpondin motifs (ADAMTS)-4 and ADAMTS-5, cyclooxygenase (COX)-2, MMP-3, IL-1α, IL-1β and TNF-α, were reduced upon pre-incubation with n-3 PUFAs, most potently by EPA. In contrast, the n-6 PUFA arachidonic acid had no visible effect on the expression of the investigated inflammatory markers[24]. Adler et al. supplemented canine chondrocytes with n-3 PUFAs, showing reduced IL-induced gene expression of inducible nitric oxide synthase (NOS). This decrease was also observed after incubation with the n-6 PUFA arachidonic acid, however, arachidonic acid additionally exerted pro-inflammatory effects, namely increased expression of ADAMTS-5 and release of prostaglandin E (PGE)[25]. In contrast, stimulation of mouse primary chondrocytes with the SFA stearic acid resulted in increased protein stability and transcription of hypoxia-inducible factor (HIF)1-α, a marker of oxidative stress[23].

In summary, stimulation of chondrocytes of animal origin with n-3 PUFAs has been shown to reduce the expression of inflammatory markers, proteinases involved in cartilage degradation and markers of oxidative stress. Additionally, IL-α induced cell death seemed to be delayed by stimulation with the n-3 PUFA EPA. In contrast, stimulation with the n-6 PUFA arachidonic acid was shown to increase makers of cartilage degradation and inflammation and SFA increased makers of oxidative stress.

Lower n-6:n-3 PUFA ratios may protect against osteoarthritis

In a mouse model able to endogenously convert n-6 PUFAs to n-3 PUFAs (Fat-1 mice), it was shown that a reduced n-6:n-3 ratio gave a modest reduction of IL-6 and TNF-α production, compared to wild-type mice. However, the authors did not see any effects on idiopathic osteophyte development, synovial hyperplasia or cartilage degeneration, or on subchondral bone features[27]. Huang et al. utilized the same mouse model, however additionally induced OA by meniscectomy. In Fat-1 mice with surgically induced OA, they found a decrease in cartilage loss, less abrasion and less osteophytes, when compared to wild-type operated mice. They also observed a decrease in MMP-13 and ADAMTS-5 protein expression in mice with enhanced n-3 PUFA synthesis[26].

Increasing n-3 PUFA levels, and thereby decreasing the n-6:n-3 PUFA ratio, might reduce expression of inflammatory makers and structural damage compared to controls in mice OA models.

Supplementation of different fatty acid types yields opposite effects

In mice with surgically induced OA, a diet high in n-3 PUFA showed less cartilage degradation, synovial hypertrophy, macrophage infiltration and heterotopic ossification, when compared to mice on diets high in SFA or n-6 PUFA. Also, n-3 PUFA fed mice showed lower bone mineral density (BMD) compared to mice on the other diets and controls[28]. In line, in OA prone guinea pigs, dietary n-3 PUFAs ameliorated OA associated histologic features[29]. In addition to their work on chondrocytes, Miao et al. also investigated the effect of a high-fat diet on plasma levels of inflammatory markers in mice. They observed increased production of IL-6, TNF-α and IL-1β compared in mice on a high-fat diet, to mice on a normal diet[23]. Sekar et al. investigated the relative effect of SFA chain length, and observed that a diet with longer chain SFAs, such as palmitic acid and stearic acid, induced greater cartilage degeneration, increased MMP-13 and collagenase (COL)10 expression and increased apoptosis, compared to mice fed a diet with shorter chain SFAs[30].

A cross-over study with cats with radiographically diagnosed OA, showed increased owner-reported activity and less stiffness upon a fish oil diet high in n-3 PUFA, compared to cats on a corn oil diet high in n-6 PUFA. However, no wash-out period was used between the diets tested[31]. The duration of the effect of dietary supplemented fatty acids is not well known, therefore effects of the first diet may have interfered with the effects of the second diet. Randomised controlled, double blinded trials in dogs have shown that dogs with OA showed less lameness and discomfort[32,33] and less functional disability when fed a n-3 rich diet compared to dogs fed a control diet[34,35]. Fritsch et al. investigated the need for the painkiller carprofen in dogs with OA, upon a diet high in n-3 PUFA compared to a control diet low in n-3 PUFA. They observed that carprofen dosages could be decreased faster in dogs receiving n-3 PUFAs[36]. A study comparing fish oil and corn oil supplementation showed improvement of markers of oxidative stress in both groups. In contrast to the other studies they failed to show a difference between diets[37].

In conclusion, various studies have investigated the effects of fatty acid enriched diets. A diet high in n-3 PUFA has been shown to reduce OA associated structural damage, compared to a diet high in SFA or n-6 PUFA. Studies also showed that n-3 supplementation resulted in owner-reported decreased dysfunction and less need for pain medication when compared to a regular control diet. In contrast, high fat diet or a diet high in SFA increased the expression of inflammatory markers, which was increased even further with SFA with longer chain length. Additionally, an increase in apoptotic chondrocytes was found upon a diet high in SFA.

Table 1a. Main outcomes of animal in-vitro studies

Author, year	Study population	FA	Methods	Main outcomes
Adler, 2017[25]	Canine chondrocytes	n-3 PUFA n-6 PUFA	Incubation with FA, stimulated with IL-1β	N-6 and n-3 PUFA reduced IL-induced iNOS gene expression and NO production. N-6 PUFA reduced MMP-3, increased ADAMTS-5 and PGE.
Huang, 2014[26]	Mouse primary chondrocytes	n-3:n-6	Incubation with FA	N-3 PUFA decreased mTORC1 activity and promoted autophagy.
Miao, 2015[23]	Mouse primary chondrocytes	SFA	Stimulation with SFA	SFA increased protein levels, stability and transcription activity of HIF1a in primary chondrocytes.
Zainal, 2009[24]	Bovine chondrocytes	n-3 PUFA n-6 PUFA	Pre-incubation with FA followed by IL-1 stimulation	EPA delayed cell death. All n-3 PUFAs reduced Il-1 induced ADAMTS-4, COX2, IL-1α and TNF-α expression. EPA and ALA reduced ADAMTS-5 expression. EPA reduced expression of MMP-3 and MMP-13. No effect on COX1 expression. N-6 PUFA showed no effect on gene expression.

ADAMTS = A Disintegrin And Metalloproteinase with ThromboSpondin motifs, ALA = alpha-linolenic acid, COL = collagenase, COX = cyclooxygenase, DHA = docosahexaenoic acid, ECM = extracellular matrix, EPA = eicosapentaenoic acid, GAG = glycosaminoglycan, HIFs = hypoxia-inducible factors, HFD = high fat diet, IL = interleukin, MA = myristic acid, MMP = matrix metalloproteinase, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n= number, PA = palmitic acid, PGE2 = prostaglandin E2, PUFA = polyunsaturated fatty acid, SA = stearic acid, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, VAS = visual analogue score, VEGF = vascular endothelial growth factor, vs = versus, WT = wild-type

Table 1b. Main outcomes of animal intervention studies

Author, year	Study population	FA	Methods	Main outcomes
Barrouin-Melo, 2016[37]	Dogs with OA, n = 77	n-3 PUFA n-6 PUFA	Fish oil vs. corn oil supplementation	Fish oil and corn oil improved markers of oxidative stress and decreased monocytes, no clear differences between the groups.
Cai, 2014[27]	Fat-1 (n= 17) vs. WT mice (n = 18), idiopathic OA	n6:n3 PUFA	n-6 PUFA enriched diet	Fat-1 expression reduced n-6:n-3 compared to WT. IL-6 and TNF-α levels were modestly reduced in Fat-1 mice. No differences in histologic changes or bone morphology.
Corbee, 2013[31]	Cats with OA, n = 16 mean age 13y	n-3 PUFA	Fish oil vs. corn oil (0% EPA and DHA)	Cats on fish oil showed higher activity, less stiffness and more interaction compared to corn oil.
Fritsch, 2010[36]	Dogs with OA, n = 131	n-3 PUFA	Fish oil vs. control low n-3 diet	Carprofen dosage decreased faster among dogs fed n-3 diet compared to control diet
Huang, 2014[26]	Fat-1 vs. WT mice, n = 24/ group, surgically induced OA.	n-3:n-6	-	Decreased n-6:n-3 reduced cartilage degeneration and osteophytes, decreased MMP-13, ADAMTS-5, reduced chondrocyte number and loss of ECM.
Knott, 2011[29]	OA prone guinea pigs, n = 10/ group	n-3 PUFA	N-3 enriched vs. standard diet	N-3 PUFA reduced histology scores, increased GAG and decreased denatured type II collagen and decreased active MMP-2, compared to standard diet. Subchondral bone parameters changed towards normal.
Mehler, 2016[32]	Dogs with OA, n= 74, mean age 7.8y	n-3 PUFA	Triglyceride n-3 oil vs. placebo oil	No differences in total joint score and improvement VAS compared to placebo. Discomfort and lameness scales improved after 84 days.
Miao, 2015[23]	Lean and obese C57BL/6 mice	-	HFD	HFD increased plasma IL-6, TNF-α, Il-1β and VEGF. No histologic OA signs. Effects partly mediated through HIF1a and TLR4.
Moreau, 2012[34]	Dogs with OA, n=15 / group, mean age 6.5y	n-3 PUFA	N-3 diet vs. control	Improvement of functional disability and performance in activities of daily living in n-3 PUFA diet group, not in controls.
Roush, 2010[33]	Dogs with OA, n = 38	n-3 PUFA	3,5% fish oil vs control	Dogs fed n-3 diet showed significant improvements in lameness and weight bearing after 3 months, compared to baseline.
Roush, 2010[35]	Dogs with OA, n =127	n-3 PUFA n-6:n-3	N-3 rich n-6:n-3 low diet vs. control diet	N-3 enriched diet improved owner-reported ability to rise, play and walk, compared to control diet.
Sekar, 2017[30]	Rats, n = 12 /group Chondrocytes	SFA	Corn starch diet vs. HFD with different SFAs	Rats fed beef tallow, SA, MA or PA diets exhibited cartilage degradation and subchondral bone changes. More cartilage degradation, MMP-13, COL10 expression and apoptosis in longer chain SFAs.
Wu, 2015[28]	Mice, n = 49, surgically induced OA	SFA n-3 PUFA n-6 PUFA	HFD SFA-rich, n-6 rich or n-3 rich vs control	N-3 PUFA enriched HFD reduced surgically-induced OA and enhanced wound repair. SFA or n-6 PUFA increased OA severity, with increased cartilage damage, thicker synovium, heterotopic ossification and were associated with macrophage infiltration.

ADAMTS = A Disintegrin And Metalloproteinase with ThromboSpondin motifs, ALA = alpha-linolenic acid, COL = collagenase, COX = cyclooxygenase, DHA = docosahexaenoic acid, ECM = extracellular matrix, EPA = eicosapentaenoic acid, GAG = glycosaminoglycan, HIFs = hypoxia-inducible factors, HFD = high fat diet, IL = interleukin, MA = myristic acid, MMP = matrix metalloproteinase, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n= number, PA = palmitic acid, PGE2 = prostaglandin E2, PUFA = polyunsaturated fatty acid, SA = stearic acid, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, VAS = visual analogue score, VEGF = vascular endothelial growth factor, vs = versus, WT = wild-type

Evidence from human studies

Beneficial effects of n-3 fatty acid supplementation on chondrocytes and synoviocytes
Six studies investigated the effect of *in vitro* stimulation of human chondrocytes and/or synoviocytes[23,38–42]. Alvarez-Garcia et al. incubated chondrocytes isolated from human articular cartilage with fatty acids. They found no effect of mono-incubation of chondrocytes with fatty acids. However, when they co-incubated chondrocytes with IL-1β, they observed an increase in caspase 3 and 7, and decreased chondrocyte viability upon incubation with the SFA palmitate. They did not see this effect upon co-incubation with oleate, a MUFA. In addition, they observed increased IL-6, COX-2 and NOS2 mRNA levels in both normal and OA chondrocytes upon incubation with palmitate, but not oleate. Incubation of human synoviocytes showed similar results. Furthermore, they found that these effects were reduced by inhibition of caspase or TLR-4 signalling[38]. In contrast, Bastiaansen-Jenniskens et al. observed a decrease in MMP-1 expression and glycosaminoglycan release upon treatment of human chondrocytes not only with oleate, but also with the SFA palmitate when co-stimulated with TNF-α. Additionally, oleate decreased prostaglandin-endoperoxide synthase (PTGS2). They did not observe effects on the investigated inflammatory and destructive markers when chondrocytes were stimulated only with SFA, MUFA or n-6 PUFA[39]. Frommer et al. investigated the effect of SFA and PUFA stimulation on IL-6 secretion, both in human chondrocytes and synovial fibroblasts. In human chondrocytes the strongest effect was observed upon stimulation with the SFA palmitic acid, while unsaturated fatty acids, especially PUFAs, had much weaker effects on IL-6 secretion. In synovial fibroblasts they found a dose-dependent increase in IL-6 secretion both upon stimulation with SFA and PUFA. The SFA stearic acid has also been shown to enhance expression of IL-6, TNF-α, IL-1β in chondrocytes, compared to controls. This study supported the finding from Alvarez-Garcia et al.[38] that the SFA induced inflammation is partially mediated by TLR-4[40]. Shen et al. treated human osteoarthritic chondrocytes for 6 days with different n-6 PUFAs, (conjugated) linoleic acid and arachidonic acid, and the n-3 PUFA EPA. They observed a lower PGE2 production in chondrocytes treated with n-3 PUFA, however not significantly. Stimulation of NO production with IL-1 and LPS was significantly reduced by all investigated PUFA treatments, compared to the control group[41].

In summary, in line with studies with chondrocytes from animal origin, most human *in vitro* studies show pro-apoptotic and pro-inflammatory actions of SFA. These actions seem to be at least partially mediated by TLR-4 signalling. Although MUFA have been studied less often, most studies show no effect of cell treatment with MUFA. N-3 PUFAs have been shown to reduce prostaglandin production by human chondrocytes, while all PUFAs reduced makers of oxidative stress. Although results are comparable to those observed in animal studies, n-3 PUFAs have been studied less often in human *in vitro* studies.

Table 2a. Main outcomes of human in-vitro studies

Author, year	Study population	FA	Methods	Main outcomes
Alvarez-Garcia, 2014[38]	Human chondrocytes and synoviocytes	SFA MUFA	Incubation with FA alone, or co-incubation with IL-β	Co-incubation of chondrocyte with SFA and IL-β increased caspase 3 and 7. SFA but not MUFA increased il6, cox2 and nos2 mRNA and IL-6 secretion. In human synoviocytes SFA, not MUFA increased IL-6 and COX2 expression. Effects reduced by inhibition of caspase or TLR-4.
Bastiaansen-Jenniskens, 2013[39]	Chondrocytes isolated from TKR material. Knee OA, n= 3, mean age 72y, mean BMI 31, 67% women	SFA MUFA n-6 PUFA	Stimulation with FA alone, or co-incubation with TNFα	Without TNF-α: no effect of FA on MMP-1, PTGS2 and PGE2, but decrease of GAG by oleic acid and PA, not LA. With TNF-α: decrease of MMP-1 gene expression by oleic acid and PA, no effect of LA. Oleic acid decreased PTGS2, no effect of LA and PA. LA increased PGE2, no effect of oleic acid and PA. Oleic acid and PA lowered GAG release, no effect of LA.
Frommer, 2013[40]	Synovial fibroblasts from RA, OA, PsA and controls. Primary chondrocytes	SFA PUFA	Stimulation with FFA	Both SFA and PUFA increased IL-6 secretion dose-dependently. Findings seem to be mediated by TLR4.
Humphries, 2012[44]	Cancellous bone matrix from OA patients	SFA MUFA n-3 PUFA n-6 PUFA	Gas chromatography of FFA profile	Subchondral bone from OA subjects had higher n-6 and n-3 PUFA than controls, no difference in n-6:n-3 ratio, or MUFA.
Lippiello, 1991[43]	Femoral heads from THR in OA patients , n=21	SFA n-6 PUFA MUFA	Gas chromatography of FFA levels	85% of total lipid content was comprised of palmitic, linoleic and oleic acid. Increasing FFA levels with increasing OA severity, no differences between FFA type.
Miao, 2015[23]	Human chondrocytes from TKR material, n=10, mean age 65y	SFA	Stimulation with SFA	SFA treated chondrocytes showed increased IL-6, TNF-α, Il-1β and VEGF mRNA levels, compared to controls.
Shen, 2004[41]	Human osteoarthritic chondrocytes	CLA, combined with n-6 or n-3 PUFA	6 days pre-incubation with FA	Compared to control, supplementation of CLA or LA alone and LA/EPA resulted in lower PGE2 production. Incubation with CLA/EPA resulted in lowest PGE2 production. LA/AA, LA/EPA, and CLA treatments decreased IL- β induced NO, LA and CLA/EPA treatments increased NO production.
Yu, 2015[42]	Human chondrocytes	n-6:n-3	Treatment with FA	Low n-6:n-3 decreased MMP-13 mRNA and protein levels in human chondrocytes. No effect on chondrocyte proliferation

AA = arachidonic acid, AdA = adrenic acid, ALA = alpha-linolenic acid, BMI = body mass index, BMD = bone mineral density, BML = bone marrow lesion, CFA = cetylated fatty acid, CLA = conjugated linoleic acid, COX = cyclooxygenase, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FFA = free fatty acid, GAG = glycosaminoglycan, IL = interleukin, JSW = joint space width, LA = linolenic acid, MMP = matrix metalloproteinase, MUFA = monounsaturated fatty acid, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n= number, PA = palmitic acid, PGE2 = prostaglandin E2, PLSDA = partial least-squares-discrimination analysis, PUFA = polyunsaturated fatty acid, RA = rheumatoid arthritis, RCT = randomized controlled trial, ROM = range of motion, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, UPLC-MC = ultra-performance liquid chromatography mass spectrometry, VEGF = vascular endothelial growth factor, vs = versus

Table 2b. Main outcomes of human epidemiological and intervention studies

Author, year	Study population	FA	Methods	Main outcomes
Baker, 2012[52]	Knee OA, n = 472, mean age 60y, mean BMI 30, 50% women	n-3 PUFA n-6 PUFA	Cross-sectional	Multivariable logistic regression controlling for age, sex and BMI, showed positive relation between plasma levels of n-3 PUFAs with the amount of patellofemoral cartilage, but not tibiofemoral cartilage or synovitis. Positive association between n-6 PUFA and synovitis.
Castro-Perez, 2010[46]	Hip and knee OA, n = 59, 100% women	-	Lipidomics UPLC-MS	Multivariable PLSDA suggested altered lipid metabolism associated with OA.
Chen, 2016[53]	Knee OA, n = 202, mean age 61y, mean BMI 29, 49% women	n-3 PUFA	RCT, double blind. High vs low dose n-3 fish oil	No differences in BMD after 2 years between high and low dose groups after adjusting for baseline BMD.
Gruenwald, 2009[48]	Hip and knee OA, n = 178, mean age 62y, mean BMI 29, 63% women	n-3 PUFA	RCT, double blind. Glucosamine sulphate + n-3 PUFA vs glucosamine sulphate alone	Greater reductions in morning stiffness and pain (WOMAC) upon combination therapy vs. glucosamine sulphate alone.
Hesslink, 2002[49]	Knee OA, n = 66, mean age 58y, mean BMI 28, 33% women	CFA	RCT, double blind. CFA vs placebo (vegetable oil)	CFA increased knee flexion, but not extension, compared to placebo. Patient-reported pain reduction and improvement of disability after 68 days compared to placebo.
Hill, 2016[50]	Knee OA, n = 202, mean age 61y, mean BMI 29, 49% women	n-3 PUFA	RCT, double blind. High dose vs low dose fish oil	Greatest reduction in WOMAC pain and disability after 2 years in low-dose group. No difference in cartilage volume, BML score, NSAID use and quality of life.
Jónasdóttir, 2017[45]	Knee OA, n = 24, Mean age 68y, mean BMI 29, 16% women	-	Targeted lipidomics LC-MS/MS	N-6 PUFAs AA, AdA, LA and the n-3 PUFAs EPA, DPA and ALA were detected in SF of OA patients.
Kraemer, 2004[51]	Knee OA, n= 40, mean age 64y, mean BMI 31, 85% women	CFA	RCT, double blind. Topical cream with CFA or placebo	CFA topical cream improved knee ROM, ability to ascend/ descend stairs, ability to rise from sitting, walk and sit down, and unilateral balance.
Lu, 2017[54]	Radiographic knee OA, n = 2092, mean age 62y, 59% women	SFA MUFA PUFA	Longitudinal	Self-reported total fat and SFA intake was positively associated with JSW loss at 48 months. Higher intakes of MUFA, PUFA and higher PUFA to SFA ratio was associated with reduced JSW loss.
Zhang, 2015[47]	Knee OA, n = 40, healthy controls. Mean age 58y, mean BMI 28, 50% women	-	Metabolomics UPLC-MS	OA-specific serum metabolic profile enabled discriminating patients with knee OA from age- and gender-matched controls and different severities of OA.

AA = arachidonic acid, AdA = adrenic acid, ALA = alpha-linolenic acid, BMI = body mass index, BMD = bone mineral density, BML = bone marrow lesion, CFA = cetylated fatty acid, CLA = conjugated linoleic acid, COX = cyclooxygenase, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FFA = free fatty acid, GAG = glycosaminoglycan, IL = interleukin, JSW = joint space width, LA = linolenic acid, MMP = matrix metalloproteinase, MUFA = monounsaturated fatty acid, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n= number, PA = palmitic acid, PGE2 = prostaglandin E2, PLSDA = partial least-squares-discrimination analysis, PUFA = polyunsaturated fatty acid, RA = rheumatoid arthritis, RCT = randomized controlled trial, ROM = range of motion, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, UPLC-MC = ultra-performance liquid chromatography mass spectrometry, VEGF = vascular endothelial growth factor, vs = versus

Fatty acid levels in OA patients

In the beginning of the nineties of the last century, Lippiello et al. investigated the distribution profile of individual fatty acids in articular cartilage of femoral heads obtained from total hip replacement surgery. They showed that total fatty acid levels, and increased arachidonic acid levels in particular, were positively associated with the severity of osteoarthritic lesions[43]. In line, higher percentages of total n-6 and n-3 PUFAs were observed in subchondral bone of OA patients, when compared to patients with fractured neck of femur, and control subjects. No differences in the ratio between n-6 and n-3 PUFAs, nor in the percentage of MUFAs was observed in the subchondral bone of the different patient groups[44].

Characterization of lipid profiles of individual patients and identification of biomarkers may aid early OA diagnosis. Jónasdóttir et al. investigated FA in synovial fluid of knee OA patients. They detected seven different PUFAs; the n-6 PUFAs adrenic acid and linoleic acid (LA) and the n-3 PUFAs EPA, DHA and docosapentaenoic acid (DPA)[45]. Castro-Perez et al. demonstrated using ultra performance liquid chromatography mass spectrometry (UPLC-MS) that they were able to differentiate between patients without structural hip or knee OA and patient with early and moderate OA. Multivariable partial least-squares-discrimination analysis (PLSDA) suggested an altered lipid metabolism in OA patients, compared to patients with knee complaints without OA abnormalities on radiography[46]. Using a similar approach, Zhang et al. discriminated knee OA patients from healthy age- and gender-matched controls and were able to stratify patients on OA severity. They observed involvement of multiple metabolic pathways, including fatty acid and lipid metabolism[47].

Investigation of the fatty acids present in OA tissues showed that in more severe osteoarthritic lesions the total fatty acid levels and in particular the n-6 PUFA levels were increased. Current lipidomic research seems promising for the development of biomarkers. However, how these findings relate to dietary fatty acid intake is difficult to establish. Unfortunately, research in this field is still rather limited. However, these studies may be valuable to identify potential biomarkers to help diagnose OA in an early disease-stage, therefore additional research is warranted.

Effect on pain and function

The efficacy of fatty acids on OA symptoms has been investigated in four human intervention studies, which investigated almost exclusively patients with knee OA[48–51]. Supplementation of cetylated fatty acids (CFA), compared to placebo consisting of vegetable oil, resulted in a significant increase in knee flexion, and a modest improvement in patient-reported outcomes. However, the consistency of the placebo vegetable oil was not described[49], therefore possible effects of the placebo treatment cannot be ruled out. CFA has also been investigated as a topical treatment. Application of cream with CFA showed improvement in range of motion of knee OA patients after 30 days, which was not observed in patients receiving placebo cream[51]. Gruenwald et al. investigated in a randomized, double blind controlled trial the effects of adding n-3 PUFAs to glucosamine sulphate therapy in hip and knee OA patients. They found a higher number of patients with reported WOMAC pain reduction after 26 weeks, compared to mono-therapy with glucosamine sulphate[48]. The beneficial effect of n-3 PUFAs is supported by Hill et al., who compared high-dose versus low-dose fish oil supplementation in knee OA patients. They observed improvement in WOMAC pain and function in both groups. However, they found a greater improvement in the low-dose fish oil group after 2 years. They did not observe any differences in NSAID use or quality of life. Of note, the low-dose fish oil supplement was a blend of fish oil and sunola oil. The effect of sunola oil on the measured

outcomes is not clear[50].

In conclusion, human intervention studies with n-3 PUFA supplementation indicate a possible beneficial effect of n-3 PUFAs on patient reported outcomes such as pain and function. However, studies struggled to include a proper control group. Placebo treatment consisted of a different kind of (vegetable) oil, of which the consistency was not always described. It is hard to predict the treatment effect of these placebo treatments. Therefore, results should be interpreted with caution.

Table 3. The effect of fatty acids on different joint tissues

Fatty acid	Cartilage	Synovium	Subchondral bone
Saturated fatty acid	Increased plasma TNF-α, Il-1β, VEGF[23] in mice, IL6 secretion in mice and humans[23, 38, 40] and il6, cox2, nos2 mRNA and caspase 3 and 7 expression in humans[38], Increase in cartilage degradation[28,30] more severe in longer chain SFA[30] in mice and rats. Associated with JSW loss in humans[54].	Synovial hypertrophy and macrophage infiltration in mice[28]. Increased IL-6 and COX2 expression in human synoviocytes[38].	Increased heterotopic ossification in mice[28].
Monounsaturated fatty acid	Increased IL6 expression[40], no effect on il6, cox2 and nos2 mRNA[38] in human chondrocytes. Associated with reduced JSW loss in humans[54].	Not reported	Not reported
Polyunsaturated fatty acid Omega-6	Increased expression of ADAMTS-5, iNOS and PGE in canine chondrocytes[25]. Reduced NO production in animal and human chondrocytes[25,41]. Increased cartilage degradation in mice[28] and with reduced JSW loss[54] in humans.	Synovial hypertrophy and macrophage infiltration in mice[28]. In humans a positive association with MRI assessed synovitis was found[52]	Not reported
Omega-3	Reduced NO and iNOS production in canine chondrocytes[25]. Reduced expression of ADAMTS-4[24] and ADAMTS-5[24,26], COX-2, MMP-3, IL-1 and TNF-α[24] in chondrocytes of animal origin. Delayed cell death[24]. Reduced PGE2, NO[41] and MMP-13 production[42] in human chondrocytes. Reduced cartilage degradation[26] in mice. In humans a reduced amount of patellofemoral, but not tibiofemoral cartilage[52] and reduced JSW loss[54], but not all showed effect on cartilage volume[50].	In humans no effect observed[52].	In mice less osteophytes were observed upon lowering n-6:n-3 ratio[26], which was not seen by others[27]. In humans no effect on BMLs[50] or BMD was observed[53].

ADAMTS = A Disintegrin And Metalloproteinase with ThromboSpondin motifs, BMD = bone mineral density, BML = bone marrow lesion, COX = cyclooxygenase, IL = interleukin, JSW = joint space width, MMP = matrix metalloproteinase, MRI = magnetic resonance imaging, NOS = nitric oxide synthase, PGE = prostaglandin E, SFA = saturated fatty acid, TNF = tumour necrosis factor, VEGF = vascular endothelial growth factor

Fatty acids and structural OA abnormalities

Additionally, four human studies have investigated structural abnormalities upon fatty acid supplementation (table 3)[50,52–54]. In multivariable models adjusting for several potential confounding factors, including BMI, weight changes and baseline disease severity, Lu et al. observed effects of reported dietary intake of fatty acids on joint space width (JSW) loss over 4 years' time. Total fatty acid intake and SFA intake was positively associated with JSW loss. Opposite effects were found for MUFA and PUFA. Higher unsaturated fatty acid intake was associated with reduced JSW loss[54]. Baker et al. cross-sectionally investigated the association of plasma n-6 and n-3 PUFAs with semi-quantitatively scored synovial thickening and cartilage amount in knee OA patients in the MOST study. In multivariable models adjusting for age, sex and BMI, they observed an positive relation between n-3 PUFAs and amount of

patellofemoral cartilage, but not tibiofemoral cartilage or synovial thickness. In addition, they found a positive association between the n-6 PUFA arachidonic acid and synovial thickness[52]. However, in a randomized controlled trial by Hill et al., no differences in cartilage volume or BML scores assessed by MRI were found between patients receiving high-dose or low-dose fish oil supplementation[50]. Another randomized, double blind clinical trial investigated the effect of high-dose and low-dose n-3 supplementation on BMD measured by DEXA. They found no differences in BMD between the groups after 2 years[53].

The effect of fatty acids on structural abnormalities in OA has been investigated rarely. To be able to draw more robust conclusions additional research is warranted.

Discussion and conclusion

Animal *in vitro* and intervention studies suggest unfavourable effects of SFA, with increased production of pro-inflammatory and pro-apoptotic markers. Conversely, n-3 PUFAs have been shown to decrease markers of inflammation and cartilage degradation. Multiple animal intervention studies have shown a beneficial effect of a diet high in n-3 PUFAs or with a low n-6:n-3 PUFA ratio, with a decrease in cartilage degradation and less osteophyte formation. However, the study designs and research methods employed often preclude an unequivocal interpretation of the results. Studies utilized owner-reported outcomes, which are difficult to interpret regarding reliability. In addition, due to unknown effects of other supplementation diets, appropriateness of control treatment was questionable in most designs. Moreover, negative results have been reported very infrequent, which may indicate publication bias.

Studies in humans have been rather limited so far. An adverse effect of total fatty acids and SFAs has been observed on structural abnormalities. In addition, n-3 PUFAs may be associated with less structural damage and improvement in pain and function in knee and hip OA patients. Due to a variety in the regular diet to which the fatty acids are supplemented, fatty acid intake is less well controlled in human studies compared to animal studies. However, this will also be the case in real-life, and is therefore well generalizable to daily-practise. Unfortunately, current research in OA patients is limited to patients with knee and hip OA. It would be of great interest to see if similar results can be obtained in patients with hand OA, since in particular in hand OA patients systemic factors, such as fatty acid levels, are very likely involved in the development and progression of the disease.

In conclusion, fatty acids appear to have effects on both symptoms and structural abnormalities associated with osteoarthritis. The different fatty acid types exert distinct effects. N-3 PUFAs seem to reduce inflammatory markers and cartilage degradation. In contrast, opposed effects where observed of SFAs and n-6 PUFAs. However, to be able to draw indisputable conclusions, additional research with high quality methods, in a broader array of osteoarthritis patients will be needed.

Research agenda

- More high-quality human intervention studies investigating the effect of fatty acid supplementation on OA are needed to draw firm conclusions.
- The involvement of fatty acids needs to be investigated in different OA phenotypes, especially in hand OA.
- The effect of MUFAs on OA requires further investigation.

References

1. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388, 1545–1602 (2016).
2. Bijlsma, J. W. J., Berenbaum, F. & Lafeber, F. P. J. G. Osteoarthritis: an update with relevance for clinical practice. *Lancet* 377, 2115–2126 (2011).
3. Sellam, J. & Berenbaum, F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 80, 568–573 (2013).
4. Dell'Isola, A., Allan, R., Smith, S. L., Marreiros, S. S. P. & Steultjens, M. Identification of clinical phenotypes in knee osteoarthritis: a systematic review of the literature. *BMC Musculoskelet Disord* 17, 425 (2016).
5. Felson, D. T., Anderson, J. J., Naimark, A., Walker, A. M. & Meenan, R. F. Obesity and knee osteoarthritis. The Framingham Study. *Ann. Intern. Med.* 109, 18–24 (1988).
6. Visser, A. W. *et al.* The role of fat mass and skeletal muscle mass in knee osteoarthritis is different for men and women: the NEO study. *Osteoarthr. Cartil.* 22, 197–202 (2014).
7. Visser, A. W. *et al.* The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. *Ann. Rheum. Dis.* 74, 1842–1847 (2015).
8. Visser, A. W. *et al.* Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. *Arthritis Res. Ther.* 16, R19 (2014).
9. Davis, M. A., Ettinger, W. H. & Neuhaus, J. M. Obesity and osteoarthritis of the knee: evidence from the National Health and Nutrition Examination Survey (NHANES I). *Semin. Arthritis Rheum.* 20, 34–41 (1990).
10. Stürmer, T., Günther, K. P. & Brenner, H. Obesity, overweight and patterns of osteoarthritis: the Ulm Osteoarthritis Study. *J Clin Epidemiol* 53, 307–313 (2000).
11. Radin, E. L., Paul, I. L. & Rose, R. M. Role of mechanical factors in pathogenesis of primary osteoarthritis. *Lancet* 1, 519–522 (1972).
12. Yusuf, E. *et al.* Association between weight or body mass index and hand osteoarthritis: a systematic review. *Ann. Rheum. Dis.* 69, 761–765 (2010).
13. Hart, D. J., Doyle, D. V. & Spector, T. D. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J. Rheumatol.* 22, 1118–1123 (1995).
14. Monira Hussain, S. *et al.* Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin. Arthritis Rheum.* 43, 429–436 (2014).
15. Yoshimura, N. *et al.* Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthr. Cartil.* 20, 1217–1226 (2012).
16. Dahaghin, S., Bierma-Zeinstra, S. M. A., Koes, B. W., Hazes, J. M. W. & Pols, H. a. P. Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. *Ann. Rheum. Dis.* 66, 916–920 (2007).
17. Garessus, E. D. G., de Mutsert, R., Visser, A. W., Rosendaal, F. R. & Kloppenburg, M. No association between impaired glucose metabolism and osteoarthritis. *Osteoarthr. Cartil.* 24, 1541–1547 (2016).
18. Kornaat, P. R. *et al.* Positive association between increased popliteal artery vessel wall thickness and generalized osteoarthritis: is OA also part of the metabolic syndrome? *Skeletal Radiol.* 38, 1147–1151 (2009).
19. Richter, M., Trzeciak, T., Owecki, M., Pucher, A. & Kaczmarczyk, J. The role of adipocytokines in the pathogenesis of knee joint osteoarthritis. *Int Orthop* 39, 1211–1217 (2015).
20. Hotamisligil, G. S. Inflammation, metaflammation and immunometabolic disorders. *Nature* 542, 177–185 (2017).
21. Cordain, L. *et al.* Origins and evolution of the Western diet: health implications for the 21st century. *Am. J. Clin. Nutr.* 81, 341–354 (2005).
22. Bagga, D., Wang, L., Farias-Eisner, R., Glaspy, J. A. & Reddy, S. T. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1751–1756 (2003).
23. Miao, H. *et al.* Stearic acid induces proinflammatory cytokine production partly through activation of lactate-HIF1α pathway in chondrocytes. *Sci Rep* 5, 13092 (2015).
24. Zainal, Z. *et al.* Relative efficacies of omega-3 polyunsaturated fatty acids in reducing expression of key proteins in a model system for studying osteoarthritis. *Osteoarthr. Cartil.* 17, 896–905 (2009).
25. Adler, N., Schoeniger, A. & Fuhrmann, H. Polyunsaturated fatty acids influence inflammatory markers in a cellular model for canine osteoarthritis. *J Anim Physiol Anim Nutr (Berl)* 102, e623–e632 (2018).
26. Huang, M.-J. *et al.* Enhancement of the synthesis of n-3 PUFAs in fat-1 transgenic mice inhibits mTORC1 signalling and delays surgically induced osteoarthritis in comparison with wild-type mice. *Ann Rheum Dis* 73, 1719–1727 (2014).
27. Cai, A. *et al.* Metabolic enrichment of omega-3 polyunsaturated fatty acids does not reduce the onset of idiopathic knee osteoarthritis in mice. *Osteoarthritis Cartilage* 22, 1301–1309 (2014).
28. Wu, C.-L. *et al.* Dietary fatty acid content regulates wound repair and the pathogenesis of osteoarthritis following joint injury. *Ann Rheum Dis* 74,

2076–2083 (2015).
29. Knott, L., Avery, N. C., Hollander, A. P. & Tarlton, J. F. Regulation of osteoarthritis by omega-3 (n-3) polyunsaturated fatty acids in a naturally occurring model of disease. *Osteoarthritis Cartilage* 19, 1150–1157 (2011).
30. Sekar, S. *et al.* Saturated fatty acids induce development of both metabolic syndrome and osteoarthritis in rats. *Sci Rep* 7, 46457 (2017).
31. Corbee, R. J., Barnier, M. M. C., van de Lest, C. H. A. & Hazewinkel, H. A. W. The effect of dietary long-chain omega-3 fatty acid supplementation on owner's perception of behaviour and locomotion in cats with naturally occurring osteoarthritis. *J Anim Physiol Anim Nutr (Berl)* 97, 846–853 (2013).
32. Mehler, S. J., May, L. R., King, C., Harris, W. S. & Shah, Z. A prospective, randomized, double blind, placebo-controlled evaluation of the effects of eicosapentaenoic acid and docosahexaenoic acid on the clinical signs and erythrocyte membrane polyunsaturated fatty acid concentrations in dogs with osteoarthritis. *Prostaglandins Leukot Essent Fatty Acids* 109, 1–7 (2016).
33. Roush, J. K. *et al.* Evaluation of the effects of dietary supplementation with fish oil omega-3 fatty acids on weight bearing in dogs with osteoarthritis. *J Am Vet Med Assoc* 236, 67–73 (2010).
34. Moreau, M. *et al.* Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis. *J Anim Physiol Anim Nutr (Berl)* 97, 830–837 (2013).
35. Roush, J. K. *et al.* Multicenter veterinary practice assessment of the effects of omega-3 fatty acids on osteoarthritis in dogs. *J Am Vet Med Assoc* 236, 59–66 (2010).
36. Fritsch, D. A. *et al.* A multicenter study of the effect of dietary supplementation with fish oil omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. *J Am Vet Med Assoc* 236, 535–539 (2010).
37. Barrouin-Melo, S. M. *et al.* Evaluating oxidative stress, serological- and haematological status of dogs suffering from osteoarthritis, after supplementing their diet with fish or corn oil. *Lipids Health Dis* 15, 139 (2016).
38. Alvarez-Garcia, O., Rogers, N. H., Smith, R. G. & Lotz, M. K. Palmitate has proapoptotic and proinflammatory effects on articular cartilage and synergizes with interleukin-1. *Arthritis Rheumatol* 66, 1779–1788 (2014).
39. Bastiaansen-Jenniskens, Y. M. *et al.* Monounsaturated and Saturated, but Not n-6 Polyunsaturated Fatty Acids Decrease Cartilage Destruction under Inflammatory Conditions: A Preliminary Study. *Cartilage* 4, 321–328 (2013).
40. Frommer, K. W. *et al.* Free fatty acids: potential proinflammatory mediators in rheumatic diseases. *Ann Rheum Dis* 74, 303–310 (2015).
41. Shen, C.-L., Dunn, D. M., Henry, J. H., Li, Y. & Watkins, B. A. Decreased production of inflammatory mediators in human osteoarthritic chondrocytes by conjugated linoleic acids. *Lipids* 39, 161–166 (2004).
42. Yu, H. *et al.* A low ratio of n-6/n-3 polyunsaturated fatty acids suppresses matrix metalloproteinase 13 expression and reduces adjuvant-induced arthritis in rats. *Nutr Res* 35, 1113–1121 (2015).
43. Lippiello, L., Walsh, T. & Fienhold, M. The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. *Metabolism* 40, 571–576 (1991).
44. Humphries, J. M., Kuliwaba, J. S., Gibson, R. J. & Fazzalari, N. L. In situ fatty acid profile of femoral cancellous subchondral bone in osteoarthritic and fragility fracture females: implications for bone remodelling. *Bone* 51, 218–223 (2012).
45. Jónasdóttir, H. S. *et al.* Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans. *Osteoarthr. Cartil.* 25, 1150–1160 (2017).
46. Castro-Perez, J. M. *et al.* Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. *J Proteome Res* 9, 2377–2389 (2010).
47. Zhang, Q., Li, H., Zhang, Z., Yang, F. & Chen, J. Serum metabolites as potential biomarkers for diagnosis of knee osteoarthritis. *Dis Markers* 2015, 684794 (2015).
48. Gruenwald, J., Petzold, E., Busch, R., Petzold, H.-P. & Graubaum, H.-J. Effect of glucosamine sulfate with or without omega-3 fatty acids in patients with osteoarthritis. *Adv Ther* 26, 858–871 (2009).
49. Hesslink, R. J., Armstrong, D. 3rd, Nagendran, M. V., Sreevatsan, S. & Barathur, R. Cetylated fatty acids improve knee function in patients with osteoarthritis. *J Rheumatol* 29, 1708–1712 (2002).
50. Hill, C. L. *et al.* Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. *Ann. Rheum. Dis.* 75, 23–29 (2016).
51. Kraemer, W. J. *et al.* Effect of a cetylated fatty acid topical cream on functional mobility and quality of life of patients with osteoarthritis. *J Rheumatol* 31, 767–774 (2004).
52. Baker, K. R. *et al.* Association of plasma n-6 and n-3 polyunsaturated fatty acids with synovitis in the knee: the MOST study. *Osteoarthr. Cartil.* 20, 382–387 (2012).
53. Chen, J. S. *et al.* Supplementation with omega-3 fish oil has no effect on bone mineral density in adults with knee osteoarthritis: a 2-year randomized controlled trial. *Osteoporos Int* 27, 1897–1905 (2016).
54. Lu, B. *et al.* Dietary Fat Intake and Radiographic Progression of Knee Osteoarthritis: Data From the Osteoarthritis Initiative. *Arthritis Care Res (Hoboken)* 69, 368–375 (2017).

3

The association of plasma fatty acids with hand and knee osteoarthritis: the NEO-study

Marieke Loef, Andreea Ioan-Facsinay, Dennis Mook-Kanamori,
Ko Willems van Dijk, Renée de Mutsert, Margreet Kloppenburg, Frits Rosendaal

Osteoarthritis and Cartilage 2020;28:223-30

Abstract

Objective To investigate the association of postprandial and fasting plasma saturated fatty acid (SFAs), monounsaturated fatty acid (MUFAs) and polyunsaturated fatty acid (PUFAs) concentrations with hand and knee osteoarthritis (OA).

Design In the population-based NEO study clinical hand and knee OA were defined by the ACR classification criteria. Structural knee OA was defined on MRI. Hand and knee pain was determined by AUSCAN and KOOS, respectively. Plasma was sampled fasted and 150 minutes after a standardized meal, and subsequently analysed using a nuclear magnetic resonance platform. Logistic regression analyses were used to investigate the association of total fatty acid, SFA, MUFA, total PUFA, omega-3 PUFA and omega-6 PUFA concentrations with clinical hand and knee OA, structural knee OA and hand and knee pain. Fatty acid concentrations were standardized (mean 0, SD 1). Analyses were stratified by sex and corrected for age, education, ethnicity and total body fat percentage.

Results Of the 5,328 participants (mean age 56 years, 58% women) 7% was classified with hand OA, 10% with knee OA and 4% with concurrent hand and knee OA. In men, postprandial SFAs (OR (95% CI)) 1.23 (1.00; 1.50), total PUFAs 1.26 (1.00; 1.58) and omega-3 PUFAs 1.24 (1.01; 1.52) were associated with hand OA. SFAs and PUFAs were associated with structural, but not clinical knee OA. Association of fasting fatty acid concentrations were weaker than postprandial concentrations.

Conclusion Plasma postprandial SFA and PUFA levels were positively associated with clinical hand and structural knee OA in men, but not in women.

Introduction

In the past decades, the prevalence and burden of osteoarthritis (OA) have increased significantly. This development is likely to continue due to ageing of the population and rising numbers of obese individuals[1]. The association between obesity and OA was for a long time believed to be explained by increased mechanical loading[2]. More recently, the role of systemic factors is becoming increasingly recognized, especially in non-weightbearing joints[3,4].

Nutrient excess may lead to systemic lipid overload with increased levels of circulating fatty acids and lipotoxicity[5]. Previous studies investigating the role of fatty acids in OA have indicated a detrimental effect of saturated fatty acids (SFAs) and omega-6 polyunsaturated fatty acids (PUFAs) on chondrocytes, via induction of prostaglandins and upregulation of gene expression related to apoptosis and cartilage degradation[6,7]. In contrast, incubation of chondrocyte cultures with omega-3 PUFAs resulted in a reduction of cartilage proteinase mRNA levels and inflammatory cytokines[8]. However, human studies are few. Baker et al. showed that high omega-3 PUFA levels were associated with a greater amount of patellofemoral cartilage, but not with tibiofemoral cartilage, and higher omega-6 PUFA levels were associated with an increased severity of synovitis. However, these associations were only observed for the highest levels of omega-3 PUFAs[9]. In addition, a recent randomized trial found a decrease in pain and function in patients with knee OA after fish oil supplementation (containing high levels of omega-3 PUFAs). However, the effect was paradoxically most profound in the low dose group and no effects were seen on cartilage loss[10].

Overall, evidence supports an effect of fatty acid concentrations on OA. However, human studies investigating the effect of the different fatty acids types on OA are scarce and inconsistent, and limited to structural knee OA. Moreover, due to regular food intake at meal times and frequent snacking, humans are in a postprandial state during most of the day. Hence, postprandial concentrations may be a better representative of long-term exposure. Therefore, our primary aim was to investigate the association of postprandial plasma SFAs, monounsaturated fatty acid (MUFAs), omega-6 and omega-3 PUFAs with clinically defined hand and knee OA. Furthermore, we assessed the association of postprandial fatty acids with structural knee OA, and hand and knee pain. In addition, we investigated the association of fasting fatty acids with hand and knee OA.

Materials and methods

Study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study, with an oversampling of individuals with overweight or obesity. Detailed description of study design and data collection has been described elsewhere[11]. In short, men and women between 45 and 65 years with a self-reported body mass index (BMI) ≥ 27 kg/m^2 living in the greater area of Leiden (The Netherlands) were eligible to participate. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population[12]. In total, 6,671 participants were included in the NEO study. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent. The present study is a cross-sectional analysis of baseline measurements. We excluded participants who reported

to have inflammatory rheumatic disease or fibromyalgia, with missing physical examination, who were non-fasting at baseline or reported using lipid-lowering medication.

General and disease specific questionnaires
Prior to the study visit, participants completed questionnaires on demographic and clinical information; including self-reported presence of rheumatic disease other than OA and the use of lipid lowering medication. In addition, participants completed the Knee Injury and Osteoarthritis Outcome Score (KOOS)[13,14] and the Australian/Canadian Hand Osteoarthritis Index (AUSCAN)[15]. Since the relevance in difference in pain score may depend on the level of the score, we dichotomized the AUSCAN and KOOS pain subscales to represent relevant elevations in pain using cut-offs determined in benchmark studies[16,17]. Hand pain was defined as present when the AUSCAN pain subscale was equal to or above 5 points in men, and equal to or above 10 points in women[16]. Knee pain was present when the KOOS pain subscale was equal to or below 84 in men, and equal to or below 97 in women[17].

Clinical assessment
Body weight (kg) and total body fat (%) were measured by bioelectrical impedance balance (TBF-310; Tanita Europe BV, Amsterdam, The Netherlands). BMI was calculated from measured body weight and height (kg/m^2). In addition, extensive physical examination of the hands and knees was performed by trained research nurses, using a standardized scoring form. Of both hands, bony and soft swellings and deformities of distal interphalangeal, proximal interphalangeal, metacarpophalangeal, carpometacarpal and wrist joints were assessed. Regarding the knees, presence of bony swellings, palpable pain and warmth, crepitus and movement restriction were assessed. Clinical hand and knee OA was defined according to the American College of Rheumatology (ACR) clinical classification criteria[18,19], and was present in 7% and 10%, respectively.

Structural knee OA diagnosis
At the baseline visit participants completed a screening form to identify contraindications to undergo magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia or a body circumference of more than 1.70m). A random sample of 1,285 participants without contra-indications underwent MRI of the right knee. Imaging was performed on a MR system operating at a 1.5T field strength (Philips, Medical Systems, Best, The Netherlands), using a dedicated knee coil and a standardized scanning protocol as described earlier[20]. All MRI images were analyzed using the validated semi-quantitative knee OA scoring system (KOSS)[21] as described previously[20] to obtain a structural knee OA phenotype, which was present in 12% of participants. Joint effusion and bone marrow lesions (BMLs) were investigated separately. We compared BMLs with a grade 2 or higher versus smaller or absent in due to the lack of clinical relevance of small BMLs shown in previous research[22].

Lipid metabolites
Blood samples were obtained after an overnight fast. Within 5 minutes after the fasting blood draw, a standardized liquid mixed-meal was consumed containing 600kCal, with 16% of energy (En%) derived from protein, 50 En% from carbohydrates and 34 En% from fat. Subsequently, after 150 minutes postprandial blood samples were drawn. EDTA-plasma samples were analysed using a high-throughput proton nuclear magnetic resonance (NMR) metabolomics platform[23] (Nightingale Health Ltd., Helsinki, Finland) to quantify 159 lipid

and metabolite measures. The NMR spectroscopy was conducted at the Medical Research Council Integrative Epidemiology Unit (MRC IEU) at the University of Bristol (Bristol, United Kingdom), and processed by Nightingale's biomarker quantification algorithms (version 2014). Details of the experimentation and applications of the NMR metabolomics platform have been described previously[23], as well as CVs for the metabolic biomarkers[24]. For the present analyses the concentrations of total fatty acids, SFAs, MUFAs, PUFAs, omega-6 PUFAs and omega-3 PUFAs in mmol/l were used, assessed in a fasting state and 150 minutes after the standardized meal.

Statistical analysis

The NEO study was designed to investigate pathways that lead to obesity-related diseases and conditions. Participants were recruited in two phases. At first participants with a BMI ≥ 27 kg/m^2 were oversampled. Secondly, a reference population was recruited with a BMI distribution similar to the Dutch general population. In this study we aimed to make inferences on the associations in the general population, and the over-representation of overweight and obese participants may induce bias due to the skewed BMI distribution. To represent distributions and associations in the general population correctly, adjustment for this oversampling was made by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality (n = 1,671)[25,26], whose BMI distribution was similar to the general Dutch population[12]. All results were based on weighted analyses, using probability weights. Consequently, results apply to a population-based study without oversampling. For our primary analysis we performed logistic regression analyses to investigate the associations of postprandial total fatty acid, SFAs, MUFAs, PUFAs, omega-6 PUFAs and omega-3 PUFAs concentrations with clinical hand and knee OA. Secondly, we investigated the association of fasting fatty acid concentrations with clinical hand and knee OA, and of fasting and postprandial fatty acid concentrations with structural knee OA, and hand and knee pain. All fatty acid concentrations were standardized by rescaling them to a mean of zero and a standard deviation of one, to ensure a similar interpretation of the estimated effect. Therefore, the odds ratio (OR) can be interpreted as the increased odds on the outcome per standard deviation of the studied fatty acid concentration. In order to make etiological inferences about the associations, all analyses were corrected for age, education, ethnicity and total body fat, according to the causal diagram illustrated in figure 1. Inclusion of the potential confounding variables in the model was based on current knowledge and expert opinion. Total body fat was included as a confounder in the model to eliminate the mechanical effect of excess body weight. As shown in figure 1, we believe that the fatty acid concentration is a result of body fat, and is therefore not in the causal path between fatty acid concentration and OA. Education and ethnicity are used as proxy for social economic status (SES). Based on previous research results by our group, we stratified our analyses by sex in order to account for differences in body composition between men and women[20,27]. A sensitivity analysis was performed without exclusion of participants using lipid lowering medication. Since the fatty acid classes were strongly correlated (Pearson correlation coefficients between 0.99 and 0.47), no multiple testing corrections were performed. STATA V14.1 (StataCorp LP, TX, USA) was used for all analyses.

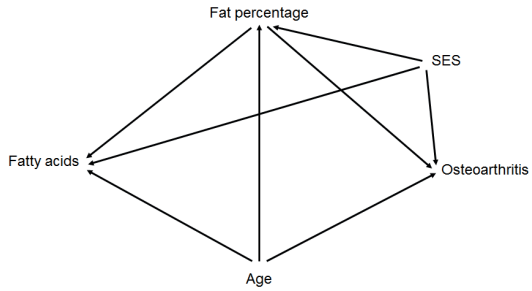

Figure 1. Causal diagram illustrating confounding of the association between fatty acids and osteoarthritis by age, total body fat and social economic status (SES).

Results

Study population

The population consisted of 6,671 participants. After exclusion of participants with missing physical examination (n = 14), who reported the presence of concomitant other rheumatic diseases (n = 323) or were non-fasting at baseline (n = 28), as well as those who used lipid-lowering medication (n = 978), the population for analysis consisted of 5,328 participants (see supplementary figure S1 for a flow chart of excluded participants). Table 1 shows the demographic characteristics stratified by clinical OA phenotype. Seven percent of participants fulfilled the ACR clinical criteria for hand OA, 10% was classified with clinical knee OA, and an additional 4% of participants fulfilled the ACR clinical criteria for both hand and knee OA. Hand OA and concurrent hand and knee OA occurred more often in women. Compared with participants without OA, participants with OA were less educated, particularly those with knee OA. Median (IQR) AUSCAN pain scores were 0 (0-0) in participants without OA and 3 (0-6) in participants with hand OA. Median (IQR) KOOS pain scores were 100 (97-100) in participants without OA and 86 (69-94) in participants classified with knee OA.

Plasma fatty acids levels and clinically defined osteoarthritis

Plasma postprandial fatty acid levels are shown in figure 2. Mean concentrations of both fasting and postprandial fatty acids can be found in supplementary table S1. Unstratified adjusted analyses showed positive associations (OR (95% CI) per SD concentration) of total PUFA concentrations (1.21 (1.02; 1.42)), omega-3 (1.17 (0.99; 1.38)) and omega-6 PUFA concentrations (1.19 (1.01; 1.40)) with clinical hand OA, but not with clinical or structural knee OA. Stratification by sex resulted in positive associations of total fatty acid, SFA, PUFA and omega-3 PUFA concentrations with hand OA only in men, with OR (95% CI) of 1.24 (1.01; 1.53), 1.23 (1.00; 1.50), 1.26 (1.00; 1.58) and 1.24 (1.01; 1.52), respectively. Similar effect estimates were observed for men with concurrent hand and knee OA. Total fatty acid (0.93 (0.78; 1.12)), SFA (0.99 (0.83; 1.19)), MUFA (0.92 (0.76; 1.12), PUFA (0.90 (0.74; 1.09)) omega-3 PUFA (1.06 (0.86;1.29)) and omega-6 PUFA (0.87 (0.72; 1.06)) concentrations were not associated with clinical knee OA alone. In women, no associations were seen for any of the fatty acids with clinical hand or knee OA (table 2). Univariable analyses can be found in supplementary table S2. Analyses of the association between fasting fatty acid levels and clinical hand and knee OA showed similar results, with slightly lower ORs and wider confidence intervals (supplementary table S3). Furthermore, a sensitivity analysis was performed without exclusion of participants using lipid-lowering medication, showing similar results (supplementary table S4).

Table 1. Characteristics of the NEO study population (n = 5,328), stratified by clinical OA phenotype

	No OA	Hand OA	Knee OA	Hand and knee OA
	79%	7%	10%	4%
General patient characteristics				
Age (year)	54.8 (6.1)	57.7 (5.3)	56.8 (5.1)	57.9 (4.5)
Women (%)	54	76	63	90
Ethnicity (% Caucasian)	95	93	95	91
Education (% high)	49	42	39	38
Body morphology measures				
Height (cm)	174 (10)	170 (9)	172 (10)	168 (7)
Weight (kg)	78.2 (15.5)	75.8 (16.0)	81.8 (17.4)	76.7 (15.1)
BMI (kg/m^2)	25.8 (4.1)	26.2 (4.6)	27.5 (5.2)	27.0 (4.8)
Total body fat (%)	30.5 (8.5)	34.4 (7.7)	33.8 (9.3)	37.3 (7.2)
Pain scores				
AUSCAN subscale pain †	0 (0-0)	3 (0-6)	0 (0-2)	5 (3-9)
KOOS subscale pain †	100 (97-100)	100 (94-100)	83 (64-97)	86 (69-94)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 5,328). Patients with missing physical examination, who were non-fasting at baseline, reported inflammatory rheumatic diseases or fibromyalgia or using lipid lowering medication are excluded. Numbers represent mean (SD) unless otherwise specified. † = median (IQR), BMI= body mass index.

Table 2. Association between postprandial plasma fatty acids and clinical OA phenotypes

		Clinical		
		Hand OA	Knee OA	Hand and knee OA
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Total FA	(SD=2.41)			
		1.10 (0.94; 1.29)	0.92 (0.81;1.05)	0.86 (0.65; 1.13)
	Men	1.24 (1.01; 1.53)	0.93 (0.78; 1.12)	1.21 (0.76; 1.90)
	Women	1.05 (0.85; 1.30)	0.88 (0.74; 1.05)	0.83 (0.61; 1.13)
SFA	(SD=0.85)			
		1.09 (0.93; 1.29)	0.94 (0.82; 1.07)	0.80 (0.61; 1.04)
	Men	1.23 (1.00; 1.50)	0.99 (0.83; 1.19)	1.19 (0.80; 1.75)
	Women	1.05 (0.84; 1.31)	0.86 (0.72; 1.03)	0.76 (0.56; 1.04)
MUFA	(SD=0.99)			
		1.02 (0.87; 1.19)	0.95 (0.84; 1.08)	0.80 (0.60; 1.05)
	Men	1.20 (0.96; 1.50)	0.92 (0.76; 1.12)	1.12 (0.64; 1.98)
	Women	0.98 (0.80; 1.20)	0.92 (0.78; 1.08)	0.81 (0.60; 1.10)
PUFA	(SD=0.75)			
		1.21 (1.02; 1.42)	0.90 (0.78; 1.03)	0.95 (0.74; 1.21)
	Men	1.26 (1.00; 1.58)	0.90 (0.74; 1.09)	1.31 (0.84; 2.04)
	Women	1.13 (0.91; 1.41)	0.88 (0.73; 1.06)	0.86 (0.64; 1.14)
Omega-3 PUFA	(SD=0.13)			
		1.17 (0.99; 1.38)	0.94 (0.82; 1.09)	1.02 (0.81; 1.29)
	Men	1.24 (1.01; 1.52)	1.06 (0.86; 1.29)	1.24 (0.83; 1.85)
	Women	1.13 (0.90; 1.40)	0.85 (0.70; 1.04)	0.96 (0.74; 1.26)
Omega-6 PUFA	(SD=0.67)			
		1.19 (1.01; 1.40)	0.90 (0.78; 1.03)	0.94 (0.73; 1.20)
	Men	1.24 (0.98; 1.56)	0.87 (0.72; 1.06)	1.29 (0.85; 1.97)
	Women	1.12 (0.91; 1.39)	0.89 (0.74; 1.08)	0.85 (0.64; 1.13)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 5,328). Plasma fatty acid levels have been standardized (mean = 0, SD = 1), ORs represent increased odds of OA per SD of fatty acid concentration. Analyses have been adjusted for age, fat percentage, education and ethnicity. Abbreviations: SFA= saturated fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid.

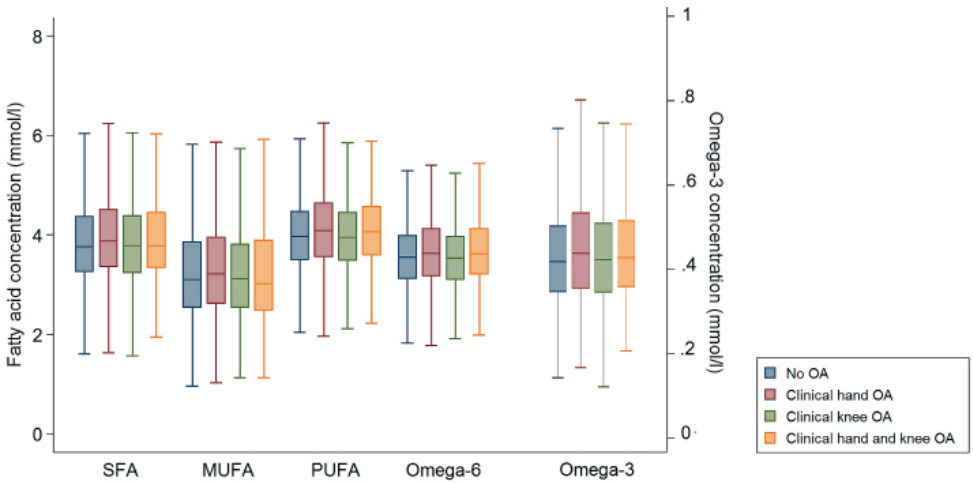

Figure 2. Postprandial fatty acid concentrations stratified by clinical OA phenotype.
Results are based on analyses weighted towards the BMI distribution of the general population. Boxes and bars represent median and interquartile range respectively. Abbreviations: SFA= saturated fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid.

Plasma postprandial fatty acids levels and structural knee osteoarthritis

In a random subset of participants an MRI of the right knee was obtained for determination of structural knee OA. Characteristics of the MRI subgroup were comparable to the whole study population, and structural knee OA was present in 14% of men and 12% of women. In men, postprandial total PUFA and omega-6 PUFA levels were significantly associated with structural knee OA with OR (95% CI) of 1.45 (1.02; 2.05) and 1.48 (1.04; 2.12), respectively. Total fatty acid and SFA concentrations were also positively, although not significantly, associated with structural knee OA. Similar results were found for the association of fatty acids with on MRI defined joint effusion. Omega-3 PUFA concentrations were negatively associated with BMLs in men. In women, no associations were found between any of the fatty acids and structural knee OA, effusion or BMLs (table 3).

Plasma postprandial fatty acids levels were not associated with hand and knee pain

The presence of hand and knee pain was determined by dichotomizing the AUSCAN and KOOS questionnaires, respectively. Hand pain was present in 6% of men and 5% of women. Knee pain was present in 11% of men and 10% of women. None of the fatty acids were associated with hand or knee pain in men or women (table 3).

Table 3. Association of postprandial plasma fatty acids with hand and knee pain, and structural knee OA

		Structural			Pain	
		Knee OA	Effusion	BML	Hand	Knee
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total FA	(SD=2.41)					
Men		1.28 (0.90; 1.81)	1.25 (0.87; 1.78)	0.86 (0.62; 1.19)	1.09 (0.87; 1.35)	0.88 (0.74; 1.05)
Women		0.87 (0.61; 1.25)	0.99 (0.70; 1.39)	1.06 (0.80; 1.41)	1.04 (0.82; 1.32)	1.12 (0.95; 1.33)
SFA	(SD=0.85)					
Men		1.33 (0.95; 1.87)	1.20 (0.83; 1.74)	0.89 (0.63; 1.26)	1.13 (0.93; 1.38)	0.90 (0.75; 1.07)
Women		0.98 (0.71; 1.36)	1.01 (0.72; 1.42)	1.11 (0.82; 1.51)	0.94 (0.76; 1.15)	1.09 (0.92; 1.30)
MUFA	(SD=0.99)					
Men		1.11 (0.76; 1.62)	1.13 (0.80; 1.61)	0.84 (0.61; 1.15)	1.05 (0.84; 1.30)	0.89 (0.75; 1.06)
Women		0.84 (0.57; 1.26)	1.00 (0.70; 1.44)	1.10 (0.82; 1.47)	1.01 (0.80; 1.29)	1.08 (0.91; 1.27)
PUFA	(SD=0.75)					
Men		1.45 (1.02; 2.05)	1.42 (1.01; 2.00)	0.90 (0.64; 1.26)	1.07 (0.85; 1.34)	0.88 (0.74; 1.06)
Women		0.84 (0.60; 1.18)	0.98 (0.71; 1.37)	1.02 (0.77; 1.34)	1.04 (0.81; 1.32)	1.12 (0.95; 1.32)
Omega-3 PUFA	(SD=0.13)					
Men		1.15 (0.88; 1.50)	1.08 (0.82; 1.41)	0.67 (0.50; 0.92)	1.10 (0.89; 1.37)	0.95 (0.80; 1.12)
Women		0.75 (0.57; 0.98)	1.10 (0.85; 1.42)	1.08 (0.78; 1.51)	1.05 (0.84; 1.31)	0.95 (0.79; 1.13)
Omega-6 PUFA	SD=0.67)					
Men		1.48 (1.04; 2.12)	1.47 (1.03; 2.09)	0.96 (0.68; 1.35)	1.05 (0.83; 1.33)	0.88 (0.73; 1.06)
Women		0.89 (0.62; 1.27)	0.96 (0.70; 1.33)	1.00 (0.76; 1.33)	1.03 (0.81; 1.30)	1.14 (0.97; 1.34)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 5,328). Plasma fatty acid levels have been standardized (mean = 0, SD = 1), ORs represent increased odds of OA per SD of fatty acid concentration. Analyses have been adjusted for age, fat percentage, education and ethnicity. Abbreviations: BML= bone marrow lesions, SFA= saturated fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid.

Discussion

In the present study, we aimed to gain insight in the association of plasma fatty acid levels with hand and knee OA in a large population-based cohort study. After correction for variables that may confound this association, we found positive associations of total fatty acids, SFA and PUFA concentrations with clinically defined hand OA in men. We did not see these associations with clinically defined knee OA, however we did observe positive associations of SFA and PUFA concentrations with structural knee OA in men. In women none of these associations were found. Furthermore, none of the fatty acids were associated with joint pain in men or women.

The positive associations we have observed of postprandial total fatty acids and SFA, as well as of PUFA concentrations with clinical hand OA in men are rather unexpected. Based on previous research, focussed mainly on fatty acids in *in vitro* and in animal studies, we hypothesized an opposing effect of these particular fatty acid types. *In vitro* studies have shown that treatment of chondrocytes with SFA increased expression of inflammatory cytokines[7,28,29] and apoptosis markers[7]. In addition, SFA-rich diets resulted in increased structural OA changes in rats and mice[30,31]. Contrastingly, the omega-3 PUFAs eicosapentaenoic acid and α-linolenic acid have been shown to have anti-inflammatory actions *in vitro*[8]. In addition, mice that preferentially convert omega-6 to omega-3 PUFA (transgenic Fat-1 mice) showed a reduction in pro-inflammatory cytokines; however, effects on structural changes were inconsistent[32,33]. To our knowledge, we are the first to investigate the association of fatty acid levels in patients with clinical hand and knee OA. As we cannot compare our results to previous human studies, future research is essential to verify these results.

We found that SFA concentration is associated with increased odds of structural knee OA, which is in line with previous research by Lu et al. They observed that self-reported total fat and SFA intake was positively associated with joint space width loss after 2-year follow-up. In addition, they found that higher intakes of MUFA and PUFA had an opposing effect, with a reduction of joint space width loss[34]. This is in contrast to our results, which showed a positive association between postprandial PUFA concentrations and structural knee OA. Although the study by Lu et al. has the advantage that it is longitudinal, they used self-reported dietary intake as measure of exposure, which is likely biased[35]. In the current study, omega-6 PUFAs were positively associated with structural knee OA and joint effusion in men. This is in line with previous findings, which showed a positive association of plasma omega-6 PUFA with the presence of synovitis[9]. In addition, we observed an association between postprandial omega-3 PUFA concentrations and presence of BMLs, but not between omega-3 PUFA concentrations and structural knee OA. A recent randomized controlled trial supports the latter finding; they observed no effects of fish oil supplementation (containing high levels of omega-3 PUFAs) on structural knee OA changes[10]. Possibly, PUFAs have an effect on inflammation rather than on structural damage.

We measured fatty acids after a standardized mixed-meal, which may provide additional insights in the association between fatty acids and OA. We are in a postprandial state during most of the day, therefore these levels might be a better reflection of the involvement of plasma fatty acids in the development of chronic diseases. However, it must be noted that the effects of long-term fatty acid intake and plasma fatty acid exposure may differ from postprandial measures after a standardized meal. We found that the associations between fatty acid levels in postprandial state, after a standardized meal, and OA were stronger compared to fasting fatty acid concentrations. Interestingly, the observed associations were only present in men. This might indicate that the uptake, metabolism or clearance of lipids differs between sex.

We did not observe associations of any of the fatty acids with hand or knee pain. This is in contrast with an animal study that found that an omega-3 PUFA enriched diet in dogs with OA reduced discomfort, and improved lameness, functional disability and weight bearing, compared to dogs on a control diet[36–39]. In humans, in the randomized controlled trial by Hill et al., reductions in pain and disability were observed after 2 years fish oil supplementation. Paradoxically, the greatest effects were seen in the low-dose group, and no effect was seen on NSAID use, which sheds doubt on the validity of these findings[40].

One of the major strengths of our study is the objective and quantitative method we used to measure plasma fatty acid concentrations in a population-based cohort of substantial size. Another great advantage of our study is that we are the first to investigate the association of plasma fatty acid levels in individuals with hand OA, which might be the most relevant phenotype when investigating systemic factors in OA[3]. We want to stress that the reported effect estimates are ORs. The OR is often an overestimation of the relative risk. However, since in our cohort the prevalence of hand OA is low (7%), fulfilling the rare disease assumption (prevalence <10%), and the observed effect sizes are low, this overestimation of the effect is likely limited. Furthermore, it is worth noting that there were large variations in fatty acid concentrations, varying considerably between participants. This might partially

reflect measurement error, however since this is a population-based study, this phenomenon also likely represents the natural variation found in the general population. Another point to consider is that we did not have information on intake of dietary supplements. However, the concentrations of supplements available are probably too low to really affect the disease course and thus our results. Due to the cross-sectional design we cannot exclude reversed causation. The observational nature our study hinders causal inferences, as exchangeability is hard to achieve due to the possibility of unmeasured or residual confounding. Lastly, our study does not give insight in how changing fatty acid levels over time may influence development and progression of OA. Future longitudinal analyses may elucidate the observed associations. At the moment 10-year follow-up measurements of the NEO study are being planned.

More research is warranted to draw firm conclusions. For future studies it may be valuable to investigate the role of fatty acids via more extensive lipidomic platforms and to investigate the role of downstream bioactive metabolites. One could argue that the individual fatty acids within a class have distinct modes of action and specific metabolic or signalling roles that are opposing to other fatty acids within the same class. Therefore investigating the effect of an entire fatty acid class might not be appropriate[41]. Unfortunately, we did not have information on individual fatty acids concentrations. Furthermore, the Nightingale platform only gives information on the total fatty acid concentration, no matter if this represents bound or free fatty acids. Also, we do not know if plasma fatty acids are a good representation of the potential local effects fatty acids may have on the joint. Perhaps more clear, or different, associations might be found when addressing the role of local fatty acids levels in for example synovial fluid. In addition, the fatty acids are metabolized to bioactive mediators, such as eicosanoids originating from the omega-6 PUFA arachidonic acid and resolvins and protectins from the omega-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid. At the moment the relevance of these bioactive lipid mediators in OA in humans is not known; no previous studies have investigated the effect of lipid mediators on OA incidence or progression in humans.

In conclusion, by investigating plasma fatty acid levels, we found positive associations of postprandial SFA and PUFA concentrations with clinically defined hand OA and structurally defined knee OA in men. These associations were not found in women. The fatty acids were not associated with joint pain in men nor women. We recommend that future research should focus on determining causal relations and the investigation of the role of individual fatty acids and their bioactive mediators in OA development and progression.

References

1. Bijlsma, J. W. J., Berenbaum, F. & Lafeber, F. P. J. G. Osteoarthritis: an update with relevance for clinical practice. *Lancet* **377**, 2115–2126 (2011).
2. Radin, E. L., Paul, I. L. & Rose, R. M. Role of mechanical factors in pathogenesis of primary osteoarthritis. *Lancet* **1**, 519–522 (1972).
3. Visser, A. W. *et al.* The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. *Ann. Rheum. Dis.* **74**, 1842–1847 (2015).
4. Yusuf, E. *et al.* Association between weight or body mass index and hand osteoarthritis: a systematic review. *Ann. Rheum. Dis.* **69**, 761–765 (2010).
5. Ertunc, M. E. & Hotamisligil, G. S. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J. Lipid Res.* **57**, 2099–2114 (2016).
6. Bastiaansen-Jenniskens, Y. M. *et al.* Monounsaturated and Saturated, but Not n-6 Polyunsaturated Fatty Acids Decrease Cartilage Destruction under Inflammatory Conditions: A Preliminary Study. *Cartilage* **4**, 321–328 (2013).
7. Alvarez-Garcia, O., Rogers, N. H., Smith, R. G. & Lotz, M. K. Palmitate has proapoptotic and proinflammatory effects on articular cartilage and synergizes with interleukin-1. *Arthritis Rheumatol* **66**, 1779–1788 (2014).
8. Zainal, Z. *et al.* Relative efficacies of omega-3 polyunsaturated fatty acids in reducing expression of key proteins in a model system for studying osteoarthritis. *Osteoarthr. Cartil.* **17**, 896–905 (2009).
9. Baker, K. R. *et al.* Association of plasma n-6 and n-3 polyunsaturated fatty acids with synovitis in the knee: the MOST study. *Osteoarthr. Cartil.* **20**, 382–387 (2012).
10. Hill, C. L. *et al.* Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. *Ann Rheum Dis* **75**, 23–29 (2016).
11. de Mutsert, R. *et al.* The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur. J. Epidemiol.* **28**, 513–523 (2013).
12. Ministerie van VWS. Hoeveel mensen hebben overgewicht? *www.rivm.nl/nldemaat* **2013**,.
13. Roos, E. M., Roos, H. P., Lohmander, L. S., Ekdahl, C. & Beynnon, B. D. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. *J Orthop Sports Phys Ther* **28**, 88–96 (1998).
14. de Groot, I. B., Favejee, M. M., Reijman, M., Verhaar, J. A. N. & Terwee, C. B. The Dutch version of the Knee Injury and Osteoarthritis Outcome Score: a validation study. *Health Qual Life Outcomes* **6**, 16 (2008).
15. Bellamy, N. *et al.* Dimensionality and clinical importance of pain and disability in hand osteoarthritis: Development of the Australian/Canadian (AUSCAN) Osteoarthritis Hand Index. *Osteoarthr. Cartil.* **10**, 855–862 (2002).
16. Kroon, F. P. B. *et al.* Reference curves for the Australian/Canadian Hand Osteoarthritis Index in the middle-aged Dutch population. *Rheumatology (Oxford)* **56**, 745–752 (2017).
17. Paradowski, P. T., Bergman, S., Sundén-Lundius, A., Lohmander, L. S. & Roos, E. M. Knee complaints vary with age and gender in the adult population. Population-based reference data for the Knee injury and Osteoarthritis Outcome Score (KOOS). *BMC Musculoskelet Disord* **7**, 38 (2006).
18. Altman, R. *et al.* The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. *Arthritis Rheum.* **33**, 1601–1610 (1990).
19. Altman, R. *et al.* Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum.* **29**, 1039–1049 (1986).
20. Visser, A. W. *et al.* The role of fat mass and skeletal muscle mass in knee osteoarthritis is different for men and women: the NEO study. *Osteoarthr. Cartil.* **22**, 197–202 (2014).
21. Kornaat, P. R. *et al.* MRI assessment of knee osteoarthritis: Knee Osteoarthritis Scoring System (KOSS)--inter-observer and intra-observer reproducibility of a compartment-based scoring system. *Skeletal Radiol.* **34**, 95–102 (2005).
22. Felson, D. T. *et al.* The association of bone marrow lesions with pain in knee osteoarthritis. *Ann Intern Med* **134**, 541–549 (2001).
23. Soininen, P., Kangas, A. J., Würtz, P., Suna, T. & Ala-Korpela, M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* **8**, 192–206 (2015).
24. Kettunen, J. *et al.* Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun* **7**, 11122 (2016).
25. Lumley, T. Analysis of compex survey samples. *http:/www.jstatsoft.org/v09/i08/paper* (2004).
26. Korn, E. L. & Graubard, B. I. Epidemiologic studies utilizing surveys: accounting for the sampling design. *Am J Public Health* **81**, 1166–1173 (1991).
27. Visser, A. W. *et al.* Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. *Arthritis Res. Ther.* **16**, R19 (2014).
28. Miao, H. *et al.* Stearic acid induces proinflammatory cytokine production partly through activation of lactate-HIF1α pathway in chondrocytes. *Sci Rep* **5**, 13092 (2015).
29. Frommer, K. W. *et al.* Free fatty acids: potential proinflammatory mediators in rheumatic diseases. *Ann Rheum Dis* **74**, 303–310 (2015).

30. Sekar, S. *et al.* Saturated fatty acids induce development of both metabolic syndrome and osteoarthritis in rats. *Sci Rep* **7**, 46457 (2017).
31. Wu, C.-L. *et al.* Dietary fatty acid content regulates wound repair and the pathogenesis of osteoarthritis following joint injury. *Ann Rheum Dis* **74**, 2076–2083 (2015).
32. Cai, J. *et al.* Association Between Infrapatellar Fat Pad Volume and Knee Structural Changes in Patients with Knee Osteoarthritis. *J Rheumatol* **42**, 1878–1884 (2015).
33. Huang, M.-J. *et al.* Enhancement of the synthesis of n-3 PUFAs in fat-1 transgenic mice inhibits mTORC1 signalling and delays surgically induced osteoarthritis in comparison with wild-type mice. *Ann Rheum Dis* **73**, 1719–1727 (2014).
34. Lu, B. *et al.* Dietary Fat Intake and Radiographic Progression of Knee Osteoarthritis: Data From the Osteoarthritis Initiative. *Arthritis Care Res (Hoboken)* **69**, 368–375 (2017).
35. Kipnis, V. *et al.* Bias in dietary-report instruments and its implications for nutritional epidemiology. *Public Health Nutr* **5**, 915–923 (2002).
36. Fritsch, D. A. *et al.* A multicenter study of the effect of dietary supplementation with fish oil omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. *J Am Vet Med Assoc* **236**, 535–539 (2010).
37. Mehler, S. J., May, L. R., King, C., Harris, W. S. & Shah, Z. A prospective, randomized, double blind, placebo-controlled evaluation of the effects of eicosapentaenoic acid and docosahexaenoic acid on the clinical signs and erythrocyte membrane polyunsaturated fatty acid concentrations in dogs with osteoarthritis. *Prostaglandins Leukot Essent Fatty Acids* **109**, 1–7 (2016).
38. Roush, J. K. *et al.* Multicenter veterinary practice assessment of the effects of omega-3 fatty acids on osteoarthritis in dogs. *J Am Vet Med Assoc* **236**, 59–66 (2010).
39. Roush, J. K. *et al.* Evaluation of the effects of dietary supplementation with fish oil omega-3 fatty acids on weight bearing in dogs with osteoarthritis. *J Am Vet Med Assoc* **236**, 67–73 (2010).
40. Hill, C. L. *et al.* Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. *Ann. Rheum. Dis.* **75**, 23–29 (2016).
41. Calder, P. C. Functional Roles of Fatty Acids and Their Effects on Human Health. *JPEN J Parenter Enteral Nutr* **39**, 18S-32S (2015).

4

Reproducibility of targeted lipidome analyses (Lipidyzer™) in plasma and erythrocytes over a 6-week period

Marieke Loef, Mohan Ghorasaini, Joost von Hegedus, Andreea Ioan-Facsinay, Dennis Mook-Kanamori, Martin Giera, Margreet Kloppenburg

Metabolites. 2020;11:26.

Abstract

It is essential to measure lipid biomarkers with a high reproducibility to prevent biased results. We compared the lipid composition and inter-day reproducibility of lipid measurements in plasma and erythrocytes. Samples from 42 individuals (77% women, mean age 65 years, mean BMI 27 kg/m2), obtained non-fasted at baseline and after six weeks, were used for quantification of up to 1000 lipid species across 13 lipid classes with the Lipidyzer^{TM} platform. Intraclass correlation coefficients (ICCs) were calculated to investigate the variability of lipid concentrations between timepoints. The ICC distribution of lipids in plasma and erythrocytes were compared using Wilcoxon tests. After data processing, the analyses included 630 lipids in plasma, and 286 in erythrocytes. From these, 230 lipids overlapped between sample types. In plasma, 78% of lipid measurements were reproduced well to excellently, compared to 37% in erythrocytes. The ICC score distribution in plasma (median ICC 0.69) was significantly better than in erythrocytes (median ICC 0.51) (p-value<0.001). At class-level, reproducibility in plasma was superior for triacylglycerols and cholesteryl esters while ceramides, diacylglycerols, (lyso)phosphatidylethanolamines and sphingomyelins showed better reproducibility in erythrocytes. Although in plasma overall reproducibility was superior, differences at individual- and class-level may favor the use of erythrocytes.

Introduction

Lipidomics analysis involves the identification and quantification of molecular lipid species. Lipids as part of the cellular membrane play vital roles in cellular function and signaling. Furthermore, the lipidome is a highly dynamic pool of molecules, constantly adapting to physiological and pathological conditions [1]. In turn, lipidomics analysis has become a valuable technology for understanding physiological and pathological mechanisms and the identification of candidate biomarkers. Biomarkers are indicators of normal or pathogenic biological processes to help understand the pathogenesis of diseases or to measure disease presence and predict disease progression. In addition, biomarkers may be used to assess or to predict the individual response to pharmacological treatments and may therefore play an important role in drug development [2–4]. For the correct assessment and interpretation of biomarkers, it is essential that the measurement of potential biomarkers is reliable and reproducible. It is often assumed that measurements taken on a single day are representative of the metabolic status of an individual. However, fluctuations in biomarker concentration may occur due to sampling techniques, assay variation or biological variability [5]. Biological variability, defined as naturally occurring within-subject fluctuations in repeated measurements, may lead to bias towards the null when estimating the association between biomarkers and a disease or treatment [6]. To estimate the variability of the measurement, information regarding the stability of the metabolite levels over time is essential.

Data regarding the reproducibility of lipid metabolite measurements are scarce [7–12]. Of the available studies, most investigated technical reproducibility rather than biological variability [7,10,11], or used metabolomic platforms evaluating a relatively small number or variety of lipid metabolites [7–10,12]. The Lipidyzer™ platform is a commercially available targeted lipidomics platform with the potential to measure >1000 individual lipid species. While other studies thus far have mainly focused on inter-laboratory and cross-platform comparisons [13–15], to our knowledge no biological reproducibility studies using plasma and erythrocytes derived from the same subjects over an extended time span (6 weeks) have yet been performed using the Lipidyzer™ platform, or other lipid platforms of comparable extent. In a clinical setting, serum, and different types of plasma are the most commonly used sample types for metabolomic studies. However, when investigating chronic alterations of the lipidome other sample types might be more representative. In this respect, erythrocytes display minimal cellular metabolism and have a long half-life of approximately 120 days, their membrane lipids may therefore reflect the long-term exposure of an individual, particularly relevant when studying chronic diseases or prolonged treatments. However, a detailed study comprehensively and quantitatively describing the biological reproducibility of erythrocytes has not yet been established.

Therefore, the aim of our research was twofold. Firstly, we aimed to investigate the clinically relevant inter-day reproducibility (a combination of biological and technical variability) of lipid measurements over a period of six weeks in plasma and erythrocytes using the Lipidyzer™ platform. Secondly, we compared the variety and abundance of lipid species, as well as their reproducibility between the two different sample types.

Materials and Methods

Study design and patients

The current study included placebo treated patients included in the Hand Osteoarthritis Prednisolone Efficacy (HOPE) study. The HOPE study is a blinded, randomized placebo-controlled trial that investigated the effect of prednisolone treatment in patients with painful, inflammatory hand osteoarthritis. Full description of patient inclusion and procedures is described elsewhere [20]. Briefly, patients fulfilling the American College of Rheumatology criteria [21], and presenting signs of inflammation in the interphalangeal joints were included. Exclusion criteria involved chronic inflammatory rheumatic diseases, psoriasis, uncontrolled serious comorbidities, malignancy, infectious disease, and immune modulating drug use within 90 days before baseline. The HOPE study (Netherlands Trial Registry (NTR5263)) was approved by the local medical ethics committees and conducted in accordance with Good Clinical Practice guidelines and Declaration of Helsinki. All patients provided written informed consent.

Blood sampling and lipidomics analysis

Blood samples were obtained non-fasted at baseline and after 6 weeks in EDTA-tubes, following a standardized protocol. The blood samples were centrifuged for 10 minutes at 2200 x g to separate plasma from the cellular fraction. Erythrocytes were isolated by ficoll density gradient centrifugation and washed 3x with PBS. Samples were stored at -80°C topped with argon until further analyses [22]. The Lipidyzer™ platform (Sciex) was used to quantify total lipid content in plasma (nmol/mL) and erythrocytes (nmol/mL). Lipid extraction was performed using methyl-tert-butylether as described by Matyash et al., with some modifications [23]. To 25 µL of erythrocyte sample or plasma the following was added: 160 µL MeOH, 50µL internal standard solution (Lipidyzer™ internal standard kit, containing >50 labeled internal standards for 13 lipid classes), and 550 µL methyl-tert-butylether. Samples were vortexed and left at room temperature for 30 minutes. Subsequently, 200 µL water was added for phase separation and the samples were centrifuged at 13.100 × g. The upper layer was transferred to a glass vial and lipid extraction was repeated by adding 300 µL methyl-tert-butylether, 100 µL MeOH and 100 µL water. The organic extracts were combined and dried under a gentle stream of nitrogen. Lipidyzer running buffer consisting of 10 mM ammonium acetate in 50:50 (v/v) dichloromethane:methanol (250 µL) was added and samples were transferred to a glass vial with insert for injection. Briefly, the Lipidyzer™ platform is a flow-injection based ion-mobility triple quadrupole system consisting of a Sciex 5500 QTrap equipped with SelexIon technology coupled to a Shimadzu Nexera series UHPLC system used for injection and delivering running buffer at 7 µL/min. Fifty µL of the resuspended samples were injected using two dedicated methods. Method #1 operated with active DMS separation under the following conditions, DMS temperature low, modifier (propanol) composition low, separation voltage 3500 V, DMS resolution enhancement low. For method #2 the DMS cell was not activated. The MS operated under the following conditions: curtain gas 17, CAD gas medium, ion spray voltage 4100 V in ESI+ mode and −2500 V in ESI− mode, temperature 200 °C, nebulizing gas 17, and heater gas 25. First, PC, PE, LPC, LPE, and SM lipid classes were analyzed. Next, FFA, TAG, DAG, CER, DCER, LCER, HCER, and CE lipids were analyzed applying method #2 Further technical detail including a list of all monitored transitions and detailed experimental setting can be found elsewhere [24–26]. In the erythrocyte samples, lipid concentrations were corrected for the erythrocyte protein pellet content, which was quantified using a Micro BCA Protein Assay

Kit (Thermo Scientific, Waltham, MA, USA). Samples were measured in a randomized batch controlled fashion. The plasma samples were measured in 2 consecutive batches and the erythrocyte samples were spread across 4 consecutive batches, with baseline and follow-up samples of each patient included in the same batch. Four quality controls (QC) consisting of a commercial freeze dried plasma reference were added to each measurement batch. For both plasma and erythrocyte measurements QCs consisting of pooled reference plasma were used.

Data processing

Data from both sample types were pre-processed in the same standardized manner. Lipid species were excluded from further analysis if the relative standard deviation (RSD) of the QCs was >20% within each batch or >25% between batches. Approximately 10% of the detected lipids in erythrocytes were not detected in the QCs, hampering exclusion based on the RSD. However, these lipids were also not detected in most of the patient samples, and therefore automatically excluded in our last processing step in which we excluded lipid species that were not detected in >75% of observations or when they were observed only in a single batch. Percentage missing values was 3% and 18% in plasma and erythrocyte measurements, respectively. All remaining missing observations in plasma and erythrocyte samples were imputed with the minimum measured value divided by two. As a measure of technical variation, the RSDs of the QCs of the lipid classes, containing the lipid species included in the analyses, are provided in supplementary table S1.

Statistical analyses

Prior to the analyses all lipid variables were logarithmically transformed due to a non-normal distribution. We calculated the intra-class correlation coefficients (ICC) and corresponding 95% confidence interval (CI) for each lipid, separately for plasma and erythrocyte samples. We fitted linear mixed-effects models with restricted maximum likelihood, including patients as random effects (random-intercept) (Stata command: mixed `lipid' time || patient ID:, reml var). For each lipid, ICCs were calculated as the ratio of the between-subject variance to the total variance composed of the sum of between- and within-subject variance using the postestimation command estat icc in Stata. The reproducibility was categorized based on the ICCs as follows: excellent ≥0.80, good <0.80 - ≥0.60, moderate >0.60 - ≥0.40, and poor <0.40. The ICC score distributions were compared between sample types by two-sided paired signed-ranks Wilcoxon tests with exact probabilities (command: signrank). Stata V16.1 (StataCorp LP, TX, USA) was used for all analyses.

Data availability and lipid nomenclature

The data underlying this article cannot be shared publicly due to the privacy of the participants of the HOPE study and legal reasons (HOPE study participants did not sign informed consent to make their data publicly available). The data is available upon request to interested qualified researchers. Data requests should be sent to the corresponding author. As the Lipidyzer™ is not capable of specifying the exact sn-position of the FA side chains, we adopted the lipid short-hand notation as described by Liebisch et al. [27]. Additionally, it has to be noted that for TAG lipids the Lipidyzer™ platform is capable of defining one of the three FA side chains. Hence, a TAG lipid specified as for example TG 54:6-FA 18:1 would refer to a TAG lipid with 54 carbons, 6 double bonds and one side chain being FA 18:1.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Technical variability of quality controls, stratified by lipid class. Table S2: Intra-class correlation

Results

Study population and samples

The study population consisted of 42 individuals. The population was predominantly composed of women (77%), with a mean (SD) age of 64.9 (8.3) years and a mean (SD) BMI of 27.1 (5.2) kg/m2.

At baseline and week six, respectively 31 and 33 plasma samples, and 35 and 29 erythrocyte samples were available for analyses. The Lipidyzer™ platform is an integrated system with the potential to quantify over 1000 lipids across 13 lipid classes. We detected and quantified 778 distinct lipid species in plasma, while in erythrocyte 916 lipids were quantified. Processing of the data resulted in 630 lipids in plasma, and 286 lipids in erythrocytes remaining for further analyses. Of these, 230 lipids were measured both in plasma and erythrocytes. The pre-processing steps and exclusion numbers are shown in figure 1.

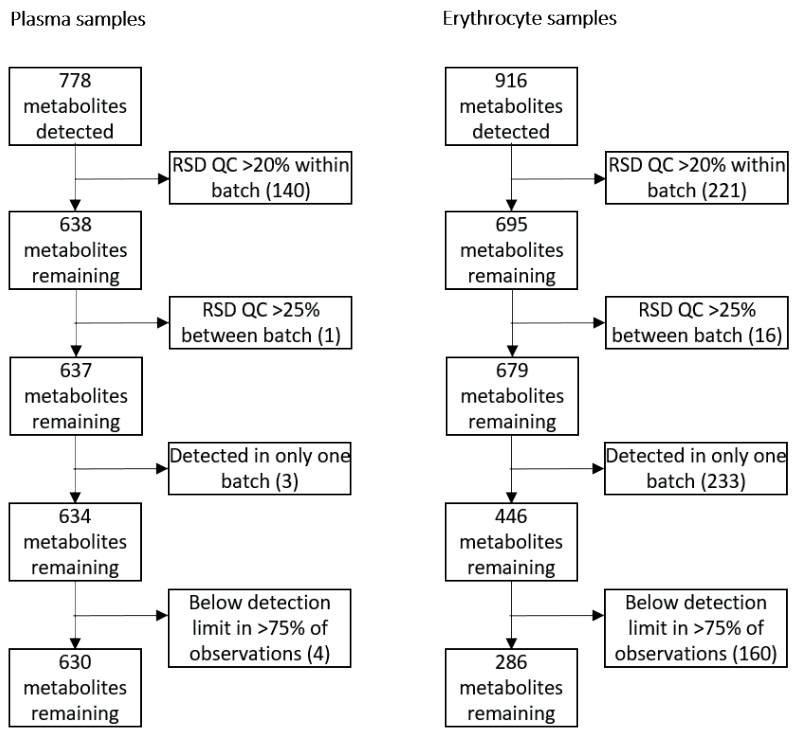

Figure 1. Pre-processing steps and exclusion numbers of Lipidyzer™ variables. Lipid metabolite variables were excluded if the relative standard deviation (RSD) of the quality control (QC) was >20% within a batch, or >25% between separate batches, if a lipid was detected in one batch only, or not detected in >75% of the observations.

Table 1. Number of individual lipids per class and class concentrations in plasma and erythrocytes.

	Plasma		Erythrocytes	
	Number of lipid species	*Class concentration (nmol/mL)*	*Number of lipid species*	*Class concentration (nmol/mL)*
Triacylglycerols	482	1579.4 (1064.9-3195.2)	134	6.5 (5.6-9.4)
Diacylglycerols	9	13.3 (8.4-22.2)	10	5.8 (4.7-6.2)
Free fatty acids	20	745.3 (552.0-1202.9)	20	486.9 (379.2-669.2)
Cholesteryl esters	24	4571.6 (4065.1-5521.3)	5	1.2 (0.9-1.7)
Phosphatidylcholines	31	4013.7 (3203.1-4661.6)	42	3899.2 (3723.0-4296.6)
Phosphatidylethanolamines	26	156.2 (120.9-180.3)	42	3954.6 (3721.9-4323.3)
Lysophosphatidylcholines	9	385.9 (335.6-442.9)	7	119.8 (109.7-168.9)
Lysophosphatidylethanolamines	2	4.2 (3.5-4.9)	4	8.6 (6.8-9.7)
Sphingomyelins	12	1204.6 (1037.0-1351.9)	8	2695.8 (2434.8-2815.6)
Ceramides	6	14.1 (11.9-17.4)	7	163.0 (133.3-186.4)
Dihydroceramides	2	1.0 (0.8-1.3)	1	1.8 (1.4-2.1)
Hexosylceramides	5	5.1 (4.7-5.9)	4	5.6 (5.0-7.4)
Lactosylceramides	2	3.4 (2.7-3.8)	2	23.8 (20.6-33.5)

Numbers represent median (interquartile range) unless otherwise specified. Data represents baseline measurements.

Lipid composition in plasma and erythrocytes

Major differences were observed between the sample types in the number of lipids per lipid class and the total concentration of the lipids within a class (table 1). Triacylglycerols (TAG) were much more abundant in plasma; 482 individual TAGs were quantified with a median concentration of 1579.4 nmol/mL. In contrast, in erythrocytes 134 TAGs were quantified with a median concentration of 6.5 nmol/mL. In addition, 24 cholesteryl esters (CE) (4571.6 nmol/mL) were quantified in plasma, while only 5 CEs (1.2 nmol/mL) were quantified in erythrocytes. Conversely, phosphatidylcholines (PC) (n=31, 4013.7 nmol/mL vs. n=42, 3899.2 nmol/mL), phosphatidylethanolamines (PE) (n=26, 156.2 nmol/mL vs. n=42, 3954.6 nmol/mL), sphingomyelins (SM) (n=12, 1204.6 nmol/mL vs n=8, 2695.8 nmol/mL) and ceramides (CER) (n=6, 14.1 nmol/mL vs. n=7, 163.0 nmol/mL) were less abundant in plasma compared to erythrocytes. Figure 2 presents the relative composition of the lipid classes in the two sample types.

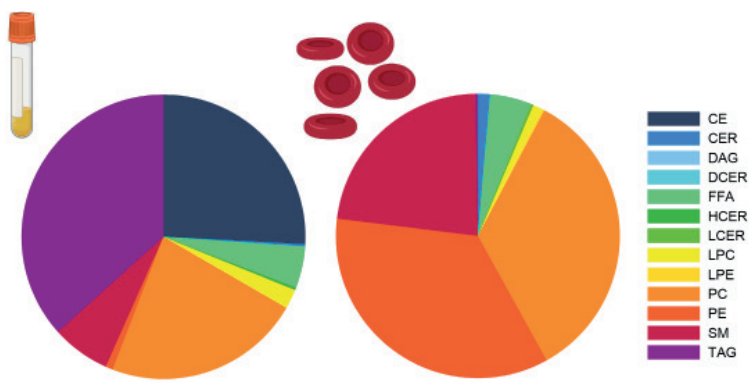

Figure 2. Plasma and erythrocyte lipid class composition. Lipid class composition in plasma (left) and erythrocytes (right) as a percentage of the total lipid concentration. Abbreviations: CE = cholesteryl ester, CER = ceramide, DAG = diacylglycerol, DCER = dihydroceramide, FFA = free fatty acid, HCER = hexosylceramide, LCER = lactosylceramide, (L)PC = (lyso)phosphatidylcholines , (L)PE = (lyso)phosphatidylethanolamine, SM = sphingomyelin, TAG = triacylglycerol.

The majority of lipid species show good reproducibility in plasma

Reproducibility in plasma varied greatly between lipids, with ICCs ranging from ICC=1.1*10-25 for DG 16:0_20:4, to excellent, with the highest ICC of 0.93 for SM 24:0. Generally, reproducibility was good, with a median ICC of 0.69. Categorization of the individual lipids according to their degree of reproducibility, showed that in plasma only 5% of lipids was poorly reproducible and 18% was moderate, while 70%, and 8% was categorized as having a good to excellent reproducibility, respectively. At class-level, the lipid classes showing best reproducibility were: SMs (median ICC=0.77) and TAGs (median ICC=0.72). In figure 3, the range in ICCs of the individual lipids within each class, as well as the median of the individual lipids for each class are given (plasma measurements are shown in blue). The ICCs and corresponding 95% CI, as well as the mean change in concentration over time of all individual lipids are provided in supplementary table S2.

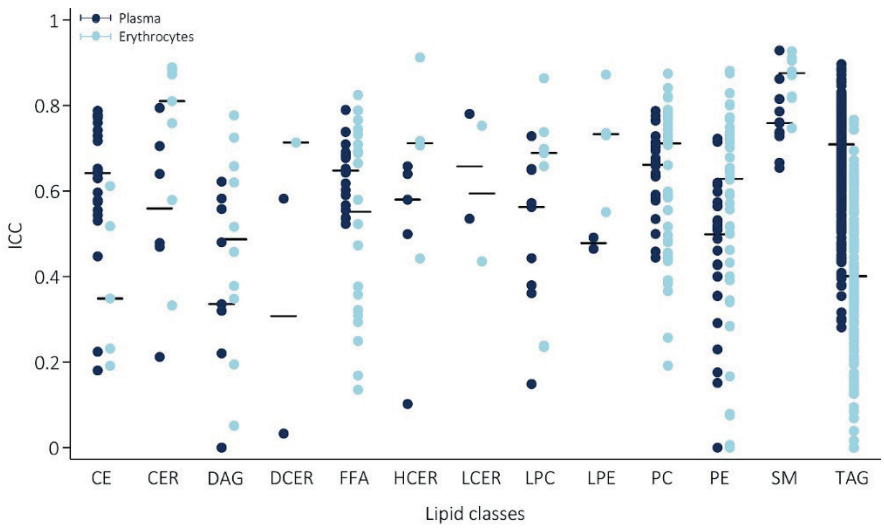

Figure 3. Reproducibility of the lipids over a 6-week period, stratified by lipid class. Reproducibility of individuals lipids within each class, in plasma (blue) and erythrocytes (red), bars represent the median ICC of the lipid class. Abbreviations: ICC = intraclass correlation coefficient, CE = cholesteryl ester, CER = ceramide, DAG = diacylglycerol, DCER = dihydroceramide, FFA = free fatty acid, HCER = hexosylceramide, LCER = lactosylceramide, (L)PC = (lyso) phosphatidylcholines, (L)PE = (lyso)phosphatidylethanolamine, SM = sphingomyelin, TAG = triacylglycerol.

Comparison of erythrocyte lipid reproducibility

Similarly to plasma, reproducibility of lipid measurements in erythrocytes varied greatly, with the worst reproducibility observed for TG 54:2-FA 16:0 (ICC=1.7*10-24) and best reproducibility for SM 26:1 (ICC=0.93). Compared to plasma, a considerably larger amount of lipids were poorly reproducible (34%). Reproducibility was moderate in 28%, good in 28%, and excellent in 9% of lipids measured in erythrocytes. Figure 4 shows the ICC score distribution of the lipids overlapping between the sample types. Comparison of the overall reproducibility of the lipids measured in both sample types showed a significantly higher variability in erythrocytes (median ICC=0.51), compared to plasma (median ICC=0.70), with Wilcoxon signed-rank test p-value<0.001.

However, while overall reproducibility was better in plasma, this was not observed for all lipid classes, as is evident from figure 3. In erythrocytes, reproducibility at class-level (median ICC of the individual lipids within a class) was notably better in CERs (median ICC=0.81 vs ICC=0.56 in plasma), DAGs (median ICC=0.49 vs ICC=0.34 in plasma), LPEs (median ICC=0.73 vs ICC=0.48 in plasma), PEs (median ICC=0.63 vs 0.50 in plasma) and SMs (median ICC=0.88 vs 0.76 in plasma). In contrast, in plasma reproducibility was better of TAGs (median ICC=0.71 vs ICC=0.40 in erythrocytes), CEs (median ICC=0.64 vs ICC=0.35 in erythrocytes) and free fatty acids (FFAs) (median ICC=0.65 vs ICC=0.55 in erythrocytes). Supplementary table S3 provides an additional comparison of the median ICCs of each lipid class considering only the lipids species overlapping between the two sample types (n = 230).

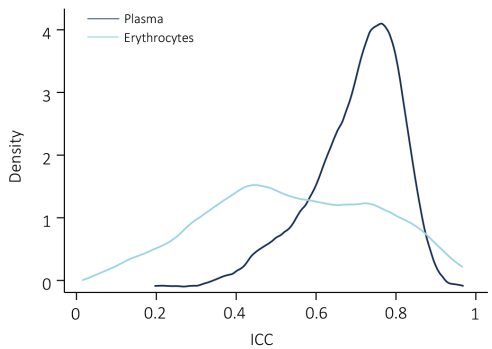

Figure 4. Density plot of the ICC score distribution of lipids measured in plasma and erythrocytes. The Wilcoxon signed-rank test showed a significant difference in the ICC distributions between plasma and erythrocytes, with a p-value<0.001.

Discussion

In the current study we aimed to investigate the clinically relevant inter-day reproducibility of lipid measurements with the Lipidyzer™ platform over a six weeks' period in plasma and erythrocytes. We analyzed 630 and 286 individual lipid species in both matrices, respectively. In plasma, overall reproducibility of lipid measurements was good, and 78% of lipids were reproduced well to excellently. At individual lipid levels and class level major differences in reproducibility were observed, with the best reproducibility in SMs and TAGs. Comparison of variability between plasma and erythrocytes showed a significantly better overall reproducibility in plasma. Furthermore, only 37% of lipid measurements were reproduced well to excellently in erythrocytes. However, sample type preference should be based on the individual lipids or lipid class of interest due to differences in reproducibility between sample types at individual lipid- and class-level.

We presented a standardized manner to pre-process the Lipidyzer™ data based on the RSD of the quality control samples included in each measurement run. We observed that although less lipid species were initially quantified in plasma samples, after data processing approximately twice the number of lipids was available for further analyses in plasma compared to erythrocytes. Approximately a third of the measured lipids overlapped

between plasma and erythrocytes. As expected, the quantity of lipids, both in number and concentration, varied between sample types. In plasma, the most abundant lipids were CEs, TAGs and PCs, which is in line with the National Institute of Standards and Technology (NIST) interlaboratory lipidomics comparison study [13]. Compared to erythrocytes, CEs and TAGs were more abundant in plasma. Conversely, PC, PE, SM and CER were in higher number and concentrations present in erythrocytes. The higher abundance of TAG and CE in plasma likely reflects liver lipid metabolism and lipid transport through the body [16,17]. The high concentration of PC, PE, SM and CER in erythrocytes reflects cell membrane composition, and is also in line with previous findings [18].

We showed great variability in the reproducibility between individual lipids and lipid classes, from very poor to excellent, in both sample types. Although overall the reproducibility of lipid measurements in plasma surpassed the reproducibility in erythrocytes, this was lipid- and class-specific. We observed that lipids that were most abundant within a sample type showed the best reproducibility. For example, in erythrocytes the more abundantly present lipids such as PC, PE, SM and CER, were markedly better reproduced in erythrocytes compared to plasma, which suggests that long-term cellular membranes lipid composition may preferable be measured in erythrocytes [19].

Our results on the quantity and reproducibility of the lipid measurements imply that the preference of sample type for lipid measurements depends on the research objectives and the lipids under investigation. Research focused on lipid metabolism and transport best make use of plasma samples, while research regarding the long term effects of interventions on cellular membrane lipid composition may benefit from the use of erythrocyte samples.

There are some notable strengths and limitations to our study. As the Lipidyzer™ platform is a targeted, triple quadrupole-based technology, the here investigated lipids are predefined by the actual analytical method. In turn, other lipid classes as for examples glycosphingolipids or phosphatidylserines known to be particularly present in erythrocytes could not be targeted in our study. We used data from a randomized controlled trial, which offered the benefits of standardized hospital visits and strict monitoring, which contributed to high data quality. Although this restricted our sample size, the study population was still relatively large compared to most lipidomic studies. Our study population consisted of hand osteoarthritis patients, who were predominantly elderly women. Since hand osteoarthritis is very common in the elderly population this likely had little influence on the observed lipid concentrations. However, the observed lipid profile may not be representative of the general population, or of younger individuals. Furthermore, data on lipid lowering medication was not available. Besides being a reflection of variances in dietary intake and lipid metabolism, reproducibility may be influenced by variances in blood drawing, processing and laboratory handling. In the current study, samples were obtained using a standardized operating procedure, and plasma and erythrocyte samples originated from the same initial test tube. Differences may have occurred in sample handling before and during lipid measurement, as the erythrocyte samples were measured by a different analyst (JvH), than the plasma samples (MG), approximately one year apart. However, the two timepoints from each sample type on which the ICCs are based have had the same sample handling. Therefore, we expect any potential effects this may have on the presented results to be limited. However, the erythrocyte measurements have been corrected for protein pellet, which may have introduced measurement error to some extent. Furthermore, we observed a higher RSD of the QCs in a large amount of

lipid species in our erythrocyte measurements, which indicates somewhat higher technical imprecision and resulted in the exclusion of more lipid species in the erythrocyte samples. We performed the blood sampling non-fasted, with variable sampling timepoints due to differences in scheduled hospital visits, which may be viewed as a limitation. Despite this, we have shown that a large proportion of the lipid metabolites had a high reproducibility. This is encouraging, as this procedure reflects better the daily practice, and limits patient burden. Moreover, identification of biomarkers that do not required fasted sampling will greatly increase feasibility and implementation in large epidemiological studies.

In conclusion, we have shown that inter-day reproducibility is good to excellent for the majority of lipids in plasma. Although overall the reproducibility was better in plasma compared to erythrocytes, notable differences were observed at the individual lipid level and at the lipid class level that may favor the use of erythrocyte samples. In the evaluation of dietary intervention studies it is essential to measure lipids with high reproducibility to prevent biased results. The presented findings may guide methodological considerations in future lipid biomarker studies.

References

[1] Wymann MP, Schneiter R. Lipid signalling in disease. Nat Rev Mol Cell Biol 2008;9:162–76. https://doi.org/10.1038/nrm2335.

[2] Laaksonen R. Identifying new Risk Markers and Potential Targets for Coronary Artery Disease: The Value of the Lipidome and Metabolome. Cardiovasc Drugs Ther 2016;30:19–32. https://doi.org/10.1007/s10557-016-6651-8.

[3] Biomarkers Definitions Working group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical Pharmacology and Therapeutics 2001;69. https://doi.org/10.1067/mcp.2001.113989.

[4] U.S. Food and drug administration. The BEST Resource: Harmonizing Biomarker Terminology 2016.

[5] Kohler I, Verhoeven A, Derks RJ, Giera M. Analytical pitfalls and challenges in clinical metabolomics. Bioanalysis 2016;8:1509–32. https://doi.org/10.4155/bio-2016-0090.

[6] Copeland KT, Checkoway H, McMichael AJ, Holbrook RH. Bias due to misclassification in the estimation of relative risk. Am J Epidemiol 1977;105:488–95.

[7] Breier M, Wahl S, Prehn C, Fugmann M, Ferrari U, Weise M, et al. Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples. PLoS ONE 2014;9:e89728. https://doi.org/10.1371/journal.pone.0089728.

[8] Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62:572–8. https://doi.org/10.1093/ajcn/62.3.572.

[9] Floegel A, Drogan D, Wang-Sattler R, Prehn C, Illig T, Adamski J, et al. Reliability of serum metabolite concentrations over a 4-month period using a targeted metabolomic approach. PLoS ONE 2011;6:e21103. https://doi.org/10.1371/journal.pone.0021103.

[10] Yu Z, Kastenmüller G, He Y, Belcredi P, Möller G, Prehn C, et al. Differences between human plasma and serum metabolite profiles. PLoS ONE 2011;6:e21230. https://doi.org/10.1371/journal.pone.0021230.

[11] Khan MJ, Codreanu SG, Goyal S, Wages PA, Gorti SKK, Pearson MJ, et al. Evaluating a targeted multiple reaction monitoring approach to global untargeted lipidomic analyses of human plasma. Rapid Commun Mass Spectrom 2020:e8911. https://doi.org/10.1002/rcm.8911.

[12] Li-Gao R, Hughes DA, Cessie S le, Mutsert R de, Heijer M den, Rosendaal FR, et al. Assessment of reproducibility and biological variability of fasting and postprandial plasma metabolite concentrations using 1H NMR spectroscopy. PLOS ONE 2019;14:e0218549. https://doi.org/10.1371/journal.pone.0218549.

[13] Bowden JA, Heckert A, Ulmer CZ, Jones CM, Koelmel JP, Abdullah L, et al. Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in Frozen Human Plasma. J Lipid Res 2017;58:2275–88. https://doi.org/10.1194/jlr.M079012.

[14] Cajka T, Smilowitz JT, Fiehn O. Validating Quantitative Untargeted Lipidomics Across Nine Liquid Chromatography-High-Resolution Mass Spectrometry Platforms. Anal Chem 2017;89:12360–8. https://doi.org/10.1021/acs.analchem.7b03404.

[15] Thompson JW, Adams KJ, Adamski J, Asad Y, Borts D, Bowden JA, et al. International Ring Trial of a High Resolution Targeted Metabolomics and Lipidomics Platform for Serum and Plasma Analysis. Anal Chem 2019;91:14407–16. https://doi.org/10.1021/acs.analchem.9b02908.

[16] Robinson DS. Plasma triglyceride metabolism. Journal of Clinical Pathology 1973;s1-5:5–10. https://doi.org/10.1136/jcp.s1-5.1.5.

[17] Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31:1149–72.

[18] Leidl K, Liebisch G, Richter D, Schmitz G. Mass spectrometric analysis of lipid species of human circulating blood cells. Biochim Biophys Acta 2008;1781:655–64. https://doi.org/10.1016/j.bbalip.2008.07.008.

[19] Han X. Lipidomics for studying metabolism. Nat Rev Endocrinol 2016;12:668–79. https://doi.org/10.1038/nrendo.2016.98.

[20] Kroon FPB, Kortekaas MC, Boonen A, Böhringer S, Reijnierse M, Rosendaal FR, et al. Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. The Lancet 2019;394:1993–2001. https://doi.org/10.1016/S0140-6736(19)32489-4.

[21] Altman R, Alarcón G, Appelrouth D, Bloch D, Borenstein D, Brandt K, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990;33:1601–10.

[22] Jonasdottir HS, Brouwers H, Toes REM, Ioan-Facsinay A, Giera M. Effects of anticoagulants and storage conditions on clinical oxylipid levels in human plasma. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863:1511–22. https://doi.org/10.1016/j.bbalip.2018.10.003.

[23] Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res 2008;49:1137–46. https://doi.org/10.1194/jlr.D700041-JLR200.

[24] Chouvarine P, Giera M, Kastenmüller G, Artati A, Adamski J, Bertram H, et al. Trans-right ventricle and transpulmonary metabolite gradients in human pulmonary arterial hypertension. Heart 2020;106:1332–41. https://doi.org/10.1136/heartjnl-2019-315900.

[25] Alarcon-Barrera JC, von Hegedus JH,

Brouwers H, Steenvoorden E, Ioan-Facsinay A, Mayboroda OA, et al. Lipid metabolism of leukocytes in the unstimulated and activated states. Anal Bioanal Chem 2020;412:2353–63. https://doi.org/10.1007/s00216-020-02460-8.

[26] Contrepois K, Mahmoudi S, Ubhi BK, Papsdorf K, Hornburg D, Brunet A, et al. Cross-Platform Comparison of Untargeted and Targeted Lipidomics Approaches on Aging Mouse Plasma. Sci Rep 2018;8:17747. https://doi.org/10.1038/s41598-018-35807-4.

[27] Liebisch G, Vizcaíno JA, Köfeler H, Trötzmüller M, Griffiths WJ, Schmitz G, et al. Shorthand notation for lipid structures derived from mass spectrometry. J Lipid Res 2013;54:1523–30. https://doi.org/10.1194/jlr.M033506

5

The association of the lipid profile with knee and hand osteoarthritis severity: the IMI-APPROACH cohort

Marieke Loef, Lotte van de Stadt, Stefan Böhringer, Anne-Christine Bay-Jensen, Ali Mobasheri, Jonathan Larkin, Floris PJG Lafeber, Francisco J Blanco, Ida K Haugen, Francis Berenbaum, Martin Giera, Andreea Ioan-Facsinay, Margreet Kloppenburg.

Osteoarthritis and Cartilage. 2022;30:1062-1069

Abstract

Objective To investigate the association of the lipidomic profile with osteoarthritis (OA) severity, considering the outcomes radiographic knee and hand OA, pain and function.

Methods We used baseline data from the APPROACH cohort, comprising persons with knee OA fulfilling the clinical American College of Rheumatology classification criteria. Radiographic knee and hand OA severity was quantified with Kellgren-Lawrence sum scores. Knee and hand pain and function were assessed with validated questionnaires. We quantified fasted plasma higher order lipids and oxylipins with LC-MS/MS-based platforms. Using penalised linear regression, we assessed the variance in OA severity explained by lipidomics, with adjustment for clinical covariates (age, sex, BMI and lipid lowering medication), measurement batch and clinical centre.

Results In 216 participants (mean age 66 years, mean BMI 27.3 kg/m^2, 75% women) we quantified 603 higher order lipids (triacylglycerols, diacylglycerols, cholesteryl esters, ceramides, free fatty acids, sphingomyelins, phospholipids) and 28 oxylipins. Lipidomics explained 3% and 2% of the variance in radiographic knee and hand OA severity, respectively. Lipids were not associated with knee pain or function. Lipidomics accounted for 12% and 6% of variance in hand pain and function, respectively. OA severity outcomes were associated with the lipidomic fraction of bound and free arachidonic acid, bound palmitoleic acid, oleic acid, linoleic acid and docosapentaenoic acid.

Conclusion Within the APPROACH cohort lipidomics explained a minor portion of the variation in OA severity, which was most evident for the outcome hand pain. Our results suggest that eicosanoids may be involved in OA severity.

Introduction

Osteoarthritis (OA) is a prevalent rheumatic musculoskeletal disorder, which occurs most commonly in knees, hands and hips. The prevalence and burden of OA has surged in the past decades, affecting approximately 300 million people globally in 2017. This rise in prevalence is accompanied by an increase in years lived with disability of more than 30% since 2007 (1). Next to age and sex, the most important risk factor for OA is obesity. Consequently, OA prevalence is expected to increase even further in the coming years due to ageing of the population and the increasing prevalence of obesity (2).

In addition to increased mechanical stress (3,4), obesity is associated with metabolic dysregulation and a release of proinflammatory systemic factors, which are likely involved in the pathophysiology of OA (5,6). Metabolomics, a holistic approach of the metabolic response, may be used to identify pathophysiological conditions. In particular, lipidomics, the identification and quantification of molecular lipid species, may be relevant as lipid levels rapidly fluctuate in response to pathophysiological situations. Lipids, as part of the chondrocyte cellular membrane, are essential for cartilage physiology and for structural maintenance. In addition, in physiological situations lipids are essential for the protection of the cartilage surface by being major components of articular cartilage boundary lubricant. Furthermore, lipids are involved in many intracellular signalling pathways, including the regulation of bone metabolism by influencing osteoblast and osteoclast function and survival. These actions may be either beneficial or detrimental, depending on the type of lipid involved (7,8). In pathological settings, lipids may assist inflammatory responses, which is suggested by an increase in phospholipid concentration and high levels of phospholipase A2 in synovial fluid in patients with OA, and changes in cartilage lipid composition corresponding to OA severity (9). Hence, lipids may be involved in the OA disease process in many ways. Indeed, previous lipid profiling studies in patients with knee and hip OA have suggested an altered lipid metabolism (10,11). However, human studies have been few, and patient numbers were small. In addition, the association between the lipid profile and hand OA has not been investigated, and evidence regarding the association with patient-reported outcomes is scarce.

Therefore, we investigated the association of the lipid profile with radiographic knee and hand OA severity, as well as with joint pain and function, using a large lipidomics platform capable of measuring up to a 1000 higher order lipid species, as well as a platform for the measurement of oxylipins.

Methods

Study design

The Applied Public-Private Research enabling OsteoArthritis Clinical Headway (APPROACH) is an exploratory, European, 5-centre, 2-year prospective follow-up cohort study (registered under NCT03883568). Selection and study design have been described in detail elsewhere (12,13). Briefly, persons were (pre-)selected from five existing European observational cohorts using machine learning models trained to increase the inclusion of persons with a high likelihood of structural and/or pain progression. Participating centres included: University Medical Centre Utrecht (UMCU), Utrecht, The Netherlands; Leiden University Medical Centre (LUMC), Leiden, The Netherlands; Diakonhjemmet Hospital, Oslo, Norway; Sorbonne

Université APHP hôpital Saint-Antoine, Paris, France; Complexo Hospitalario Universitario de A Coruña (CHUAC), A Coruña, Spain. The current study describes cross-sectional analyses of the baseline data. Ethical approval was obtained locally in the involved centres. All participants provided written informed consent.

Patient and public involvement

A patient council has contributed to the design of the clinical study and helped shape the project to ensure consideration of the interests of study participants. The patient council has been in close contact with the study researchers throughout the project (14).

Patient selection and clinical assessments

During the screening visit inclusion and exclusion criteria were verified, and parameters were collected that were subsequently used in a machine learning algorithm for the final participant selection based on the likelihood of disease progression. For inclusion, patients needed to fulfil the American College of Rheumatology (ACR) clinical classification criteria for knee OA, were able to walk unassisted and capable of understanding study protocol. Patients were excluded if they had recent surgery or had planned surgery of the index knee during follow-up, in case of secondary knee OA, alternative causes of joint pain or if a generalized pain syndrome was present. Full in- and exclusion criteria were published previously (13) The presence of knee OA was defined based on the ACR clinical classification criteria (15). If both knees were equally affected, the right knee was selected as the index knee. General characteristics included: age, sex, and measured body weight (kg) and height (cm) for calculation of the body mass index (BMI) (kg/m^2). Of the index knee, pain, stiffness and function were assessed with the Knee Injury and Osteoarthritis Outcome Score (KOOS) and joint space width was assessed on radiographs. Persons fulfilling the ACR criteria for knee OA with the highest likelihood of progression were included, and invited for a baseline visit within 9 weeks after screening.

At the baseline visit a medical history regarding comorbidities (Charlson index (16)) and current medication use were obtained. Regarding the knees, palpable warmth, effusion (positive patellar tap), passive ranges of flexion and extension, grinding in the patellofemoral joint, and knee alignment (presence of valgus or varus) were assessed. Of both hands, bony and soft swellings, and deformities of distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), carpometacarpal (CMC) joints were assessed. Participants were asked for presence of pain in the hands on most days of the past month. Clinical hand OA was defined according to the ACR classification criteria (17).

Radiographic osteoarthritis assessment

Radiographs of the index and contralateral knee, and of both hands, were obtained using the same standard operating procedure in all centres. Radiographic OA severity was assessed according to the Kellgren-Lawrence (KL) scoring method on a scale of 0-4 per joint (18). In addition to the knees, DIP, PIP, 1st interphalangeal (IP), MCP and CMC joints were scored of both hands. KL sum scores were calculated to assess knee and hand OA severity on person level. For the knee, KL scores of the index and contralateral knee were summed to a scale of 0-8 points per participant. Summing of the KL scores of the hand joints resulted in a scale of 0-120 per participant. All radiographs were scored by one reader (ML) blinded for study participant characteristics. An intra-class correlation coefficient (ICC) was calculated on a random sample of 10% of index knees and pairs of hand radiographs to evaluate intra-reader

reliability. The ICC for KL scoring of the index knee and hand radiographs was 0.89 and 0.92, respectively.

Osteoarthritis-specific disease burden
Prior to the study visit, participants completed questionnaires, including the KOOS, the Functional Index for Hand OsteoArthritis (FIHOA) and a numeric rating scale (NRS) hand pain for assessment of index knee and hand OA-specific disease burden, respectively. From the KOOS the pain (9 items) and function in activities of daily living (ADL) (17 items) subscales were used. Items were scored on a 5-point Likert scale. Subscale scores were calculated according to the KOOS user's guide as the sum of the items included, and subsequently transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems (19). The FIHOA consist of 10 items, completed on a 4-point Likert scale. The total score ranges from zero, representing no functional impairment, to 30, representing maximal impairment (20). The NRS pain was acquired regarding pain in the past week for each hand separately, ranging from 0 (no pain) to 10 (maximum pain). A NRS hand pain sum score was calculated, ranging from 0 to 20.

Blood sampling
Fasting blood samples were obtained in EDTA-tubes. Fasting conditions were defined as no meals, (including no sugar, tea, or coffee) for at least 8 hours. Fasting condition and time since last meal were recorded. Due to logistical reasons, blood could not be sampled fasted in the centre in Spain, and in some participants in the centre in Utrecht, The Netherlands. Blood samples were centrifuged at 2500 x g in a refrigerated centrifuge for 15 minutes. Subsequently, 0.25mL of plasma was transferred into red cap cryotubes for Lipidyzer™ analyses. In addition, for oxylipin measurements 0.2mL of plasma was aliquoted in glass vials, and 0.588mL of LC-MS Chormasolv grade methanol (Honeywell, 349661L), and 12µL of internal standard solution (containing: 500pg/mL PGE2-d4, 5ng/mL DHA-d5, 500pg/mL LTB_4-d4 and 500pg/mL 15S-HETE-d8) was added (21). Samples were stored at -80°C at the local centres facilities until shipment to the LUMC for further analyses.

Lipidyzer™ measurements
Total plasma lipid content was quantified with the Lipidyzer™ platform (Sciex) in nmol/mL. Lipid extraction was performed using methyl-tert-butylether as described by Matyash *et al*., with some modifications (22). To 25µL of sample the following was added: 160µL MeOH, 25µL internal standard solution (Lipidyzer™ internal standard kit, containing >50 labelled internal standards for 13 lipid classes), and 550µL methyl-tert-butylether. Samples were vortexed and left at room temperature for 30 minutes. Subsequently, all samples were centrifuged at 18.213 × g for 5 minutes at 20°C. For each sample 750µL of the supernatant was transferred to a 2mL Eppendorf safe-lock tube. The extraction was repeated with the original samples by adding 300µL of methyl-tert-butylether and 100µL of methanol. Samples were vortexed and centrifuged at 18.213 × g for 15 minutes at 20°C. 350µL of supernatant was transferred to the 2mL Eppendorf tube. 300µL of LC-MS grade water was added to the combined organic extracts and the samples were centrifuged at 18.213 × g for 5 minutes at 20°C. From the upper (organic) layer 700µL of supernatant was transferred to a 1.5mL glass vial. Samples were dried under a gentle stream of nitrogen. After drying, all samples were reconstituted in 250µL Lipidyzer running buffer (50:50 MeOH:DCM, 10mM ammonium acetate). Samples from the different centres were randomised over the consecutive measurement batches,

and pooled samples were measured in each batch to assess measurement variability. The Lipidyzer™ platform quantified 838 distinct lipid species (nmol/mL). We excluded lipid species from further analysis if the relative standard deviation (RSD) of the pooled samples was >20% within each batch or >25% between batches, or if measurement values were below the detection limit in >75% of observations, resulting in 603 lipid species remaining for analyses. A flowchart of the cleaning steps and exclusion numbers can be found in supplementary figure S1. Figure S2 shows a correlation matrix of the lipids included in the analyses.
We used the lipid nomenclature corresponding to the raw data from the Lipidyzer™ platform, which deviates slightly from the recommendations of LipidMaps.

Oxylipin measurements
Liquid-chromatography combined with mass spectrometry (LC-MS/MS) analysis was used to measure free fatty acids and their precursors and downstream metabolites in plasma as described previously (23). Samples from the different centres were randomised over the consecutive measurement batches, and pooled samples were measured in each batch to assess measurement variability. Lipids were identified using specific tandem mass spectrometric transitions and relative retention times. Only peaks with a signal to noise (S/N) > 10 were included. A total number of 45 lipid metabolites were identified. Lipids were excluded if the RSD of the pooled samples was >25%, or if measurement values were below the detection limit in >75% of observations, resulting in 28 oxylipins for further analyses. Oxylipins are presented as area ratios. A flowchart of the cleaning steps and exclusion numbers can be found in supplementary figure S1.

Statistical analyses
We used penalised linear regression to evaluate the association of the lipid profile with radiographic OA (KL sum score knees and KL sum score hands) and with pain (KOOS pain score, NRS pain hands) and function (KOOS ADL function score, FIHOA score). We used elastic net to estimate the regression models (R-package glmnet). The mean squared error (MSE) and 30-fold cross-validation were used to select the penalty parameter (lambda). The mixing parameter alpha for elastic net was not tuned, but chosen as either 1 (primary analyses) or 0.1 (sensitivity analyses) to control the sparsity of the model. Missing lipid measurements were mean-imputed, specifics on number of missings are shown in figure 1. To correct for skewness, all lipid measurements were log-transformed prior to entering the variables in the analyses. To evaluate the relative importance of clinical characteristics, the R-squared (R^2) of the models was computed for only lipids and for lipids including the clinical covariates age, sex, BMI and lipid lowering medication use. The former computation was performed by first computing a predicted value for the clinical variables not used for R^2 computation and entering these as an offset in the penalised regression. Including the clinical variables in the offset of the analyses results in a constant value for these variables and thereby controls for their possible confounding effects. In addition, to control for possible batch and centre effects, the variables identifying the lipid measurement batch and centre of origin were included in the offset in all analyses. We excluded all participants who were not fasted at blood sampling.

Data availability
Data may be obtained from a third party and are not publicly available. In order to gain and govern access to the central APPROACH databases, tranSMART and XNAT, access has to be approved by the APPROACH Steering Committee.

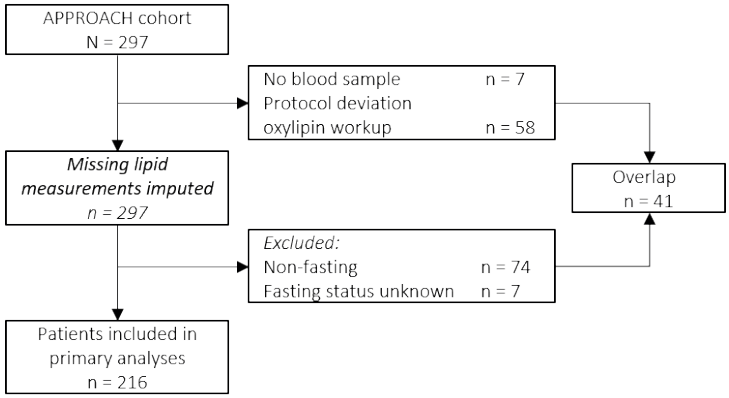

Figure 1. Study populations and exclusions.
In 65 participants there were either missing blood samples, or deviations to the work-up protocol, leading to complete missing lipid data (n=7) or missing oxylipin data (n=58). Since these measurements were regarded as missing completely at random, we mean imputed all these missing lipid measurements. In a second step, we excluded measurements that were taken non-fasting, or with unknown fasting status, resulting in 216 patients included in the analyses. In total, 11% of the lipid measurements have been imputed.

Results

Study population

The total APPROACH baseline cohort consisted of 297 participants. In 7 participants blood samples were not acquired due to logistical reasons. In addition, blood samples of 58 participants were excluded based on protocol deviations during sampling or processing of the samples. In these cases, lipid measurements were imputed, as these samples were regarded missing completely at random, leading to no reduction of numbers for the statistical analyses. Secondly, we excluded participants who were non-fasting (n = 74) or of whom fasting status was unknown at time of sampling (n = 7), resulting in a study population of 216 participants (figure 1). Missing values in covariates are provided in supplementary table S1. The population had a mean (SD) age of 66 (8) years, and 162 (75%) were women (table 1). Due to the eligibility criteria, all included participants fulfilled the clinical ACR classification criteria for knee OA for the index knee. In just over half of the participants the KL score of the index knee was ≥2. Mean KL sum score of the knees was 2.8 (range 0-8). Mean (SD) KOOS subscale scores (range 0-100) were 68.0 for pain, and 70.9 for ADL function. In addition, 43 (20%) of participants fulfilled the clinical ACR classification criteria for hand OA, 83% of participants had a KL score of ≥ 2 in at least 2 hand joints hands, and mean KL sum score of the hands was 7.0 (range 0-120). Hand function was mildly reduced with a mean FIHOA score of 4.8 (range 0-30) and hand pain was scored 6.0 (out of 20, sum score for both hands) on average.

Table 1. Baseline characteristics of the study population

	All (n = 297)	Included in analyses (n = 216)
General characteristics		
Age (year)	67 (7)	66 (8)
Women, n (%)	230 (77)	162 (75)
Body mass index (kg/m^2)	28.1 (5.3)	27.3 (5.2)
Ethnicity, white (%)	283 (95)	203 (94)
Lipid lowering medication use, n (%)	70 (24)	45 (21)
Knee osteoarthritis		
Index knee KL ≥ 2, n (%)	152 (51)	113 (52)
Contralateral knee KL ≥ 2, n (%)	100 (34)	76 (35)
KL sum score (0-8)	2.8 (1.9)	2.8 (1.9)
KOOS pain (0-100)	66.1 (18.8)	68.0 (19.1)
KOOS ADL function (0-100)	68.8 (19.1)	70.9 (19.4)
Hand osteoarthritis		
Clinical hand OA, n (%)	49 (17)	43 (20)
Number of joints with KL ≥ 2	7.5 (5.9)	7.0 (5.8)
KL ≥ 2 in ≥ 2 joints, n (%)	254 (86)	180 (83)
KL sum score (0-120)	27.1 (16.7)	25.2 (16.0)
FIHOA score (0-30)	5.5 (6.0)	4.8 (5.6)
NRS hand pain (0-20)	6.9 (5.6)	6.0 (5.2)

Numbers represent mean (SD) unless otherwise specified. ADL = activities daily living, BMI = body mass index, FIHOA = Functional Index Hand OsteoArthritis, KL = Kellgren Lawrence, KOOS = Knee Injury and Osteoarthritis Outcome Score, n = number, SD = standard deviation

Lipids associated with OA severity

Lipidomics accounted for 3% and 2% of the variance in radiographic knee and hand OA severity, respectively (table 2). Arachidonic acid containing lipids associated with both radiographic knee and hand OA severity. We observed that higher palmitic acid levels associated with more severe radiographic knee OA, while palmitoleic acid levels were inversely associated with radiographic hand OA severity. No oxylipins were selected by the models as explanatory variables of the variance in radiographic knee and hand OA severity.

Lipids associated with joint pain and function

Lipidomics accounted for 12% and 6% of variance in severity of hand pain and functional impairment, respectively (table 2). Higher palmitoleic acid and lower linoleic acid levels were associated with more hand pain and functional impairment. Higher arachidonic acid levels were associated with more functional impairment, while docosapentaenoic acid levels were associated with more hand pain. In addition, the saturated fatty acids margaric acid and palmitic acid were positively associated with hand pain, while lauric acid levels were negatively associated with hand pain. We observed no association between lipid levels and knee pain or function. No oxylipins were selected by the models as explanatory variables of the variance in joint pain and function.

Table 2. Lipids associated with knee and hand OA severity

Lipid	Coefficient*	↑/↓	R^2	Lipid	Coefficient*	↑/↓	R^2
Radiographic knee OA				*Radiographic hand OA*			
TAG(54:8)-FA(20:4)	-0.1791546	↓	0.03	LPC(20:4)	1.7704056	↑	0.02
DAG(16:1/16:1)	-0.0613229	↓		TAG(56:1)-FA(16:0)	0.1935335	↑	
Knee function				*Hand function*			
-^	NA	NA	NA	FFA(20:4)	0.7301019	↑	0.06
				DAG(16:1/16:1)	0.3176602	↑	
				TAG(50:5)-FA(16:1)	0.3166805	↑	
				PC(18:1/18:2)	-0.1158988	↓	
				TAG(54:6)-FA(16:1)	0.0664009	↑	
Knee pain				*Hand pain*			
-^	NA	NA	NA	PC(17:0/18:2)	-0.9668823	↓	0.12
				FFA(17:0)	0.5551604	↑	
				PC(18:0/22:5)	0.4633670	↑	
				TAG(38:0)-FA(12:0)	-0.3404136	↓	
				CE(16:1)	0.3338632	↑	
				PC(18:1/18:2)	-0.3188525	↓	
				TAG(58:6)-FA(16:0)	0.2505877	↑	
				TAG(50:4)-FA(16:1)	0.2136476	↑	
				TAG(42:2)-FA(12:0)	-0.1398661	↓	
				TAG(50:5)-FA(16:1)	0.0687346	↑	

The variables age, sex, BMI, lipid lowering medication use, centre and batch were kept constant in the analyses. Only fasted samples were included. Lipids were log transformed and mean-scaled prior to the analyses.
Abbreviations: CE = cholesterol ester, DAG = diacylglycerol, FFA = free fatty acid, NA = not applicable, OA = osteoarthritis, (L)PC = (lyso)phosphatidylcholine, SM = sphingomyelin, TAG = triacylglycerol
↑/↓ higher/lower concentrations were associated with more severe radiographic OA, more hand pain or functional impairment, and less knee pain or functional impairment.
*Penalised regression coefficients.
^No lipids were associated with knee pain and function.

Table 3. Percentage explained by variables in the model

	Knee			**Hand**		
	Radiographic OA	Pain	Function	Radiographic OA	Pain	Function
Lipids	0.03	0	0	0.02	0.12	0.06
Lipids + clinical variables	0.28	0	0.20	0.51	0.18	0.17

Numbers represent the R^2 of the elastic net regularised regression analyses, using an alpha of 1. In the lipids only model, clinical variables and batch and centre were kept constant by including them in the offset. In the lipids + clinical variables model, both lipids and clinical variables were included as explanatory variables, while keeping batch and centre constant.

Amount of variation in OA severity accounted for by lipids and clinical variables

Including the clinical variables age, sex, BMI and lipid lowering medication as explanatory variables in the model next to the lipid variables, increased the amount of variation in OA severity accounted (table 3). This was most evident for radiographic knee and hand OA severity. Combining lipidomics with clinical variables accounted for 28% and 51% of severity in radiographic knee and hand OA, respectively, in comparison to 3% in radiographic knee OA severity and 2% of radiographic hand OA severity accounted for by lipidomics only.

Sensitivity analyses
In our sensitivity analyses, we set the tuning parameter alpha to 0.1 to allow for an increase in included explanatory variables. Although this resulted in a large increase in lipids included as explanatory variables, we observed no increase in amount of variation of OA severity accounted for by the included variables (supplementary table S2-S5).

Discussion

We investigated the association of the lipid profile with knee and hand OA severity, considering the outcomes radiographic OA, joint pain and function, in a multi-centre European prospective cohort study. We observed that in participants included in this cohort, lipidomics accounted for a part of the variation in OA severity, albeit minor. Of the investigated outcomes, lipidomics showed strongest associations with hand pain.

We observed that OA severity was associated with the unsaturated fatty acids arachidonic acid, palmitoleic acid, oleic acid, linoleic acid and docosapentaenoic acid, and the saturated fatty acids lauric acid, margaric acid and palmitic acid. The association of lipidomics was most evident for hand pain severity. Our results suggest that eicosanoids may be involved in OA severity. Fatty acids, and in particular arachidonic acid are metabolised by cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 (CYP450) to produce prostaglandins, leukotrienes, thromboxanes and lipoxins. Depending on the substrate metabolised and the cellular environment, this may result in either suppression or promotion of inflammation, among other actions (24).

Although human studies investigating the lipidomic profile in association with OA severity are scarce, the results we observed are more or less in line with other studies, such as the study by Kim *et al*. They investigated the metabolic profile of synovial fluid in patients with early (KL grade 1 or 2) versus late (KL grade 3 or 4) knee OA using gas-chromatography/time-of-flight mass spectrometry (GC/TOF MS) followed by orthogonal partial least squares discriminant analyses and hierarchical clustering analyses. In addition to other metabolites, they observed higher levels of several lipids, including arachidonic acid, palmitoleic acid, linoleic acid, oleic acid, palmitic acid and stearic acid, in patients with late stage knee OA (25). Although we did not see this association with knee OA, positive associations of most of these fatty acids were observed with hand OA severity. It is important to note that due to methodological differences such as the concentration on only a few lipids or grouping of all unsaturated and saturated fatty acids, as well as differences in analytical methods, it is difficult to directly compare previous literature to ours.

We observed no associations of lipidomics with the knee OA related outcomes pain and function. This absence of association between lipids and knee pain and function also held when we adjusted the models' mixing parameter to a more inclusive alpha of 0.1. In our cohort, participants were included based on the presence of clinical knee OA, for which knee pain is the major inclusion criterion. Perhaps this resulted in too much homogeneity in the variable knee pain and function, hampering distinguishing between participants with different knee OA severity. In addition, in approximately half of our participants the KL score of the index knee was below 2, a cut-off often used to define the presence of radiographic

knee OA. Possibly, the large number of patients in a very early radiographic disease phase may have resulted in the absence of association of knee OA with the lipid profile in our study population. Furthermore, upon including clinical characteristics to the model as explanatory variables next to lipids, we observe an variability of 16% of knee (dis)function accounted for by lipidomics combined with BMI. This suggests that the well-known association between obesity and knee OA is mostly due to mechanical factors, which has also been suggested previously (26).

Our study is strengthened by its large sample size, comprised of participants from several hospitals in North and Western Europe. Therefore, our results are well generalizable to a broad selection of European OA patients. All data has been prospectively collected using well-designed protocols followed by all participating centres, which have been frequently visited for data monitoring. Furthermore, the lipidomics measurements were executed in a single centre within a short time period to limit possible batch effects. All samples underwent only one freeze-thaw cycle. We used a large standardised lipidomics platform capable of measuring up to a 1000 higher order lipid species, as well as a platform for the measurement of oxylipins, leading to a comprehensive coverage of the lipid spectrum. In addition, we investigated multiple OA severity phenotypes. While radiographic OA is regarded as the most objective measure, this outcome correlates poorly to OA symptoms (27,28). Therefore, we also included the patient-reported outcome measures pain and function. Moreover, in addition to knee OA, which is most frequently studied, we also investigated hand OA. Despite that systemic factors may be relatively more important in hand than in knee OA, which is supported by our results, hand OA remains an OA phenotype that has been given relatively little notice in research on systemic factors.

Our study is limited by its cross-sectional design, which hinders causal inference. However, to distinguish cause from effect in a slowly progressive disease in OA will remain difficult, as this will require a long follow-up duration of prospective study designs. In addition, our study population consisted of participants included based on the presence of clinical knee OA. Inherent to the study design, all participants suffered from pain in at least one knee. We observed that the majority of participants (86%) also showed signs of radiographic hand OA. This precluded investigating knee and hand OA phenotypes separately. However, the presence of OA in knees and hands simultaneously translates well to the clinic, as patients with OA often suffer from OA in multiple joint locations (29). Furthermore, we did not correct our analyses for other medications than lipid-lowering agents. The use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) is common, and may have influenced reported pain severity, as well as the level of several lipid metabolites, which could have introduced confounding that has not been accounted for.

Our study showed promising results for future research. The APPROACH study is a 2-year prospective follow-up study that included participants with a high likelihood of structural and/or pain progression using machine learning models. The follow-up data will lend well to investigate the relationship between the patients' lipid profile and the risk of progression. Fatty acids are biochemically intertwined by the actions of desaturases and elongases (30) and hence observed concentrations can strongly correlate. In turn, compositional analysis of fatty acid patterns in conjunction with pathway analyses may help to gain mechanistic insight identifying relevant biological pathways. Since there is a pressing need for disease

modifying agents for OA, further research on the possible effect of targeted interventions on downstream bioactive lipid mediators is warranted. Careful selection of the target population is essential. OA is a heterogeneous group with a multifactorial etiopathogenesis in which both mechanic as well as inflammatory components may be involved. Additionally, in our current analyses we have treated outcomes on a linear scale. This allows for robust implementation with readily available software. We plan to investigate transformations of the outcome scale and/or alternative models, such as ordinal models, in future research.

In conclusion, within the APPROACH cohort lipidomics accounted for a small portion of the variation in OA severity, which was most evident for hand pain. The associated lipids suggest that eicosanoids may be involved in OA severity.

References

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Lond Engl. 2018 10;392(10159):1789–858.
2. Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011 Jun 18;377(9783):2115–26.
3. Yusuf E, Nelissen RG, Ioan-Facsinay A, Stojanovic-Susulic V, DeGroot J, van Osch G, et al. Association between weight or body mass index and hand osteoarthritis: a systematic review. Ann Rheum Dis. 2010 Apr;69(4):761–5.
4. Visser AW, Ioan-Facsinay A, de Mutsert R, Widya RL, Loef M, de Roos A, et al. Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. Arthritis Res Ther. 2014 Jan 22;16(1):R19.
5. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. Nat Rev Rheumatol. 2012 Dec;8(12):729–37.
6. Kroon FPB, Veenbrink AI, de Mutsert R, Visser AW, van Dijk KW, le Cessie S, et al. The role of leptin and adiponectin as mediators in the relationship between adiposity and hand and knee osteoarthritis. Osteoarthritis Cartilage. 2019 Dec;27(12):1761–7.
7. Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. Nat Rev Mol Cell Biol. 2018 May;19(5):281–96.
8. During A, Penel G, Hardouin P. Understanding the local actions of lipids in bone physiology. Prog Lipid Res. 2015 Jul;59:126–46.
9. Villalvilla A, Gómez R, Largo R, Herrero-Beaumont G. Lipid Transport and Metabolism in Healthy and Osteoarthritic Cartilage. Int J Mol Sci. 2013 Oct 16;14(10):20793–808.
10. Castro-Perez JM, Kamphorst J, DeGroot J, Lafeber F, Goshawk J, Yu K, et al. Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. J Proteome Res. 2010 May 7;9(5):2377–89.
11. Zhang Q, Li H, Zhang Z, Yang F, Chen J. Serum metabolites as potential biomarkers for diagnosis of knee osteoarthritis. Dis Markers. 2015;2015:684794.
12. Widera P. A machine learning "APPROACH" to recruitment in OA. Osteoarthritis Cartilage. 2019 Apr 1;27:S15.
13. Helvoort EM van, Spil WE van, Jansen MP, Welsing PMJ, Kloppenburg M, Loef M, et al. Cohort profile: The Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) study: a 2-year, European, cohort study to describe, validate and predict phenotypes of osteoarthritis using clinical, imaging and biochemical markers. BMJ Open. 2020 Jul 1;10(7):e035101.
14. Taylor J, Dekker S, Jurg D, Skandsen J, Grossman M, Marijnissen A-K, et al. Making the patient voice heard in a research consortium: experiences from an EU project (IMI-APPROACH). Res Involv Engagem. 2021 May 10;7(1):24.
15. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986 Aug;29(8):1039–49.
16. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373–83.
17. Altman R, Alarcón G, Appelrouth D, Bloch D, Borenstein D, Brandt K, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum. 1990 Nov;33(11):1601–10.
18. Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. Ann Rheum Dis. 1957 Dec 1;16(4):494–502.
19. The 2012 User's Guide to Knee injury and Osteoarthritis Outcome Score KOOS. www.koos.nu. 2012.
20. Dreiser RL, Maheu E, Guillou GB, Caspard H, Grouin JM. Validation of an algofunctional index for osteoarthritis of the hand. Rev Rhum Engl Ed. 1995 Jun;62(6 Suppl 1):43S-53S.
21. Jonasdottir HS, Brouwers H, Toes REM, Ioan-Facsinay A, Giera M. Effects of anticoagulants and storage conditions on clinical oxylipid levels in human plasma. Biochim Biophys Acta Mol Cell Biol Lipids. 2018 Dec;1863(12):1511–22.
22. Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res. 2008 May;49(5):1137–46.
23. Jónasdóttir HS, Ioan-Facsinay A, Kwekkeboom J, Brouwers H, Zuurmond A-M, Toes R, et al. An Advanced LC–MS/MS Platform for the Analysis of Specialized Pro-Resolving Lipid Mediators. Chromatographia. 2015 Mar 1;78(5):391–401.
24. Araújo AC, Wheelock CE, Haeggström JZ. The Eicosanoids, Redox-Regulated Lipid Mediators in Immunometabolic Disorders. Antioxid Redox Signal. 2018 Jul 20;29(3):275–96.
25. Kim S, Hwang J, Kim J, Ahn JK, Cha H-S, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. Joint Bone Spine. 2017 Oct;84(5):605–10.
26. Visser AW, de Mutsert R, le Cessie S, den Heijer M, Rosendaal FR, Kloppenburg M, et al. The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. Ann Rheum Dis. 2015 Oct;74(10):1842–7.

27. Altman RD. Criteria for classification of clinical osteoarthritis. J Rheumatol Suppl. 1991 Feb;27:10–2.
28. Hart DJ, Spector TD, Brown P, Wilson P, Doyle DV, Silman AJ. Clinical signs of early osteoarthritis: reproducibility and relation to x ray changes in 541 women in the general population. Ann Rheum Dis. 1991 Jul;50(7):467–70.
29. Nelson AE, Smith MW, Golightly YM, Jordan JM. "Generalized osteoarthritis": a systematic review. Semin Arthritis Rheum. 2014 Jun;43(6):713–20.
30. Guillou H, Zadravec D, Martin PGP, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog Lipid Res. 2010 Apr;49(2):186–99.

6

The lipid profile for the prediction of prednisolone treatment response in patients with inflammatory hand osteoarthritis: The HOPE study

Marieke Loef, Tariq Faquih, Joost von Hegedus, Mohan Ghorasaini, Andreea Ioan-Facsinay, Dennis Mook-Kanamori, Féline Kroon, Martin Giera, Margreet Kloppenburg

Osteoarthritis and Cartilage OPEN. 2021

Abstract

Objective To explore the use of lipidomics for prediction of prednisolone treatment response in patients with inflammatory hand osteoarthritis.

Design The Hand Osteoarthritis Prednisolone Efficacy (HOPE) study included patients (n=92) with symptomatic inflammatory hand osteoarthritis, fulfilling the ACR criteria. The present analyses comprised only patients randomized to prednisolone treatment (10 mg daily, n=40). Response to prednisolone treatment was defined according to the OARSI-OMERACT responder criteria at six weeks. Baseline blood samples were obtained non-fasted. Lipid species were quantified in erythrocytes with the Lipidyzer™ platform (Sciex). Oxylipins were analysed in plasma using an in-house LC-MS/MS platform. Elastic net regularized regression was used to predict prednisolone treatment response based on common patient characteristics alone and including the patients' lipid profile. ROC analyses with 1,000 bootstrapped area under the curve (AUC) was used to determine the discriminatory accuracy of the models.

Results Among included patients, 78% fulfilled the OARSI-OMERACT responder criteria. From the general patient characteristics, elastic net selected baseline hand function as only predictor of treatment response, with an AUC of 0.78 (0.56; 0.97). Addition of lipidomics resulted in an AUC of 0.92 (0.78; 0.99) and 0.85 (0.65; 0.98) for inclusion of the Lipidyzer™ platform and oxylipin platform, respectively.

Conclusion Our results suggest that the patients' lipid profile may improve the discriminative accuracy of the prediction of prednisolone treatment response in patients with inflammatory hand osteoarthritis compared to prediction by commonly measured patient characteristics alone. Hence, lipidomics may be a promising field for biomarker discovery for prediction of anti-inflammatory treatment response.

Introduction

Hand osteoarthritis (OA) is one of the most prevalent OA phenotypes, and it is associated with pain, stiffness, functional impairment and a loss in quality of life [1–4]. Currently, there is a high unmet need for disease modifying drugs for the treatment of osteoarthritis (OA). The role of inflammation in hand OA and its association with pain [5,6] has sparked increasing interest for targeting inflammation in therapeutic research. To this regard, the Hand Osteoarthritis Prednisolone Efficacy (HOPE) study was set up. The HOPE study is a blinded, randomized placebo-controlled trial, that investigated the effect of prednisolone treatment in patients with painful, inflammatory hand OA. The HOPE study showed a clinically relevant decrease in pain in patients using prednisolone [7]. Since pharmacological treatments usually show marked variation in treatment response, it is important to carefully select patients who will most likely benefit from treatment, to maximize the desired therapeutic effect, and minimize overtreatment and potential adverse effects. Metabolomics may aid the identification of biomarkers of therapeutic responsiveness [8].

Lipids are essential for joint physiology [9,10]. However, to maintain normal physiology, a tight control of lipid species is warranted. In addition, various lipids and their metabolites are involved in pathophysiological settings, in particular in inflammation. Moreover, they have been shown to play an important role in inflammation in auto-immune diseases [11], as well as in OA [12,13]. Therefore, lipidomics, involving the identification and quantification of lipid metabolites, may be particularly relevant as biomarker of therapeutic responsiveness to anti-inflammatory medication. In addition, previous lipid profiling studies have suggested an altered lipid metabolism in patients with OA [14–16]. In particular, associations between differing levels of phospholipids and OA have been observed [16–18]. Hence, the patients' lipid profile may be predictive of response to anti-inflammatory treatment in patients with inflammatory hand OA. To our knowledge, the use of lipidomics for prediction of treatment response in patients with OA has not previously been studied.

Therefore, we explored the patients' lipid profile for the prediction of prednisolone treatment response in patients with inflammatory hand OA.

Methods

Study design

The HOPE study included patients with symptomatic hand OA, fulfilling the American College of Rheumatology criteria [19] and presenting signs of inflammation in the distal and proximal interphalangeal (DIP/PIP) joints. Full description of patient inclusion and procedures can be found elsewhere [7]. Briefly, patients were required to have: finger pain of ≥30 mm on a 100 mm visual analogue scale (VAS) and flaring upon 48-hour NSAID washout (defined as ≥20 mm worsening), ≥4 DIP/PIP joints with osteoarthritic nodes, ≥1 DIP/PIP joints with soft swelling or erythema, and ≥1 DIP/PIP joints with positive power Doppler signal or synovitis grade ≥2 on ultrasound. Patients were excluded from participation in case of chronic inflammatory rheumatic diseases, psoriasis, uncontrolled serious comorbidities, malignancy, infectious disease, and immune modulating drug use within 90 days before baseline. Patients (n = 92) were randomly assigned (1:1) to receive 10 mg prednisolone daily, or placebo, for six weeks. The present study comprised of patients randomized to prednisolone treatment only (n = 40). Treatment adherence has been reported previously [7]. The HOPE study (Netherlands Trial

Registry (NTR5263)) was approved by the local medical ethics committees and conducted in accordance with Good Clinical Practice guidelines and Declaration of Helsinki. All patients provided written informed consent.

Patient reported outcomes
At baseline and week six, patients completed a VAS for finger pain and VAS global assessment on a 0-100 mm scale, and the Australian/Canadian Hand Osteoarthritis Index (AUSCAN) pain (scored as 0-20) and function (scored as 0-36) subscales (higher scores are worse). At week six, fulfilment of the OMERACT-OARSI responder criteria was assessed, which was defined as a relative improvement ≥50% and absolute change ≥20/100 in AUSCAN pain or function, or a relative improvement ≥20% and absolute change ≥10/100 in ≥2 of the following: AUSCAN pain, AUSCAN function or VAS patient global assessment [20]. In the OMERACT-OARSI criteria, the AUSCAN pain and function subscale scores are used on a 0-100 scale. The AUSCAN pain and function subscale scores were rescaled from 0-20 and 0-36, respectively, to 0-100. We calculated absolute change as the baseline score minus the follow-up score, and relative change as the absolute change divided by the baseline score.

Baseline imaging
All interphalangeal and metacarpophalangeal joints were assessed on baseline radiographs of both hands (30 joints). Radiographic OA severity was investigated using the Kellgren and Lawrence (KL) grading system on a 0-4 scale [21]. Erosive OA was defined as having ≥1 joint in the erosive or remodelling phase according to the Verbruggen-Veys score [22]. Synovial thickening was assessed on ultrasound on a 0-3 scale [6]. A sum score adding the scores of all investigated joints was calculated for KL (0-120) and synovitis (0-90). The reliability of all scoring methods was good [7].

Lipidomics measurements
Blood samples were obtained non-fasted at baseline at various time points during the day in EDTA-tubes, following a standardized protocol. The blood samples were centrifuged for 10 minutes at 2200 x g to separate plasma from the cellular fraction. Erythrocytes were isolated by ficoll density gradient centrifugation and washed 3x with PBS. Plasma samples were quenched using 600µL MeOH (Honeywell, 349661L), and 8µL IS was added (containing: 500pg/mL PGE2-d4, 5ng/mL DHA-d5, 500pg/mL LTB4-d4 and 500pg/mL 15S-HETE-d8). Samples were stored at-80°C topped with argon until further analyses [23].
The Lipidyzer™ platform (Sciex) was used to quantify total lipid content in erythrocytes (nmol/mL). Lipid extraction was performed using methyl-tert-butylether as described by Matyash et al., with some modifications [24]. To 25µL of erythrocyte sample the following was added: 160µL MeOH, 50µL internal standard solution (Lipidyzer™ internal standard kit, containing >50 labeled internal standards for 13 lipid classes), and 550µL methyl-tert-butylether. Samples were vortexed and left at room temperature for 30 minutes. Subsequently, 200µL water was added for phase separation and the samples were centrifuged at 13.100 × g. The upper layer was transferred to a glass vial and lipid extraction was repeated by adding 300µL methyl-tert-butylether, 100µL MeOH and 100µL water. The organic extracts were combined and dried under a gentle stream of nitrogen. Lipidyzer running buffer (250µL) was added and samples were transferred to a glass vial with insert for injection. Briefly, the Lipidyzer platform is a flow-injection-based ion-mobility triple quadrupole system consisting of a Sciex 5500 QTrap equipped with SelexIon technology coupled to a Shimadzu Nexera series UHPLC

system used for injection and delivering running buffer at 7 µL/min. Two methods were used for the injection of a total of 50 µL of the resuspended samples. First, PC, PE, (L)PC, (L)PE, and SM lipid classes were analyzed using method 1, operating with active DMS separation under the following conditions: DMS temperature low, modifier (propanol) composition low, separation voltage 3500 V, DMS resolution enhancement low. Next, FFA, TAG, DAG, CER, dihydroceramide (DCER), lactosylceramide (LCER), hexosylceramide (HCER), and CE lipids were analyzed applying method 2, for which the DMS cell was not activated. The MS operated under the following conditions: curtain gas 17, CAD gas medium, ion spray voltage 4100 V in ESI + mode and −2500 V in ESI− mode, temperature 200 °C, nebulizing gas 17, and heater gas 25. Further technical detail can be found elsewhere [25–27]. Lipid concentrations were corrected for the erythrocyte protein pellet content, which was quantified using a Micro BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Samples were measured in a randomized batch controlled fashion. The lipid concentrations were corrected for the erythrocyte protein pellet content. After preprocessing of the Lipidyzer™ data (Supplementary file, figure S1), 286 lipid species were available for further analyses (Supplementary file, table S1).
Oxylipins were measured in plasma, using liquid-chromatography combined with mass spectrometry (LC-MS/MS) analysis in negative electrospray ionization mode as described previously [28]. A QTrap 6500 mass spectrometer in negative ESI mode (Sciex, Nieuwerkerk aan den Ijssel, The Netherlands) was used, coupled to a LC system employing LC-30AD pumps, a SIL-30AC auto sampler, and a CTO-20AC column oven (Shimadzu, 's-Hertogenbosch, The Netherlands). A Kinetex C18 50 × 2.1 mm, 1.7 µm column, combined with a C8 pre column (Phenomenex, Utrecht, The Netherlands) was used, kept at 50 °C. A gradient of water and Methanol with 0.01% acetic acid was used. An injection volume of 40 µL was used, with a flow rate of 400 µL/min (6). Oxylipins were identified using characteristic mass transitions and relative retention times. Only peaks with a signal to noise > 10 were included, resulting in identification of 25 oxylipins. For a subset of these, synthetic standards were available, allowing for quantification (ng/mL). Area ratios were calculated for all other oxylipins.

Statistical analyses
Descriptive statistics were used for baseline patient characteristics. Two-sample t-tests and Chi-square tests were used as appropriate to assess differences in baseline general patient characteristics. We used elastic net (EN) regularized regression for selection of predictors [29]. EN uses an additional tuning parameter (alpha) to combine the properties of ridge regression and lasso by applying both L1 and L2 penalties. Thereby, it simultaneously performs automatic variable selection and continuous shrinkage, while also dealing with high correlations amongst predictors. Prior to fitting the model, lipid measurements below the detection limit were imputed with the minimum measured value divided by two, all lipid variables were logarithmically transformed due to a non-normal distribution, and were mean scaled to ensure comparability by giving the metabolites equal weight. We performed EN regularization with a logit model, defining the OARSI-OMERACT responder status as the outcome. Prior to fitting the EN models, we performed a 10-fold cross-validation (CV) for selection of the optimal tuning parameters based on the smallest CV mean prediction error. In addition, we used manual alpha selection based on the out-of-sample deviance ratio and CV mean deviance to investigate the performance of more comprehensive models. First, a model was fit with commonly assessed patient characteristics and patient reported outcomes, measured at baseline (model 1). Second, we fitted model 2 by adding the Lipidyzer™ platform lipids to model 1. Third, we fitted model 3 by adding the oxylipins to model 1. Fourth, we

combined the general patient characteristics with both lipid platforms in model 4. Lastly, we fitted a model with the predictors selected by model 2 and 3. We used the Stata command: *elasticnet logit depvar othervars, alpha(0.1(0.1)1) selection(cv, fold(10) alllambdas)*. The discriminatory accuracy of the model was estimated by receiver operating characteristic (ROC) analyses (Stata command: *rocreg*). The area under the curve (AUC) and corresponding 95% confidence intervals (CI) were calculated using 1,000 bootstrap replications. Additionally, we performed sensitivity analyses investigating the association between the lipid predictors and treatment response using univariable logistic regression. Stata V16.1 (StataCorp LP, TX, USA) was used for all analyses.

Availability of data and materials

The data underlying this article cannot be shared publicly due to the privacy of the participants of the HOPE study and legal reasons (HOPE study participants did not sign informed consent to make their data publicly available). The data is available upon request to interested qualified researchers. Data requests should be sent to the corresponding author.

Results

Study population

Baseline lipid measurements and the OARSI-OMERACT responder status at week six were available in 40 prednisolone-treated patients. Figure 1 shows a flowchart of included patients. Of these patients, 31 (78%) fulfilled the OARSI-OMERACT responder criteria. The percentage of patient fulfilling either the major criteria or a particular combination of minor criteria is presented in supplementary figure 2. Patients responding to prednisolone treatment showed statistically worse baseline AUSCAN function scores (19.6 ±6.6) than non-responders (11 ±7.5). None of the other general characteristics differed between responders and non-responders (table 1).

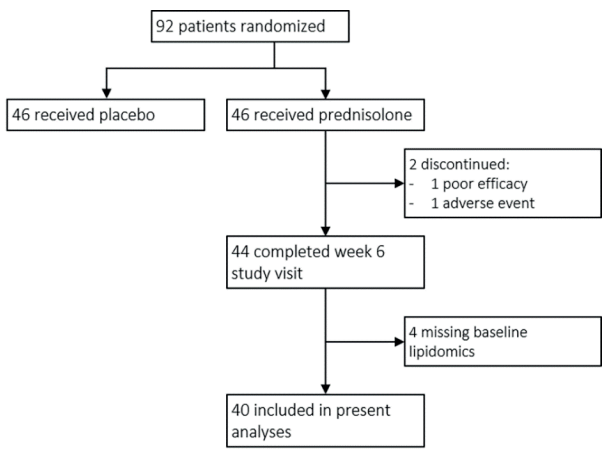

Figure 1. Flowchart of patient numbers

The present analyses included only patients randomized to prednisolone treatment. Of the 46 patients assigned, 2 discontinued the study due to poor efficacy or an adverse event. Four patients were excluded due to missing lipid measurements at baseline.

Table 1. Baseline characteristics of prednisolone-treated patients in the HOPE study

	All prednisolone treated n = 40	Responders n = 31 (78%)	Non-responders n = 9 (23%)
General characteristics			
Age, year	62.4 (9.3)	62.9 (9.4)	60.8 (9.4)
Sex, % women	85	84	89
BMI, kg/m^2	27.4 (4.4)	27.8 (4.2)	26.2 (5.0)
Education, % high	46	42	56
Disease duration	6.7 (7.1)	7.2 (7.4)	4.9 (5.8)
Erosive OA, %	71	74	56
Kellgren-Lawrence sum score, 0-120	35.1 (16.4)	34.1 (16.5)	37.5 (14.7)
Ultrasound synovitis sum score, 0-90	16.2 (6.6)	15.5 (6.4)	18.7 (7.2)
VAS global assessment, 0-100	52.3 (20.6)	54.2 (16.8)	45.6 (30.8)
AUSCAN pain, 0-20	11.0 (3.3)	11.3 (2.4)	10 (5.4)
AUSCAN function, 0-36	17.7 (7.6)	19.6 (6.6)	11 (7.5)

Numbers represent mean (SD) unless otherwise specified. Abbreviations: AUSCAN = Australian/Canadian Hand Osteoarthritis Index, BMI = body mass index, VAS = visual analogue scale

Prediction of treatment response using general patient characteristics

The general characteristics presented in table 1 were entered in model 1 as predictors of OARSI-OMERACT responder status. Only AUSCAN function was selected in the model (worse function associated with response), resulting in an AUC with 95% CI of 0.78 (0.56; 0.94). Predictors entered in the model, predictors selected by EN, and corresponding ROC curves of the models are shown in figure 2. Table 2 presents the baseline concentrations of the selected lipids. Tuning parameters and model deviances of all models are provided in table 3.

Table 2. Baseline levels of selected lipids

	All prednisolone treated n = 40	Responders n = 31 (78%)	Non-responders n = 9 (23%)
Levels selected Lipidyzer™ lipids			
DAG(16:0/16:0), nmol/mL	0.28 (0.12)	0.30 (0.12)	0.18 (0.084)
PE(O-18:0/20:4), nmol/mL	66.01 (12.20)	63.26 (10.65)	75.48 (13.04)
Levels selected oxylipins			
9-HOTrE, area rati**O**	0.12 (0.09)	0.093 (0.059)	0.20 (0.14)
5-HEPE, area ratio	0.011 (0.015)	0.014 (0.016)	0.0043 (0.0032)
10-HDHA, ng/mL	0.0039 (0.0044)	0.0046 (0.0048)	0.0019 (0.0020)

Numbers represent mean (SD). Abbreviations: DAG = diacylglycerol, PE = phosphatidylethanolamine, 9-HOTrE = 9-hydroxy-octadecatrienoic acid, 5-HEPE = 5-hydroxy-eicosapentaenoic acid, 10-HDHA = 10-hydroxy-docosahexaenoic acid.

Added value of lipidomics for prediction of treatment response - Lipidyzer™

In model 2, we added the 286 Lipidyzer™ platform lipid species to model 1. Cross-validated parameter tuning selected an alpha of 0, resulting in the inclusion of all predictors in the model with an AUC of 0.95 (0.85; 0.99). With only minor increase in deviance (CV mean deviance 1.096 vs 1.095), a model (2a) with an alpha of 1 resulted in the selection of three variables: AUSCAN function and two lipids: diacylglycerol(DAG)(16:0/16:0) (higher levels associated with response), and phosphatidylethanolamine(PE)(O-18:0/20:4) (lower levels associated with response), with an AUC of 0.92 (0.78; 0.99).

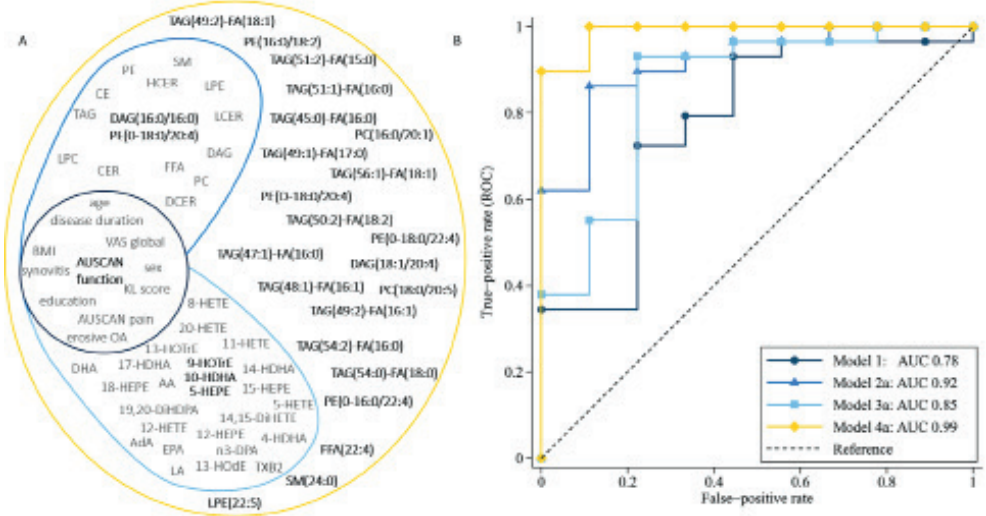

Figure 2. Prediction model characteristics.
A) shows the variables included for model fitting of the three prediction models, colours correspond to the lines of the ROC curves in B). Of model 2, only the lipid classes are shown. Variables in bold font were selected in the final models. Model 1: General patient characteristics, model 2: model 1 + Lipidyzer™ platform, model 3: model 1 + oxylipin platform, model 4: all variables included. Abbreviations: AUC = area under the curve, AUSCAN = Australian/Canadian Hand Osteoarthritis Index, CE = cholesteryl ester, CER = ceramide, DAG = diacylglycerol, DCER = dihydroceramide, FFA = free fatty acid, HCER = hexosylceramide, KL = Kellgren-Lawrence, LCER = lactosylceramide, (L)PC = (lyso)phosphatidylcholines , (L)PE = (lyso) phosphatidylethanolamine, OA = osteoarthritis, SM = sphingomyelin, TAG = triacylglycerol, VAS = visual analogue scale, 9-HOTrE = 9-hydroxy-octadecatrienoic acid, 5-HEPE = 5-hydroxy-eicosapentaenoic acid, 10-HDHA = 10-hydroxy-docosahexaenoic acid.

Added value of lipidomics for prediction of treatment response – oxylipins

In model 3, the 25 identified oxylipins were added to model 1. With automated parameter tuning an alpha of 0 was used, selecting all variables for the model, resulting in an AUC of 0.88 (0.73; 0.97). However, with only marginal inflation of the CV mean deviance (1.186 vs 1.184) a more comprehensible model (3a) could be fit, which included AUSCAN function and three oxylipin predictors: 9-hydroxy-octadecatrienoic acid (HOTrE) (lower levels associated with response), 5-hydroxy-eicosapentaenoic acid (HEPE) and 10-hydroxy-docosahexaenoic acid (HDHA) (higher levels associated with response), with an AUC of 0.85 (0.65; 0.98).

Combining all predictors

Lastly, we combined the general patient characteristics with both lipid platforms in model 4. Again, automated parameter tuning resulting in an alpha of 0. Including all 326 variables in the model resulted in an AUC of 0.97 (0.90; 1). A more comprehensive model (4a) could be fit using an alpha of 0.2, resulting in the selection of 27 predictors. This model included all previously selected predictors from models 2 and 3, as well as 21 additional higher order (Lipidyzer™) lipids (table 3), resulting in a model with an AUC of 0.99 (0.93; 1). In addition, we ran model 5 in which we included only the 6 predictors previously selected by EN in models 2 and 3. The discriminative ability of this model was only slightly less compared to the full model, with an AUC of 0.95 (0.81; 1), and significantly improved the prediction compared to a model based on general patient characteristics alone (model 1 vs model 5, p=0.03).

Table 3. Selected predictors and prediction model parameters

	Selected predictors		Tuning parameters		Out-of-sample	CV mean	AUC
			Alpha	*Lambda*	deviance ratio	deviance	(95% CI)
Model 1 General characteristics	1	AUSCAN function	1.00	0.113	0.0838	0.9770132	0.78 (0.56; 0.94)
Model 2 Model 1 + Lipidyzer™	301	All variables*	0	23.327	-0.0269	1.095027	0.95 (0.85; 0.99)
Model 2a Model 1 + Lipidyzer™ *Manual alpha selection*	3	AUSCAN function DAG(16:0/16:0) PE(O-18:0/20:4)	1.00	0.150	-0.0275	1.095633	0.92 (0.78; 0.99)
Model 3 Model 1 + oxylipins	40	All variables*	0	2.193	-0.0689	1.170265	0.88 (0.73; 0.97)
Model 3a Model 1 + oxylipins *Manual alpha selection*	4	AUSCAN function 9-HOTrE 5-HEPE 10-HDHA	0.60	0.182	-0.0835	1.186297	0.85 (0.65; 0.98)
Model 4 All variables combined	326	All variables*	0	8.853	-0.0505	1.150134	0.97 (0.90; 1)
Model 4a All variables combined *Manual alpha selection*	27	AUSCAN function DAG(16:0/16:0) DAG(18:1/20:4) FFA(22:4) LPE(22:5) PC(16:0/20:1) PC(18:0/20:5) PE(16:0/18:2) PE(O-16:0/22:4) PE(O-18:0/20:4) PE(O-18:0/22:4) SM(24:0) TAG(45:0)-FA(16:0) TAG(47:1)-FA(16:0) TAG(48:1)-FA(16:1) TAG(49:1)-FA(17:0) TAG(49:2)-FA(16:1) TAG(49:2)-FA(18:1) TAG(50:2)-FA(18:2) TAG(51:1)-FA(16:0) TAG(51:2)-FA(15:0) TAG(54:0)-FA(18:0) TAG(54:2)-FA(16:0) TAG(56:1)-FA(18:1) 9-HOTrE 5-HEPE 10-HDHA	0.2	0.475	-0.0921	1.195637	0.99 (0.93; 1)
Model 5 *Predefined model based on predictor selection of model 2a and 3a*	6	AUSCAN function DAG(16:0/16:0) PE(O-18:0/20:4) 9-HOTrE 5-HEPE 10-HDHA	0	0.079	0.2993	.7671022	0.95 (0.81; 1)

*See additional file 1, tables A1 and A2 for the included lipids. Abbreviations: AUSCAN = Australian/Canadian Hand Osteoarthritis Index, AUC = area under the curve, CI = confidence interval, CV = cross-validation, DAG = diacylglycerol, FFA = free fatty acid, (L)PE = (lyso)phosphatidylethanolamine, PC = phosphatidylcholine, SM = sphingomyelin, TAG = triacylglycerol, 9-HOTrE = 9-hydroxy-octadecatrienoic acid, 5-HEPE = 5-hydroxy-eicosapentaenoic acid, 10-HDHA = 10-hydroxy-docosahexaenoic acid.

Sensitivity analyses
The univariable associations of baseline lipid levels with prednisolone treatment response are shown in the supplementary file, tables S1 and S2. The lipids included in model 2 and 3 were univariably among the lipids most strongly associated with treatment response, supporting the selection of predictors by the EN models.

Discussion

In this exploratory study we investigated the patients' lipid profile for the prediction of prednisolone treatment response in patients with painful inflammatory hand OA. We showed that lipidomics improved the discriminative accuracy of the prediction, when compared to commonly measured patient outcomes alone. Our results suggest that lipidomics is a promising field for further biomarker discovery for the prediction of anti-inflammatory treatment response.

The added predictive value of lipidomics is an interesting finding. From the Lipidyzer™ platform, lipids containing fatty acid chains of palmitic acid (16:0), stearic acid (18:0) and arachidonic acid (20:4) were selected as predictors. Palmitic acid is the most abundant saturated fatty acid (SFA) in humans; under physiological conditions its concentration is tightly controlled by desaturation to palmitoleic acid and oleic acid, or elongation to stearic acid [30]. Pathophysiological conditions may increase SFA content, leading to activation of toll-like receptor (TLR)-4 triggered inflammatory signalling cascades via nuclear factor kappa B (NFκB) and cyclooxygenase (COX)-2, increasing proinflammatory cytokine production [31]. Arachidonic acid, an omega-6 polyunsaturated fatty acid (PUFA), is the main precursor of proinflammatory eicosanoids, although it may also give rise to anti-inflammatory mediators. In addition, hydroxylation of the omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may lead to hydroxyeicosapentaenoic acids (HEPE) and hydroxydocosahexaenoic acids (HDHA), which are precursors of anti-inflammatory and pro-resolving mediators [32]. Possibly, the lipid profile represents an indication of the patients' inflammatory state, and their likelihood to respond to anti-inflammatory treatment. However, we should be careful to avoid causal interpretations of our results since no causal inferences can be drawn from prediction analyses.

Furthermore, our results suggest that amongst other patient characteristics such as pain, radiographic OA severity and synovitis, hand function is the most contributing to the prediction of treatment response. Despite possible influences of the small sample size and patient selection, which likely resulted in a lack of predictive ability of characteristics such as age and sex, as well as regression to the mean, it implies that patients' hand function may be an important outcome to consider when making treatment decisions.

To our knowledge lipidomics for the prediction of treatment response in hand OA has not previously been investigated. A major strength of our study is the use of high-quality trial data. Furthermore, we have used lipidomics data from two different platforms, the standardized and commercially available Lipidyzer™ platform for the measurement of a large variety of higher order lipids, and an in-house developed platform for the measurement of oxylipins.

However, there are also limitations to our study. Most notable is the small sample, which has likely resulted in overfitting of the models and a higher degree of uncertainty of the estimations. Also, since no study population with comparable data was available, external validation was not possible. In addition, the analyses have been performed in a specific, carefully selected patient population, therefore results may not be generalizable to other patient populations. The blood samples were obtained non-fasted at variable time points during the day due to differences in scheduled hospital visits. Although this may be viewed as a limitation, this procedure is a good reflection of daily practice and limits patient burden. Moreover, the identification of predictions for treatment response that do not required fasted or strictly scheduled sampling will benefit the feasibility and implementation in clinical practice. However, this may have resulted in additional variability in the lipid measurements. In a recent study by our research group we described intra-day variability (ICC) of (DAG) (16:0/16:0) of 0.62 and of (PE)(O-18:0/20:4) ICC of 0.46 [33], representing moderate to good reproducibility of the lipids selected in model 2a. Furthermore, we cannot exclude *in vitro* auto-oxidation of lipid metabolites. However, as this would have occurred to a similar extend in responders and non-responders, it is unlikely this has influenced our findings. Hence, the use of lipidomics, and in particular the development of a lipid biomarker, for the prediction of prednisolone treatment response warrants further investigation.

In conclusion, this exploratory study suggests that lipidomics may prove valuable in the prediction of prednisolone treatment response in patients with inflammatory hand OA. Prediction of treatment response may aid the selection of patients with a high likelihood of treatment benefit, which is crucial to prevent overtreatment and unnecessary exposure to adverse effects.

References

[1] Zhang Y, Niu J, Kelly-Hayes M, Chaisson CE, Aliabadi P, Felson DT. Prevalence of symptomatic hand osteoarthritis and its impact on functional status among the elderly: The Framingham Study. Am J Epidemiol 2002;156:1021–7. https://doi.org/10.1093/aje/kwf141.

[2] Kwok WY, Vliet Vlieland TPM, Rosendaal FR, Huizinga TWJ, Kloppenburg M. Limitations in daily activities are the major determinant of reduced health-related quality of life in patients with hand osteoarthritis. Ann Rheum Dis 2011;70:334–6. https://doi.org/10.1136/ard.2010.133603.

[3] Kloppenburg M, Kwok W-Y. Hand osteoarthritis—a heterogeneous disorder. Nat Rev Rheumatol 2012;8:22–31. https://doi.org/10.1038/nrrheum.2011.170.

[4] Loef M, Damman W, Mutsert R de, Rosendaa FR, Kloppenburg M. Health-related quality of life in patients with hand osteoarthritis from the general population and the outpatient clinic. J Rheumatol 2019. https://doi.org/10.3899/jrheum.190781.

[5] Kortekaas MC, Kwok W-Y, Reijnierse M, Watt I, Huizinga TWJ, Kloppenburg M. Pain in hand osteoarthritis is associated with inflammation: the value of ultrasound. Ann Rheum Dis 2010;69:1367–9. https://doi.org/10.1136/ard.2009.124875.

[6] Keen HI, Wakefield RJ, Grainger AJ, Hensor EMA, Emery P, Conaghan PG. An ultrasonographic study of osteoarthritis of the hand: synovitis and its relationship to structural pathology and symptoms. Arthritis Rheum 2008;59:1756–63. https://doi.org/10.1002/art.24312.

[7] Kroon FPB, Kortekaas MC, Boonen A, Böhringer S, Reijnierse M, Rosendaal FR, et al. Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. The Lancet 2019;394:1993–2001. https://doi.org/10.1016/S0140-6736(19)32489-4.

[8] Kaddurah-Daouk R, Kristal BS, Weinshilboum RM. Metabolomics: a global biochemical approach to drug response and disease. Annu Rev Pharmacol Toxicol 2008;48:653–83. https://doi.org/10.1146/annurev.pharmtox.48.113006.094715.

[9] Villalvilla A, Gómez R, Largo R, Herrero-Beaumont G. Lipid Transport and Metabolism in Healthy and Osteoarthritic Cartilage. Int J Mol Sci 2013;14:20793–808. https://doi.org/10.3390/ijms141020793.

[10] During A, Penel G, Hardouin P. Understanding the local actions of lipids in bone physiology. Prog Lipid Res 2015;59:126–46. https://doi.org/10.1016/j.plipres.2015.06.002.

[11] Tsuge K, Inazumi T, Shimamoto A, Sugimoto Y. Molecular mechanisms underlying prostaglandin E2-exacerbated inflammation and immune diseases. Int Immunol 2019;31:597–606. https://doi.org/10.1093/intimm/dxz021.

[12] Bastiaansen-Jenniskens YM, Siawash M, van de Lest CHA, Verhaar J a. N, Kloppenburg M, Zuurmond A-M, et al. Monounsaturated and Saturated, but Not n-6 Polyunsaturated Fatty Acids Decrease Cartilage Destruction under Inflammatory Conditions: A Preliminary Study. Cartilage 2013;4:321–8. https://doi.org/10.1177/1947603513494401.

[13] Alvarez-Garcia O, Rogers NH, Smith RG, Lotz MK. Palmitate has proapoptotic and proinflammatory effects on articular cartilage and synergizes with interleukin-1. Arthritis Rheumatol Hoboken NJ 2014;66:1779–88. https://doi.org/10.1002/art.38399.

[14] Castro-Perez JM, Kamphorst J, DeGroot J, Lafeber F, Goshawk J, Yu K, et al. Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. J Proteome Res 2010;9:2377–89. https://doi.org/10.1021/pr901094j.

[15] Zhang Q, Li H, Zhang Z, Yang F, Chen J. Serum metabolites as potential biomarkers for diagnosis of knee osteoarthritis. Dis Markers 2015;2015:684794. https://doi.org/10.1155/2015/684794.

[16] Zhang W, Sun G, Aitken D, Likhodii S, Liu M, Martin G, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. Rheumatol Oxf Engl 2016;55:1566–74. https://doi.org/10.1093/rheumatology/kew207.

[17] Kosinska MK, Liebisch G, Lochnit G, Wilhelm J, Klein H, Kaesser U, et al. A lipidomic study of phospholipid classes and species in human synovial fluid. Arthritis Rheum 2013;65:2323–33. https://doi.org/10.1002/art.38053.

[18] Rockel JS, Zhang W, Shestopaloff K, Likhodii S, Sun G, Furey A, et al. A classification modeling approach for determining metabolite signatures in osteoarthritis. PLoS ONE 2018;13. https://doi.org/10.1371/journal.pone.0199618.

[19] Altman R, Alarcón G, Appelrouth D, Bloch D, Borenstein D, Brandt K, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990;33:1601–10.

[20] Pham T, van der Heijde D, Altman RD, Anderson JJ, Bellamy N, Hochberg M, et al. OMERACT-OARSI initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. Osteoarthritis Cartilage 2004;12:389–99. https://doi.org/10.1016/j.joca.2004.02.001.

[21] Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. Ann Rheum Dis 1957;16:494–502. https://doi.org/10.1136/ard.16.4.494.

[22] Verbruggen G, Veys EM. Numerical scoring systems for the anatomic evolution of osteoarthritis of the finger joints. Arthritis Rheum 1996;39:308–20. https://doi.org/10.1002/art.1780390221.

[23] Jonasdottir HS, Brouwers H, Toes REM,

Ioan-Facsinay A, Giera M. Effects of anticoagulants and storage conditions on clinical oxylipid levels in human plasma. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863:1511–22. https://doi.org/10.1016/j.bbalip.2018.10.003.
[24] Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res 2008;49:1137–46. https://doi.org/10.1194/jlr.D700041-JLR200.
[25] Chouvarine P, Giera M, Kastenmüller G, Artati A, Adamski J, Bertram H, et al. Trans-right ventricle and transpulmonary metabolite gradients in human pulmonary arterial hypertension. Heart Br Card Soc 2020;106:1332–41. https://doi.org/10.1136/heartjnl-2019-315900.
[26] Alarcon-Barrera JC, von Hegedus JH, Brouwers H, Steenvoorden E, Ioan-Facsinay A, Mayboroda OA, et al. Lipid metabolism of leukocytes in the unstimulated and activated states. Anal Bioanal Chem 2020;412:2353–63. https://doi.org/10.1007/s00216-020-02460-8.
[27] Contrepois K, Mahmoudi S, Ubhi BK, Papsdorf K, Hornburg D, Brunet A, et al. Cross-Platform Comparison of Untargeted and Targeted Lipidomics Approaches on Aging Mouse Plasma. Sci Rep 2018;8:17747. https://doi.org/10.1038/s41598-018-35807-4.
[28] Jónasdóttir HS, Ioan-Facsinay A, Kwekkeboom J, Brouwers H, Zuurmond A-M, Toes R, et al. An Advanced LC–MS/MS Platform for the Analysis of Specialized Pro-Resolving Lipid Mediators. Chromatographia 2015;78:391–401. https://doi.org/10.1007/s10337-014-2779-5.
[29] Zou H, Hastie T. Regularization and variable selection via the elastic net. J R Stat Soc Ser B Stat Methodol 2005;67:301–20. https://doi.org/10.1111/j.1467-9868.2005.00503.x.
[30] Carta G, Murru E, Banni S, Manca C. Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. Front Physiol 2017;8:902. https://doi.org/10.3389/fphys.2017.00902.
[31] Legrand P, Rioux V. The Complex and Important Cellular and Metabolic Functions of Saturated Fatty Acids. Lipids 2010;45:941–6. https://doi.org/10.1007/s11745-010-3444-x.
[32] Spite M, Clària J, Serhan CN. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. Cell Metab 2014;19:21–36. https://doi.org/10.1016/j.cmet.2013.10.006.
[33] Loef M, von Hegedus JH, Ghorasaini M, Kroon FPB, Giera M, Ioan-Facsinay A, et al. Reproducibility of Targeted Lipidome Analyses (Lipidyzer) in Plasma and Erythrocytes over a 6-Week Period. Metabolites 2020;11. https://doi.org/10.3390/metabo11010026.

7

Prednisolone treatment is associated with changes in lipid levels in patients with inflammatory hand osteoarthritis

Marieke Loef, Joost von Hegedus, Mohan Ghorasaini, Feline Kroon, Lotte van de Stadt, Martin Giera, Andreea Ioan-Facsinay, Margreet Kloppenburg

Unpublished

Abstract

Objective To investigate the effect of prednisolone treatment on the lipid profile in patients with inflammatory hand osteoarthritis (OA).

Methods In the Hand Osteoarthritis Prednisolone Efficacy (HOPE) study patients with symptomatic hand OA, fulfilling the ACR criteria, and signs of inflammation on ultrasound were included. Patients were randomized to receive 10 mg prednisolone daily or placebo for six weeks. Erythrocytes were isolated from blood samples obtained non-fasted at baseline and after 6 weeks. The Lipidyzer™ platform was used for the quantification of lipid concentrations in nmol/ml. First, we identified the lipid species that could reliably be measured over the investigated study duration in the placebo-treated patients using Pearson correlations. Second, we performed linear regression analyses to assess the differences in change in lipid concentrations of these lipids between prednisolone-treated and placebo-treated patients. We corrected for multiple testing while taking into account the correlation between variables.

Results The study population consisted of 76 (35 placebo-, and 41 prednisolone-treated) patients, with a mean age of 63.5 (8.9) years, 82% were women. We quantified 916 lipid species, which were reduced to 286 lipids after exclusions based on predefined data preprocessing steps. Of these, 94 lipids were eligible for further analyses based on their reproducibility. Compared to placebo, prednisolone-treated patients showed a significant decrease in phosphatidylcholine (PC) (18:0/20:4), PC(18:0/22:6) and PC(18:0/20:3) erythrocyte concentrations. Furthermore, the decrease in PC(18:0/20:4) and PC(18:0/22:6) concentrations was associated with a decrease in pain, and the decrease in PC(18:0/20:3) concentration with synovial thickening.

Conclusion Three phosphatidylcholines, comprised of stearic acid with either arachidonic acid, linolenic acid or docosahexaenoic acid, significantly decreased with six weeks prednisolone treatment. Our results suggest that lipids are involved in OA-related pain and inflammation, and may potentially be used as biomarkers to monitor treatment efficacy.

Introduction

Hand osteoarthritis (OA) is one of the most prevalent OA phenotypes, and it is associated with pain, stiffness, functional impairment and a loss in quality of life[1–4]. OA is a disease of the entire joint, affecting multiple tissues such as cartilage, bone and synovium[5]. Although the pathogenesis of hand OA remains incompletely understood, the involvement of inflammation has become overtly apparent from imaging studies[6–8]. Inflammatory signs such as gray-scale synovitis and power Doppler signals on ultrasonography[6], as well as synovitis and bone marrow lesions (BMLs) on MRI scans[7], are often present in patients with hand OA. Moreover, synovial inflammation is associated with clinical outcomes such as pain[6–8].

Inflammatory processes are regulated by various lipids and their metabolites. In general, saturated and omega-6 polyunsaturated fatty acids have pro-inflammatory effects via production of proinflammatory cytokines such as prostaglandins, leukotrienes and thromboxanes[9–11]. In contrast, omega-3 polyunsaturated fatty acids can be metabolized to anti-inflammatory and pro-resolving mediators, and decrease the production of prostaglandins and oxidation products[12]. Fatty acids occur in free form, as well as incorporated in higher-order lipids such as triglycerides and phospholipids. Phospholipids are key constituents of cell membranes and synovial fluid, playing an important role in the protection of cartilage surfaces via joint lubrication[13]. A tight regulation of the balance between lipid species is essential to maintain normal physiological settings, and to restore the local environment after pathophysiological triggers[13–15].

Previous lipid profiling studies have suggested an altered lipid metabolism in patients with hip and knee OA[16–18]. In particular, associations between differing levels of phosphatidylcholines (PC) and lysophosphatidylcholines (LPC) and OA have been observed[19–21]. Furthermore, increased phospholipid concentrations and high levels of phospholipase A2 were observed in the synovial fluid of patients with OA, and changes in cartilage lipid composition corresponded to OA severity[13]. However, lipidomics studies in OA are still few, and no previous studies have investigated the association of the lipid profile with hand OA. Moreover, as lipids are involved in inflammation, effect of anti-inflammatory treatment on the lipidome of patients with OA may provide additional insight of the role of lipids in OA pathogenesis. To our knowledge, the effect of anti-inflammatory treatment on the lipid profile of patients with OA has not been investigated previously.

Therefore, we investigated the effect of the anti-inflammatory drug prednisolone on the lipid profile in patients with inflammatory hand OA from the a double-blind, randomized placebo-controlled trial, the Hand Osteoarthritis Prednisolone Efficacy (HOPE) study[22]. While metabolomic studies most commonly used serum or plasma, we assessed the lipid profile in erythrocytes, representing membrane-bound lipids which likely are less affected by short-term fluctuations due to for example diet, as erythrocytes show minimal cellular metabolism and have a relatively long half-life.

Methods

Study design and patients

The HOPE study included adult patients with symptomatic hand OA, who fulfilled the American College of Rheumatology criteria[23] and had signs of inflammation in the distal and proximal interphalangeal (DIP/PIP) joints. A detailed description of patient inclusion and

procedures can be found elsewhere[22]. Briefly, patients were required to have ≥4 DIP/PIP joints with osteoarthritic nodes, ≥1 DIP/PIP joints with soft swelling or erythema and ≥1 DIP/PIP joints with positive power Doppler signal or synovitis grade ≥2 on ultrasound, in addition to finger pain of ≥30 mm on 100 mm visual analogue scale (VAS) and flaring upon 48-hour NSAID washout (defined as ≥20 mm worsening). In the case of a contraindication to NSAID, flare was assessed with paracetamol. Patients were excluded from participation in case of chronic inflammatory rheumatic diseases, immune modulating drug use within 90 days before baseline (e.g., antimalarials, systemic or local glucocorticoids), psoriasis, uncontrolled serious comorbidities, malignancy and infectious disease. The study (Netherlands Trial Registry (NTR) 5263) was approved by the local medical ethics committees, and conducted in accordance with Good Clinical Practice guidelines and Declaration of Helsinki. All patients provided written informed consent.

Treatment randomization and adherence

Patients were assigned (1:1) to receive 10mg prednisolone or placebo daily for six weeks. Patients, outcome assessors and data analysts were blinded for treatment allocation until locking of the study database. Treatment adherence was recorded by the patients in a diary. For rescue medication, paracetamol was allowed, as well as a stable dosage of chondroitin sulphate, glucosamine, bisphosphonate, tetracycline or estrogen. The use of NSAIDs and intramuscular or intra-articular glucocorticoid or hyaluronic acid injections was not allowed during the study period. Patients were discouraged to start new non-pharmacological interventions.

Outcome measures

At baseline and week 6, patients completed the Australian/Canadian Hand Osteoarthritis Index (AUSCAN) pain (scored as 0-20) and function (scored as 0-36) subscales (higher scores are worse). Synovial thickening was assessed on ultrasound on a 0-3 scale in 30 hand joints[8]. BMLs were assessed using magnetic resonance imaging (MRI) of the DIP/PIP joints 2-5 (16 joints) on a 0-3 scale[24]. Sum scores on patient level were calculated for synovitis (0-90) and BMLs (0-48).

Blood samples

Blood samples were obtained non-fasted at baseline in EDTA-tubes, following a standardized protocol. From each blood sample, 200µL was centrifuged for 10 minutes at 2200 x g to isolate erythrocytes. Erythrocytes were isolated by ficoll density gradient centrifugation and washed 3x with PBS. Samples were stored at-80°C topped with argon until further analyses[25]. All analyses were performed after one freeze-thaw cycle.

Erythrocyte lipid profile

The Lipidyzer™ platform (Sciex) was used to quantify total lipid content in erythrocytes (nmol/mL). Lipid extraction was performed using methyl tert-butyl ether as described by Matyash et al., with some modifications[26]. To 30µL of erythrocyte sample the following was added: 160µL MeOH, 50µL internal standard solution (Lipidyzer™ internal standard kit, containing >50 labelled internal standards for 13 lipid classes), and 550µL methyl tert-butyl ether. Samples were vortexed and left at room temperature for 30 minutes. Subsequently, 200µL water was added for phase separation and the samples were centrifuged at maximum speed. The upper layer was transferred to a glass vial and lipid extraction was repeated by adding 300µL methyl

tert-butyl ether, 100µL MeOH and 100µL water. Lipidyzer running buffer was added and the samples were transferred to a glass vial with insert for injection. Further technical details can be found elsewhere[27–29]. Samples from the treatment groups were randomized over the four consecutive measurement batches. The lipid concentrations were corrected for the erythrocyte protein pellet content, quantified using a Micro BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA).

Pre-processing of Lipidyzer™ data

The Lipidyzer™ platform is an integrated system to quantify over 1000 lipids. In the erythrocyte samples, we quantified 916 distinct lipid species. Lipid species were excluded from further analysis if the relative standard deviation (RSD) of the internal standard was >20% within each batch and if the RSD of the internal standards was >25% between batches, leading to exclusion of 237 lipid species. Furthermore, we excluded lipid species that were observed in <75% of observations or when they were observed only in a single batch, resulting in 286 distinct lipid species (figure 1). Subsequently, all missing values (18%) were imputed with the minimum measured value of the individual lipid divided by two.

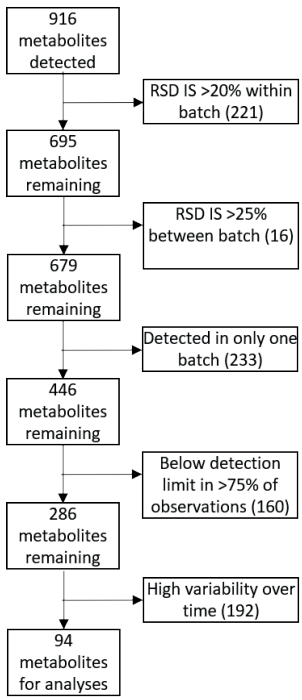

Figure 1. Cleaning steps and exclusion numbers of Lipidyzer™ variables.
Lipid metabolite variables were excluded if the relative standard deviation (RSD) of the internal standard (IS) was >20% within a batch, or >25% between separate batches, if a lipid was detected in one batch only, or if below the detection limit in >75% of the observations.

Statistical analyses

First, we investigated the reproducibility of the lipid species over the investigated study duration. We assessed Pearson correlations between lipid concentrations at baseline and week 6 in the placebo-treated patients. Lipid species were eligible for the subsequent regression analyses if their concentrations at baseline and week 6 were significantly and well correlated (correlation coefficient ≥0.60[30]), as a measure of stability in lipid concentration during the study period. To account for the large number of statistical tests and the potential correlation between the different lipid species, we calculated the effective number of independent variables required to keep the type I error at 5% according to the method by Li and Ji[31], resulting in a significance threshold of a p-value < $4.71*10^{-4}$ for the reproducibility analyses. All lipid variables were logarithmically transformed because of a non-normal distribution, and were mean-scaled to ensure comparability by giving the metabolites equal weight. Second, we performed hypothesis-free linear regression analyses to analyze the association between prednisolone (vs placebo) treatment and the change in concentration of the individual lipid species from baseline to week 6 (delta scores). Using the multiple correction method by Li and Ji[31] the significance threshold was set at a p-value below $1.02*10^{-3}$ for the regression analyses. Previous literature has described an association of the LPC and PC ratio with OA[20]. Therefore, we additionally performed linear regression analyses to test the hypothesis of an association between prednisolone (vs placebo) treatment and the change in LPC/PC ratio. Lastly, the lipid species that were significantly associated with prednisolone treatment were analyzed for the potential association with change in AUSCAN pain, synovitis and BMLs over 6 weeks, independent of intervention. The R script *matSpD* was used to calculate the multiple testing significance threshold. Stata V16.1 (StataCorp LP, TX, USA) was used for all other analyses.

Results

Study population

The study population consisted of patients with available lipid measurements at baseline and after six weeks. In total 76 patients were included, of which 35 in the placebo group and 41 patients in the prednisolone group. Overall, the population was predominantly comprised of women (82%), and the mean (SD) age was 63.5 (8.9) years. At baseline, a mean AUSCAN pain of 10.5 (3.3), synovial thickening sum score of 16.7 (6.2) and BML sum score of 9.9 (6.2) was observed. Baseline characteristics were well-balanced between the placebo and prednisolone groups (table 1).

Reproducibility of erythrocyte lipid concentrations

Of the 286 lipids used in the analyses, 94 lipids showed good to excellent reproducibility after six weeks, with correlation coefficients ranging from 0.95 (p-value $1.04*10^{-14}$) for sphingomyelin (SM)(26:1) to 0.61 (p-value $4.63*10^{-4}$) for lysophosphatidylethanolamine (LPE)(22:5). Table 2 shows the correlation coefficients of all 286 lipid variables. Lipids from all 13 lipid classes quantifiable with the Lipidyzer™ platform were represented among the 94 lipids identified for subsequent regression analyses.

Table 1. Baseline characteristics of the HOPE study population

	All	**Placebo**	**Prednisolone**
	76 (100%)	35 (46%)	41 (54%)
Demographics			
Sex, % women	82	77	85
Age, year	63.5 (8.9)	65.0 (8.4)	62.3 (9.2)
BMI, kg/m^2	27.3 (4.9)	27.2 (5.2)	27.3 (4.4)
Osteoarthritis characteristics			
AUSCAN pain (0-20)	10.5 (3.3)	10.1 (3.4)	10.9 (3.3)
Synovitis sum score (0-90)	16.7 (6.2)	17.1 (5.9)	16.3 (6.5)
Bone marrow lesions sum score (0-48)	9.9 (6.2)	9.7 (6.2)	10.2 (6.3)

Numbers represent mean (SD) unless otherwise specified. Abbreviations: AUSCAN = Australian/Canadian Hand Osteoarthritis Index, BMI = body mass index.

Table 2 Correlation between lipid species concentrations in erythrocytes measured at baseline and 6 weeks

Very strong 0.80-1			Strong 0.60-0.80			Moderate 0.40-0.60			Weak 0.20 - 0.40			Very weak 0-0.20		
Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value
SM(26:1)	0.95	1.04E-14*	PE(O-16:0/18:1)	0.79	2.81E-07*	TAG(49:1-FA18:1)	0.60	6.12E-04	FFA(18:1)	0.39	3.46E-02	TAG(54:5-FA16:0)	0.19	3.24E-01
SM(26:0)	0.92	1.10E-12*	SM(16:0)	0.79	3.53E-07*	PE(P18:0/22:6)	0.59	7.15E-04	TAG(56:1-FA18:1)	0.39	3.49E-02	TAG(54:1-FA18:1)	0.18	3.49E-01
CER(24:0)	0.91	1.40E-11*	CER(24:1)	0.79	3.96E-07*	TAG(49:1-FA16:0)	0.59	7.38E-04	FFA(20:3)	0.39	3.51E-02	TAG(55:5-FA18:1)	0.17	3.64E-01
PE(18:1/18:1)	0.90	2.54E-11*	LPC(18:0)	0.78	5.22E-07*	PE(P18:1/18:1)	0.59	7.43E-04	TAG(51:2-FA16:0)	0.39	3.54E-02	TAG(48:0-FA16:0)	0.17	3.64E-01
SM(18:1)	0.89	1.29E-10*	TAG(49:2-FA16:1)	0.77	8.66E-07*	PE(16:0/20:4)	0.59	7.62E-04	TAG(54:3-FA18:1)	0.39	3.59E-02	TAG(50:0-FA16:0)	0.17	3.74E-01
HCER(16:0)	0.89	1.53E-10*	LPE(20:3)	0.77	1.24E-06*	PE(O-16:0/22:5)	0.59	7.74E-04	TAG(54:4-FA16:0)	0.39	3.78E-02	TAG(56:5-FA18:1)	0.17	3.88E-01
SM(24:1)	0.88	3.43E-10*	PE(P-18:1/20:5)	0.77	1.33E-06*	PC(18:1/16:1)	0.59	8.02E-04	TAG(47:0-FA16:0)	0.38	4.02E-02	TAG(56:6-FA18:1)	0.17	3.88E-01
PC(16:0/22:6)	0.88	4.22E-10*	PE(16:0/18:1)	0.76	1.45E-06*	TAG(54:3-FA18:0)	0.58	9.79E-04	TAG(54:6-FA18:2)	0.38	4.04E-02	TAG(56:7-FA22:6)	0.16	4.11E-01
CER(26:0)	0.87	5.71E-10*	PC(16:0/22:4)	0.75	2.25E-06*	TAG(46:1-FA16:0)	0.58	9.85E-04	TAG(48:1-FA14:0)	0.38	4.10E-02	FFA(18:4)	0.16	4.13E-01
CER(22:0)	0.87	1.24E-09*	PC(18:2/16:1)	0.75	2.41E-06*	CER(16:0)	0.58	1.03E-03	TAG(52:2-FA16:1)	0.38	4.11E-02	PE(P-18:1/22:5)	0.14	4.74E-01
PE(18:1/16:1)	0.87	1.42E-09*	PE(P-16:0/18:2)	0.75	2.47E-06*	FFA(12:0)	0.58	1.08E-03	TAG(47:0-FA15:0)	0.38	4.17E-02	PC(18:1/22:6)	0.14	4.81E-01
PC(16:0/16:1)	0.86	3.18E-09*	PC(18:0/22:5)	0.75	2.85E-06*	TAG(52:4-FA18:2)	0.57	1.28E-03	PE(P-16:0/20:3)	0.37	4.53E-02	TAG(46:0-FA16:0)	0.13	4.85E-01
PC(16:0/18:1)	0.86	3.32E-09*	PE(18:1/18:2)	0.75	3.43E-06*	TAG(47:2-FA18:1)	0.56	1.42E-03	FFA(22:5)	0.37	4.86E-02	TAG(50:0-FA18:0)	0.13	4.92E-01
SM(14:0)	0.84	8.24E-09*	CER(20:0)	0.74	3.75E-06*	PE(P18:0/16:0)	0.56	1.47E-03	TAG(51:1-FA18:1)	0.37	4.90E-02	TAG(55:4-FA18:1)	0.13	5.03E-01
SM(24:0)	0.83	2.30E-08*	PE(P-18:0/18:1)	0.74	3.84E-06*	FFA(24:0)	0.56	1.47E-03	DAG(18:1/18:1)	0.37	4.95E-02	TAG(49:0-FA16:0)	0.13	5.04E-01
LPE(16:0)	0.82	3.79E-08*	PE(16:0/18:2)	0.74	3.93E-06*	PE(P-18:1/20:4)	0.56	1.53E-03	PE(P-18:0/22:4)	0.36	5.26E-02	TAG(44:0-FA16:0)	0.12	5.22E-01
PC(16:0/18:2)	0.82	5.34E-08*	PC(14:0/18:2)	0.74	4.02E-06*	TAG(54:2-FA18:0)	0.56	1.54E-03	TAG(54:2-FA18:2)	0.36	5.27E-02	DAG(16:1/20:0)	0.11	5.72E-01
TAG(52:2-FA18:0)	0.82	5.90E-08*	SM(18:0)	0.74	5.37E-06*	TAG(50:2-FA16:0)	0.56	1.67E-03	FFA(22:6)	0.36	5.29E-02	TAG(56:7-FA20:3)	-0.11	5.75E-01
PC(16:0/22:5)	0.81	8.48E-08*	PE(O-16-0/18:2)	0.73	8.42E-06*	TAG(46:2-FA16:1)	0.56	1.68E-03	FFA(22:1)	0.36	5.61E-02	TAG(56:4-FA18:1)	0.11	5.85E-01
PC(18:1/18:2)	0.80	1.74E-07*	PC(16:0/18:3)	0.72	8.97E-06*	TAG(52:1-FA16:0)	0.56	1.71E-03	TAG(48:2-FA18:1)	0.36	5.81E-02	TAG(46:0-FA14:0)	0.09	6.29E-01
			LPC(18:2)	0.72	9.16E-06*	TAG(49:2-FA16:0)	0.56	1.71E-03	TAG(50:1-FA16:1)	0.35	6.11E-02	TAG(54:2-FA20:1)	0.09	6.34E-01
			PC(18:0/18:2)	0.72	1.06E-05*	TAG(50:2-FA18:2)	0.56	1.77E-03	TAG(50:1-FA18:0)	0.35	6.21E-02	TAG(50:2-FA14:0)	-0.07	7.08E-01
			PC(16:0/20:3)	0.72	1.13E-05*	PE(P-18:1/22:6)	0.55	1.90E-03	DAG(18:1/20:4)	0.35	6.44E-02	TAG(56:3-FA18:0)	0.07	7.19E-01
			PC(18:0/22:6)	0.72	1.15E-05*	PC(18:0/20:5)	0.55	1.96E-03	CE(20:4)	0.35	6.48E-02	TAG(46:2-FA14:0)	0.07	7.24E-01
			PC(17:0/18:2)	0.72	1.25E-05*	TAG(49:3-FA16:1)	0.55	2.03E-03	DAG(18:0/18:1)	0.35	6.61E-02	TAG(52:0-FA16:0)	0.06	7.74E-01
			PC(18:1/20:4)	0.71	1.55E-05*	TAG(44:1-FA14:0)	0.55	2.18E-03	TAG(54:4-FA18:0)	0.34	6.69E-02	TAG(52:5-FA18:2)	0.04	8.53E-01
			PE(P-18:1/18:2)	0.71	1.58E-05*	TAG(54:5-FA18:2)	0.55	2.19E-03	TAG(47:1-FA14:0)	0.34	6.74E-02			
			DAG(16:0/18:2)	0.71	1.65E-05*	PC(16:0/20:1)	0.54	2.32E-03	TAG(49:2-FA18:1)	0.34	6.98E-02			
			PE(O-18:0/20:4)	0.71	1.85E-05*	PE(18:1/22:6)	0.54	2.32E-03	TAG(47:0-FA14:0)	0.34	7.07E-02			
			FFA(20:4)	0.70	2.08E-05*	DAG(16:0/16:0)	0.54	2.36E-03	TAG(53:2-FA18:1)	0.34	7.48E-02			
			PC(15:0/18:2)	0.70	2.14E-05*	FFA(14:0)	0.54	2.50E-03	TAG(51:1-FA16:0)	0.33	7.71E-02			
			FFA(18:3)	0.70	2.20E-05*	LPC(17:0)	0.54	2.68E-03	PE(P-16:0/22:5)	0.33	7.78E-02			
			PC(16:0/20:4)	0.70	2.37E-05*	DAG(18:0/18:2)	0.54	2.73E-03	TAG(44:1-FA16:1)	0.33	8.06E-02			
			PE(18:2/20:4)	0.70	2.51E-05*	TAG(50:3-FA18:1)	0.53	2.96E-03	PC(18:1/20:3)	0.33	8.13E-02			
			PC(18:1/18:1)	0.70	2.56E-05*	TAG(51:2-FA18:1)	0.53	3.27E-03	TAG(54:4-FA20:3)	0.33	8.29E-02			
			PC(16:0/20:5)	0.70	2.81E-05*	TAG(47:1-FA15:0)	0.53	3.39E-03	TAG(52:3-FA16:1)	0.32	9.26E-02			
			PC(16:0/14:0)	0.69	2.91E-05*	PC(16:0/16:0)	0.52	3.78E-03	TAG(50:3-FA18:2)	0.32	9.41E-02			
			TAG(46:2-FA18:1)	0.69	3.06E-05*	TAG(52:3-FA18:1)	0.52	4.14E-03	TAG(56:6-FA20:4)	0.31	9.65E-02			
			PC(18:2/18:2)	0.69	3.19E-05*	TAG(50:2-FA16:1)	0.52	4.19E-03	TAG(56:3-FA18:1)	0.31	1.02E-01			
			HCER(24:1)	0.68	4.57E-05*	TAG(52:4-FA16:0)	0.52	4.19E-03	TAG(54:3-FA18:2)	0.30	1.09E-01			
			LPC(20:4)	0.68	5.37E-05*	TAG(49:1-FA15:0)	0.52	4.20E-03	TAG(51:3-FA18:2)	0.30	1.13E-01			
			PC(18:0/20:3)	0.67	6.03E-05*	TAG(47:0-FA17:0)	0.51	4.56E-03	TAG(46:1-FA14:0)	0.30	1.13E-01			
			LCER(24:0)	0.67	6.14E-05*	CE(18:2)	0.51	4.87E-03	TAG(44:1-FA18:1)	0.30	1.20E-01			
			PE(O-16:0/20:4)	0.67	7.03E-05*	TAG(56:8-FA20:4)	0.51	4.87E-03	PE(18:1/22:5)	0.29	1.27E-01			
			PE(18:2/16:1)	0.66	9.31E-05*	PE(P-18:0/20:4)	0.51	5.01E-03	TAG(54:2-FA16:0)	-0.29	1.29E-01			
			PC(17:0/18:1)	0.66	9.31E-05*	PC(18:2/20:3)	0.51	5.06E-03	TAG(45:0-FA15:0)	0.29	1.33E-01			
			LPE(18:0)	0.66	9.77E-05*	TAG(54:5-FA18:1)	0.51	5.11E-03	TAG(56:3-FA20:1)	0.27	1.49E-01			
			LPC(18:1)	0.66	1.09E-04*	PC(14:0/20:3)	0.50	5.24E-03	TAG(56:4-FA20:4)	0.27	1.56E-01			
			TAG(52:2-FA18:2)	0.66	1.11E-04*	TAG(48:1-FA16:1)	0.50	5.30E-03	TAG(45:0-FA16:0)	0.26	1.70E-01			
			PC(15:0/18:1)	0.65	1.24E-04*	PE(O-18:0/20:3)	0.50	5.55E-03	FFA(22:2)	0.26	1.70E-01			
			PC(16:0/18:0)	0.65	1.32E-04*	PE(P-16:0/18:0)	0.50	6.26E-03	FFA(22:4)	0.26	1.74E-01			
			DAG(16:0/18:1)	0.65	1.34E-04*	TAG(56:5-FA18:0)	0.49	6.42E-03	TAG(56:4-FA18:0)	0.26	1.76E-01			
			PC(18:0/18:1)	0.65	1.43E-04*	PE(P-16:0/20:4)	0.49	6.45E-03	TAG(44:0-FA14:0)	0.25	1.97E-01			
			PC(18:0/20:4)	0.64	1.67E-04*	TAG(46:1-FA18:1)	0.49	6.48E-03	PC(18:1/20:5)	0.24	2.02E-01			

Tabe 2. *Continued*

Very strong 0.80-1			Strong 0.60-0.80			Moderate 0.40-0.60			Weak 0.20 - 0.40			Very weak 0-0.20		
Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value	Lipid	ρ	p-value
			TAG(54:1-FA18:0)	0.63	2.25E-04*	TAG(52:3-FA16:0)	0.49	7.08E-03	FFA(20:1)	0.24	2.10E-01			
			FFA(16:1)	0.63	2.32E-04*	FFA(18:2)	0.49	7.35E-03	LPC(20:3)	0.24	2.15E-01			
			CE(16:0)	0.63	2.67E-04*	PE(P-18:0/20:3)	0.49	7.51E-03	TAG(54:3-FA16:0)	0.23	2.21E-01			
			FFA(20:5)	0.63	2.76E-04*	PE(O-16:0/20:3)	0.48	8.31E-03	TAG(49:0-FA17:0)	0.22	2.46E-01			
			DCER(22:0)	0.63	2.84E-04*	TAG(50:1-FA16:0)	0.48	8.99E-03	DAG(16:0/22:5)	0.22	2.49E-01			
			PE(P-16:0/18:1)	0.62	2.97E-04*	TAG(54:4-FA18:2)	0.47	9.24E-03	TAG(55:7-FA15:0)	0.21	2.63E-01			
			TAG(48:2-FA16:1)	0.62	3.18E-04*	TAG(52:1-FA18:1)	0.47	9.49E-03	TAG(42:0-FA16:0)	0.21	2.63E-01			
			TAG(46:1-FA16:1)	0.62	3.28E-04*	TAG(51:1-FA17:0)	0.47	9.65E-03	TAG(50:3-FA16:0)	0.20	2.92E-01			
			TAG(52:0-FA18:0)	0.62	3.32E-04*	PC(18:2/20:4)	0.47	1.01E-02						
			PE(P-18:0/18:2)	0.62	3.37E-04*	TAG(52:1-FA18:0)	0.47	1.01E-02						
			PC(18:0/18:3)	0.62	3.47E-04*	TAG(50:3-FA16:1)	0.47	1.04E-02						
			PC(16:0/20:2)	0.62	3.47E-04*	TAG(52:2-FA16:0)	0.47	1.05E-02						
			DAG(18:1/18:2)	0.62	3.62E-04*	TAG(48:1-FA18:1)	0.47	1.08E-02						
			TAG(48:3-FA16:1)	0.62	3.79E-04*	PC(18:1/18:3)	0.47	1.08E-02						
			LPC(16:0)	0.61	3.87E-04*	TAG(52:2-FA18:1)	0.46	1.22E-02						
			TAG(55:5-FA20:4)	0.61	4.02E-04*	PE(18:0/20:4)	0.46	1.29E-02						
			PE(O16:0/22:4)	0.61	4.09E-04*	CE(16:1)	0.46	1.31E-02						
			TAG(49:1-FA17:0)	0.61	4.28E-04*	TAG(54:0-FA18:0)	0.45	1.37E-02						
			LPE(22:5)	0.61	4.63E-04*	TAG(47:1-FA16:0)	0.45	1.38E-02						
			PE(P-16:0/22:4)	0.60	5.44E-04	TAG(49:1-FA16:1)	0.45	1.42E-02						
			HCER(24:0)	0.60	5.58E-04	TAG(54:4-FA18:1)	0.45	1.46E-02						
						TAG(56:7-FA20:4)	0.45	1.46E-02						
						TAG(48:1-FA16:0)	0.45	1.50E-02						
						TAG(52:3-FA18:2)	0.45	1.55E-02						
						TAG(52:4-FA16:1)	0.44	1.64E-02						
						PE(P-18:1/20:3)	0.44	1.67E-02						
						FFA(16:0)	0.44	1.69E-02						
						TAG(52:4-FA18:1)	0.44	1.70E-02						
						PE(P-18:0/22:5)	0.44	1.72E-02						
						TAG(51:2-FA15:0)	0.44	1.75E-02						
						PE(O-18:0/22:4)	0.44	1.81E-02						
						HCER(22:0)	0.44	1.83E-02						
						FFA(18:0)	0.43	1.93E-02						
						TAG(56:5-FA20:4)	0.43	1.93E-02						
						CER(14:0)	0.43	1.97E-02						
						TAG(50:2-FA18:1)	0.43	2.00E-02						
						PC(18:0/18:0)	0.43	2.04E-02						
						TAG(50:1-FA18:1)	0.42	2.27E-02						
						FFA(20:2)	0.42	2.45E-02						
						TAG(54:5-FA20:4)	0.42	2.48E-02						
						TAG(48:2-FA16:0)	0.41	2.68E-02						
						TAG(54:4-FA20:4)	0.41	2.72E-02						
						LCER(16:0)	0.41	2.76E-02						
						TAG(48:2-FA14:0)	0.41	2.83E-02						
						TAG(54:2-FA18:1)	0.40	2.93E-02						
						CE(18:1)	0.40	3.13E-02						

Spearman correlation coefficients (ρ). *Below multiple correction cut-off p < 4.71E-04. Abbreviations: TAG= triacylglycerol, DAG= diacylglycerol, FFA= free fatty acid, CE= cholesterol ester, PC=phosphatidylcholine, PE= phosphatidylethanolamine, LPC= lysophosphatidylcholine, LPE= lysophosphatidylethanolamine, SM=sphingomyelin, CER= ceramide, DCER= dihydroceramide, HCER= hexosylceramide, LCER= lactosylceramide.

The effect of prednisolone on lipid concentrations

We compared the change in lipid concentration from baseline to week 6 between prednisolone-treated patients and placebo-treated patients. Of the 94 lipids investigated, the concentrations of 16 lipids significantly increased or decreased in prednisolone treated patients based on the commonly used and more liberal significance threshold of a p-value below 0.05. Of these lipids, the far majority were glycerophospholipids, either PC, LPC or PE (table 3). Taking the correction for multiple testing into account we observed a significant decrease in the concentration of three lipids, PC(18:0/20:4), PC(18:0/20:3) and PC(18:0/22:6) in patients treated with prednisolone, compared to placebo (figure 2). Results from the regression analyses of all lipids are provided in supplementary table A. We observed no change in the LPC/PC ratio in prednisolone-treated patients compared to placebo-treated patients.

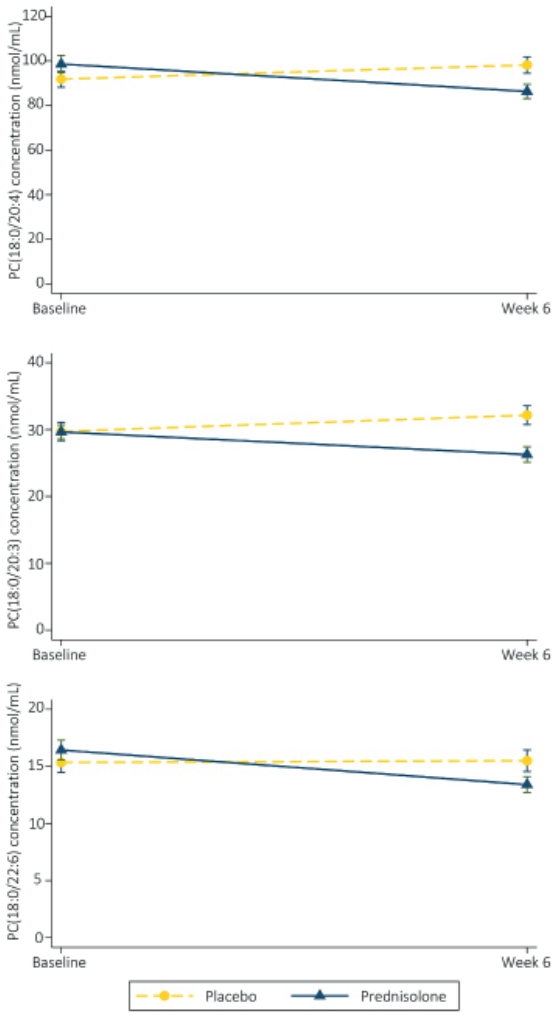

Figure 2. Change in lipid concentration of PC(18:0/20:4), PC(18:0/20:3) and PC(18:0/22:6) after 6 weeks' prednisolone treatment (blue), compared to placebo (yellow).

Table 3. Lipid species associated with 6 weeks prednisolone treatment

	Placebo		Prednisolone					
Lipid	Mean	SD	Mean	SD	**Beta^**	**95% CI**		**P value**
PC(18:0/20:4)	5.43	12.92	-12.96	14.12	-0.89	-1.21	-0.57	$7.04*10^{-7}$*
PC(18:0/20:3)	1.16	5.11	-3.83	5.16	-0.71	-1.06	-0.36	0.00012*
PC(18:0/22:6)	-0.06	3.57	-3.54	3.70	-0.65	-0.98	-0.32	0.00020*
PC(14:0/18:2)	-0.26	2.92	2.07	2.74	0.66	0.26	1.07	0.0018
LPC(18:0)	2.27	9.82	-3.45	9.20	-0.56	-0.94	-0.17	0.0054
PC(18:2/16:1)	-0.36	2.74	1.36	2.33	0.64	0.19	1.09	0.0058
PC(18:0/18:1)	11.38	21.62	-6.51	26.55	-0.53	-0.90	-0.15	0.0072
PC(18:0/18:2)	8.25	40.65	-17.65	41.40	-0.47	-0.83	-0.11	0.011
PC(16:0/14:0)	0.33	3.15	3.74	5.38	0.50	0.10	0.90	0.015
PC(16:0/22:4)	0.95	1.65	-0.17	2.30	-0.50	-0.90	-0.10	0.015
PE(O-16:0/22:4)	1.99	3.82	-0.50	4.36	-0.50	-0.92	-0.07	0.023
PE(O-16:0/20:4)	2.14	4.45	-0.75	5.57	-0.50	-0.95	-0.05	0.031
SM(24:0)	27.48	78.00	-18.35	87.06	-0.36	-0.70	-0.03	0.034
PC(16:0/16:1)	1.81	9.80	8.72	11.91	0.31	0.02	0.59	0.037
DAG(18:1/18:2)	-0.03	0.14	0.04	0.14	0.53	0.01	1.05	0.045
PC(18:1/20:4)	0.63	3.08	-0.97	3.40	-0.38	-0.76	0.00	0.049
LPC/PC ratio	-0.001	0.008	0.001	0.006	0.31	-0.11	0.72	0.142

Mean (SD) change in lipid concentration before data transformations (nmol/mL). ^Lipid concentrations have been log transformed and subsequently mean-scaled. *Below significance threshold required to keep type I error rate at 5%: 0.0010. Abbreviations: CI = confidence interval, DAG = diacylglycerol, (L)PC = (lyso)phosphatidylcholine, PE = phosphatidyletholamine, SM = sphingomyelin, SD = standard deviation

Table 4. Association of changed lipid species with pain and imaging outcomes

Lipid	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**
	AUSCAN pain		*US Synovitis*		*MRI BML*	
PC(18:0/20:4)	2.44 (1.32; 3.55)	<0.001	1.17 (-0.40; 2.75)	0.141	0.03 (-0.34; 0.40)	0.887
PC(18:0/20:3)	0.76 (-0.51; 2.02)	0.238	1.61 (-0.05; 3.17)	0.043	0.21 (-0.16; 0.58)	0.254
PC(18:0/22:6)	1.77 (0.49; 3.05)	0.007	-0.42 (-2.22; 1.18)	0.543	0.03 (-0.37; 0.43)	0.883

^Lipid concentrations have been log transformed and subsequently mean-scaled. BML= bone marrow lesion, MRI= magnetic resonance imaging, US= ultrasound.

Association of altered lipid concentration with pain and imaging outcomes

We further investigated the association of the change in the three significantly decreased PCs with change in AUSCAN pain, synovial thickening and BMLs (table 4). The observed decrease in the concentration of PC(18:0/20:4) and PC(18:0/22:6) was associated with a decrease in AUSCAN pain. Additionally, a decrease in PC(18:0/20:3) concentration was associated with a decrease in synovial thickening on ultrasound. Change in concentration of none of the lipids was associated with a change in BMLs.

Discussion

We aimed to investigate the effect of six weeks daily 10 mg prednisolone treatment on the erythrocyte lipid profile of patients with inflammatory hand OA. We observed that prednisolone treatment was associated with a significant decrease in the concentration of three PCs, all comprised of one chain of stearic acid, combined with either arachidonic acid, linolenic acid or docosahexaenoic acid. Furthermore, we showed that the change in concentration of these lipids was associated with a decrease in pain and synovial thickening, suggesting that this change in lipid concentration may have relevant clinical implications.

Our results showed a decrease in the concentration of several PCs in patients with OA treated with prednisolone. PCs are the major component of cell membranes and play an important role in membrane-associated cell signaling, and are the principal phospholipids circulating in plasma as an integral component of lipoproteins. They have been shown to regulate bone metabolism[15] and are essential for cartilage physiology[13]. To our knowledge, the effect of prednisolone treatment on the lipidome has not previously been described in patients with OA, in an *in vivo* study. In an *in vitro* study, Sluzalska et al. investigated the effect of another corticosteroid, dexamethasone, on the biosynthesis and release of phospholipids from cultured fibroblast-like synoviocytes obtained from osteoarthritic human knees. They observed a decreased biosynthesis of PC and not of LPC in treated synoviocytes vs untreated controls, which seems in accordance with our results. However, they also observed a decreased biosynthesis of PE and SM[32]. Furthermore, there are obvious methodological differences to our studies, in particular the *in vitro* study design and the association of total PC levels, opposed to our *in vivo* design and the association of the concentration of several individual PCs. The effect of prednisolone treatment on the plasma lipidome has been investigated in conditions other than OA. Pereira et al. compared plasma PC levels in patients with Crohn's disease to control subjects, and before and after 2 weeks treatment with prednisolone (0.5 mg/kg/day). They observed higher PC levels in patients with Crohn's disease compared to controls, and, in line with our findings, a decrease in PC levels after two weeks prednisolone treatment. A simultaneous improvement in disease activity was observed[33], comparable to our observations on pain and synovial thickening. Controversial results were seen in the untargeted metabolomic profiling of serum from patients with Myasthenia Gravis, before and after 12 weeks daily 20 mg prednisolone treatment. Although the majority of metabolites that were differentially expressed after treatment were glycerophospholipids[34], which is in line with our findings, the results from this study suggested an increase in glycerophospholipid synthesis[34], while we observed a decrease in levels of several PCs upon treatment with prednisolone. Unfortunately, they did not include a control arm without prednisolone treatment, hampering robust conclusions. To our knowledge, no other studies have investigated the erythrocyte lipidome, limiting comparison of our findings with previously obtained results.

Previous metabolomic profiling studies have suggested that the lipid profile, and PCs in particular, are involved in OA pathogenesis[19–21]. In synovial fluid from patients with knee OA, higher levels of PCs were observed in patients with early and late OA in a dose dependent manner[19]. In contrast, Zhang et al. observed in a cross-sectional study lower plasma PC levels in patients with end-stage knee OA compared to controls. Additionally, they found higher LPC levels, and an increased plasma LPC to PC ratio in these patients, compared to controls[20]. The role of PCs in OA is further supported by the results from a classification modelling study, which showed that individual LPCs and PC analogues in plasma were able to discriminate patients with knee OA from healthy controls[21]. While systemic factors may play role in hand OA in particular, as hand OA is likely less affected by mechanical factors due to obesity and injuries, no previous studies have investigated the association between the lipid profile and hand OA.

It is questionable whether the lipid profile changes that have been observed previously are OA specific, or rather a sign of inflammation. Similarly to the results from lipid profiling

studies in patients with OA, in comparison to control subjects lower levels of PC to LPC ratio have been observed in patients with RA[35], higher PC levels have been shown in patients with Crohn's disease[33], and higher PC and lower LPC levels in patients with asthma[36]. Likewise, the change in this ratio upon treatment may not be prednisolone specific, but may correspond to a reduction of inflammation.

The HOPE study offered the unique opportunity to investigate the effect of anti-inflammatory treatment on the lipid profile in patients with hand OA. We presented novel and timely findings, as there is a high need for disease modifying medication in OA. An increased understanding of the pathophysiology of OA and treatment effects may aid the discovery of new treatment targets and future treatment development. A major strength of our study is the use of high-quality trial data. This resulted in a tight control of both study medication as well as concurrent medication and standardized timepoints in which endpoints were measured via pre-specified protocols. In contrast to most previous research, we measured lipid concentrations in erythrocyte membranes. Although this could hamper direct comparisons with previous results, lipid concentrations in erythrocytes are, due to their relatively long half-life of approximately 3 months, likely less influenced by short-term fluctuations due to dietary intake, compared to plasma.

There are also limitations to our study. Most notable is the small study sample. In addition, the analyses have been performed in a specific, carefully selected patient population. Therefore, our results may not be generalizable to other patient populations. In the present study, our analyses were limited to the change in the lipid profile after six weeks of treatment. Possibly, the effect on the lipid profile may be more distinct after longer treatment duration. Furthermore, the primary trial analyses showed that the prednisolone treatment effect on pain levels diminished after treatment cessation. Therefore, it would be of great interest to investigate if the lipid profile changed accordingly. The Lipidyzer™ is a flow-injection based lipidomics platform, ideal for exploratory and hypothesis generating research. However, the concentration of individual lipids can be more accurately assessed using assays specifically targeted at the lipids of interest, using specific labeled internal standard materials to exclude matrix effects and minimize analytical variation. Therefore, we are currently in the process of developing a method for the targeted measurement of the three PCs associated with prednisolone treatment in the present analyses. Precise quantification of the lipids, and assessment of the change in concentration at the three consecutive timepoints, involving both start and cessation of treatment, will provide additional insight in the effect of prednisolone on the lipidome, and the possible involvement in suppression of pain and inflammation.

In conclusion, we observed a significant decrease in the concentration of several individual PCs in patients with inflammatory hand OA treated with prednisolone in comparison to placebo. These changes in lipid concentrations were associated with a decrease in pain and synovial thickening, pointing to potential clinical implications of these findings. Although additional research is warranted to support our findings, they may be a first exploratory step in the identification of biomarkers for treatment efficacy monitoring.

Reference

1 Zhang Y, Niu J, Kelly-Hayes M, Chaisson CE, Aliabadi P, Felson DT. Prevalence of symptomatic hand osteoarthritis and its impact on functional status among the elderly: The Framingham Study. Am J Epidemiol 2002; 156: 1021–7.

2 Kwok WY, Vliet Vlieland TPM, Rosendaal FR, Huizinga TWJ, Kloppenburg M. Limitations in daily activities are the major determinant of reduced health-related quality of life in patients with hand osteoarthritis. Ann Rheum Dis 2011; 70: 334–6.

3 Kloppenburg M, Kwok W-Y. Hand osteoarthritis—a heterogeneous disorder. Nature Reviews Rheumatology 2012; 8: 22–31.

4 Loef M, Damman W, Mutsert R de, Rosendaa FR, Kloppenburg M. Health-related quality of life in patients with hand osteoarthritis from the general population and the outpatient clinic. The Journal of Rheumatology 2019; published online Dec 1. DOI:10.3899/jrheum.190781.

5 Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377: 2115–26.

6 Kortekaas MC, Kwok W-Y, Reijnierse M, Watt I, Huizinga TWJ, Kloppenburg M. Pain in hand osteoarthritis is associated with inflammation: the value of ultrasound. Ann Rheum Dis 2010; 69: 1367–9.

7 Haugen IK, Christensen BS, Boyesen P, Sesseng S, van der Heijde D, Kvien TK. Increasing synovitis and bone marrow lesions are associated with incident joint tenderness in hand osteoarthritis. Annals of the Rheumatic Diseases 2016; 75: 702–8.

8 Keen HI, Wakefield RJ, Grainger AJ, Hensor EMA, Emery P, Conaghan PG. An ultrasonographic study of osteoarthritis of the hand: synovitis and its relationship to structural pathology and symptoms. Arthritis Rheum 2008; 59: 1756–63.

9 Alvarez-Garcia O, Rogers NH, Smith RG, Lotz MK. Palmitate has proapoptotic and proinflammatory effects on articular cartilage and synergizes with interleukin-1. Arthritis Rheumatol 2014; 66: 1779–88.

10 Frommer KW, Schaffler A, Rehart S, Lehr A, Muller-Ladner U, Neumann E. Free fatty acids: potential proinflammatory mediators in rheumatic diseases. Ann Rheum Dis 2015; 74: 303–10.

11 Miao H, Chen L, Hao L, et al. Stearic acid induces proinflammatory cytokine production partly through activation of lactate-HIF1α pathway in chondrocytes. Sci Rep 2015; 5: 13092.

12 Shen C-L, Dunn DM, Henry JH, Li Y, Watkins BA. Decreased production of inflammatory mediators in human osteoarthritic chondrocytes by conjugated linoleic acids. Lipids 2004; 39: 161–6.

13 Villalvilla A, Gómez R, Largo R, Herrero-Beaumont G. Lipid Transport and Metabolism in Healthy and Osteoarthritic Cartilage. Int J Mol Sci 2013; 14: 20793–808.

14 Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. Nature Reviews Molecular Cell Biology 2018; 19: 281–96.

15 During A, Penel G, Hardouin P. Understanding the local actions of lipids in bone physiology. Prog Lipid Res 2015; 59: 126–46.

16 Castro-Perez JM, Kamphorst J, DeGroot J, et al. Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. J Proteome Res 2010; 9: 2377–89.

17 Zhang Q, Li H, Zhang Z, Yang F, Chen J. Serum metabolites as potential biomarkers for diagnosis of knee osteoarthritis. Dis Markers 2015; 2015: 684794.

18 Zhang W, Sun G, Likhodii S, et al. Metabolomic analysis of human plasma reveals that arginine is depleted in knee osteoarthritis patients. Osteoarthr Cartil 2016; 24: 827–34.

19 Kosinska MK, Liebisch G, Lochnit G, et al. A lipidomic study of phospholipid classes and species in human synovial fluid. Arthritis Rheum 2013; 65: 2323–33.

20 Zhang W, Sun G, Aitken D, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. Rheumatology (Oxford) 2016; 55: 1566–74.

21 Rockel JS, Zhang W, Shestopaloff K, et al. A classification modeling approach for determining metabolite signatures in osteoarthritis. PLoS One 2018; 13. DOI:10.1371/journal.pone.0199618.

22 Kroon FPB, Kortekaas MC, Boonen A, et al. Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. The Lancet 2019; 394: 1993–2001.

23 Altman R, Alarcón G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990; 33: 1601–10.

24 Haugen IK, Ostergaard M, Eshed I, et al. Iterative development and reliability of the OMERACT hand osteoarthritis MRI scoring system. J Rheumatol 2014; 41: 386–91.

25 Jonasdottir HS, Brouwers H, Toes REM, Ioan-Facsinay A, Giera M. Effects of anticoagulants and storage conditions on clinical oxylipid levels in human plasma. Biochim Biophys Acta Mol Cell Biol Lipids 2018; 1863: 1511–22.

26 Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res 2008; 49: 1137–46.

27 Chouvarine P, Giera M, Kastenmüller G, et al. Trans-right ventricle and transpulmonary metabolite gradients in human pulmonary arterial hypertension. Heart 2020; 106: 1332–41.

28 Alarcon-Barrera JC, von Hegedus JH, Brouwers H, et al. Lipid metabolism of leukocytes in the unstimulated and activated states. Anal Bioanal Chem 2020; 412: 2353–63.

29 Contrepois K, Mahmoudi S, Ubhi BK, et al. Cross-Platform Comparison of Untargeted and Targeted Lipidomics Approaches on Aging Mouse Plasma. Sci Rep 2018; 8: 17747.
30 Evans JD. Straightforward statistics for the behavioral sciences. Pacific Grove, Calif: Brooks/Cole Publishing, 1996.
31 Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) 2005; 95: 221–7.
32 Sluzalska KD, Liebisch G, Ishaque B, Schmitz G, Rickert M, Steinmeyer J. The Effect of Dexamethasone, Adrenergic and Cholinergic Receptor Agonists on Phospholipid Metabolism in Human Osteoarthritic Synoviocytes. Int J Mol Sci 2019; 20. DOI:10.3390/ijms20020342.
33 Pereira SP, Cassell TB, Engelman JL, Sladen GE, Murphy GM, Dowling RH. Plasma arachidonic acid-rich phospholipids in Crohn's disease: response to treatment. Clin Sci (Lond) 1996; 91: 509–12.
34 Sengupta M, Cheema A, Kaminski HJ, Kusner LL, Muscle Study Group. Serum metabolomic response of myasthenia gravis patients to chronic prednisone treatment. PLoS One 2014; 9: e102635.
35 Fuchs B, Schiller J, Wagner U, Häntzschel H, Arnold K. The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis: Investigations by 31P NMR and MALDI-TOF MS. Clinical Biochemistry 2005; 38: 925–33.
36 Ried JS, Baurecht H, Stückler F, et al. Integrative genetic and metabolite profiling analysis suggests altered phosphatidylcholine metabolism in asthma. Allergy 2013; 68: 629–36.
37 Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. Biochim Biophys Acta 2012; 1818: 2465–75.
38 Law S-H, Chan M-L, Marathe GK, Parveen F, Chen C-H, Ke L-Y. An Updated Review of Lysophosphatidylcholine Metabolism in Human Diseases. Int J Mol Sci 2019; 20. DOI:10.3390/ijms20051149.

Supplementary table A. Change in lipid species associated with 6 weeks prednisolone treatment

	Placebo	Prednisolone			
Lipid	MeanSD	MeanSD	**Beta^**	**95% CI**	**P value**
PC(18:0/20:4)	5.4312.92	-12.9614.12	-0.89	-1.21-0.57	7.04E-07*
PC(18:0/20:3)	1.165.11	-3.835.16	-0.71	-1.06-0.36	0.000119*
PC(18:0/22:6)	-0.063.57	-3.543.70	-0.65	-0.98-0.32	0.000195*
PC(14:0/18:2)	-0.262.92	2.072.74	0.66	0.261.07	0.001814
LPC(18:0)	2.279.82	-3.459.20	-0.56	-0.94-0.17	0.005364
PC(18:2/16:1)	-0.362.74	1.362.33	0.64	0.191.09	0.005808
PC(18:0/18:1)	11.3821.62	-6.5126.55	-0.53	-0.90-0.15	0.007241
PC(18:0/18:2)	8.2540.65	-17.6541.40	-0.47	-0.83-0.11	0.011241
PC(16:0/14:0)	0.333.15	3.745.38	0.50	0.100.90	0.015036
PC(16:0/22:4)	0.951.65	-0.172.30	-0.50	-0.90-0.10	0.015099
PE(O-16:0/22:4)	1.993.82	-0.504.36	-0.50	-0.92-0.07	0.023209
PE(O-16:0/20:4)	2.144.45	-0.755.57	-0.50	-0.95-0.05	0.031083
SM(24:0)	27.4878.00	-18.3587.06	-0.36	-0.70-0.03	0.034155
PC(16:0/16:1)	1.819.80	8.7211.91	0.31	0.020.59	0.036657
DAG(18:1/18:2)	-0.030.14	0.040.14	0.53	0.011.05	0.045041
PC(18:1/20:4)	0.633.08	-0.973.40	-0.38	-0.760.00	0.049163
SM(24:1)	14.6045.91	-12.3055.97	-0.26	-0.530.01	0.060934
PE(O-18:0/20:4)	4.8010.63	0.1812.84	-0.36	-0.760.04	0.073161
TAG(48:3)/FA(16:1)	0.010.13	-0.030.12	-0.38	-0.810.05	0.08575
LPE(16:0)	0.210.72	-0.211.30	-0.26	-0.590.08	0.127458
PC(16:0/18:3)	1.084.45	3.347.19	0.31	-0.100.72	0.140474
PC(16:0/20:3)	10.3525.83	-0.1728.93	-0.31	-0.730.11	0.141732
SM(16:0)	56.06116.20	1.51147.16	-0.32	-0.740.11	0.142078
PC(18:2/18:2)	-0.715.19	1.025.38	0.31	-0.120.75	0.149759
PC(15:0/18:2)	-0.051.01	0.291.25	0.42	-0.161.00	0.1532
LPE(22:5)	-0.010.12	0.010.13	0.29	-0.150.74	0.188791
TAG(55:5)FA(20:4)	0.010.02	0.000.02	-0.30	-0.760.16	0.20163
PC(16:0/18:2)	32.31157.38	73.93197.77	0.25	-0.140.63	0.202625
CER(20:0)	0.160.39	0.010.45	-0.24	-0.620.14	0.208004
LPE(18:0)	0.410.91	-0.061.67	-0.32	-0.820.19	0.211382
PE(18:2/20:4)	0.684.63	-0.664.97	-0.22	-0.580.13	0.213183
PC(16:0/18:0)	2.296.79	-0.039.22	-0.31	-0.800.18	0.214681
TAG(49:2)FA(16:1)	0.010.17	-0.030.14	-0.29	-0.770.18	0.223672
PC(18:0/22:5)	0.011.03	-0.521.79	-0.25	-0.650.16	0.225464
LPC(20:4)	0.000.32	0.140.59	0.31	-0.210.83	0.237669
PC(17:0/18:1)	-0.101.34	0.331.24	0.28	-0.190.75	0.241938
DAG(16:0/18:2)	0.040.27	0.110.26	0.27	-0.190.72	0.245653
LPE(20:3)	0.010.07	-0.010.10	-0.24	-0.650.18	0.260627
PE(18:2/16:1)	0.160.82	0.410.85	0.19	-0.150.52	0.265954
TAG(54:1/FA(18:0)	0.030.09	0.010.07	-0.25	-0.690.19	0.266959
PC(15:0/18:1)	0.000.94	0.321.39	0.32	-0.310.95	0.31243
CER(22:0)	0.981.97	0.183.11	-0.18	-0.520.17	0.318358
CER(24:1)	3.7410.90	0.2114.24	-0.16	-0.470.16	0.322751
PE(P-18:0/18:2)	1.323.75	2.264.77	0.21	-0.220.65	0.331006
PE(P-18:1/18:2)	0.632.61	1.192.62	0.15	-0.190.50	0.376598
DAG(16:0/18:1)	0.100.23	0.040.30	-0.20	-0.650.26	0.394296
CER(24:0)	2.584.94	0.847.16	-0.13	-0.430.17	0.397383
PC(16:0/22:6)	-1.6219.17	-8.6619.24	-0.16	-0.530.21	0.399472
TAG(48:2)FA(16:1)	0.010.29	-0.050.24	-0.20	-0.690.29	0.426562
SM(26:0)	0.663.74	-0.043.67	-0.09	-0.330.14	0.433759
SM(26:1)	0.292.52	-0.462.67	-0.08	-0.300.13	0.434807
SM(18:1)	1.044.16	-0.305.17	-0.11	-0.410.18	0.441205
TAG(52:2)FA(18:2)	0.030.07	0.000.05	-0.18	-0.660.30	0.449792
PC(16:0/20:4)	18.9439.74	8.4155.66	-0.13	-0.490.22	0.464807
FFA(18:3)	0.140.66	-0.010.46	-0.18	-0.660.30	0.466124

Supplementary table A. *Continued*

	Placebo	Prednisolone			
Lipid	MeanSD	MeanSD	**Beta^**	**95% CI**	**P value**
TAG(46:1)FA(16:1)	0.010.16	-0.020.13	-0.17	-0.640.30	0.480382
TAG(49:1)FA(17:0)	0.010.06	-0.010.06	-0.16	-0.610.29	0.482703
CER(26:0)	0.160.42	0.040.59	-0.10	-0.390.19	0.499988
PE(16:0/18:2)	-0.3628.26	5.4726.82	0.12	-0.230.46	0.509919
TAG(46:2/FA(18:1)	0.010.03	0.000.05	-0.18	-0.770.40	0.533132
CE(16:0)	0.130.45	0.040.30	0.19	-0.420.80	0.537839
PC(16:0/20:2)	0.641.73	0.282.21	-0.12	-0.520.28	0.546432
PE(18:1/16:1)	0.832.44	1.354.01	0.09	-0.230.42	0.565479
SM(18:0)	2.6719.98	5.8022.05	0.09	-0.230.42	0.570047
PE(P-16:0/18:1)	3.3014.80	1.0120.57	-0.12	-0.560.32	0.585099
PE(P-18:1/20:5)	0.507.49	0.719.89	0.13	-0.370.63	0.597531
LPC(16:0)	5.4320.02	3.3919.94	-0.11	-0.530.31	0.603135
PE(18:1/18:1)	2.1214.42	-0.5925.97	-0.10	-0.490.29	0.621277
FFA(16:1)	-0.063.00	-1.449.08	-0.10	-0.530.32	0.624363
LCER(24:0)	0.052.04	0.243.08	0.08	-0.290.45	0.656257
LPC(18:1)	1.274.69	0.923.45	-0.09	-0.490.31	0.656918
PC(18:1/18:1)	2.757.57	1.988.17	-0.08	-0.460.31	0.688163
TAG(52:2)FA(18:0)	0.030.07	0.020.06	-0.08	-0.460.31	0.697087
PC(18:1/18:2)	0.8413.54	-0.7214.23	-0.06	-0.400.29	0.734727
PE(16:0/18:1)	7.5867.57	-1.38103.94	-0.07	-0.450.32	0.735355
PE(P-18:0/18:1)	2.4213.16	1.2517.65	-0.07	-0.520.37	0.74154
LPC(18:2)	0.521.90	0.281.83	-0.05	-0.400.31	0.790908
SM(14:0)	4.0110.93	4.9518.75	0.05	-0.300.39	0.792769
TAG(52:0)FA(18:0)	0.020.11	0.020.05	0.08	-0.520.67	0.794872
FFA(20:5)	0.050.19	0.030.27	-0.07	-0.680.55	0.826206
PE(O-16:0/18:2)	0.391.08	0.311.26	-0.05	-0.470.38	0.827176
PE(O-16:0/18:1)	0.821.83	0.773.17	-0.03	-0.440.37	0.868367
TAG(49:2)FA(15:0)	0.000.06	0.000.04	0.04	-0.460.54	0.873119
DCER(22:0)	0.130.42	0.090.57	-0.03	-0.480.42	0.903255
PE(P-16:0/18:2)	1.794.99	1.475.64	-0.02	-0.410.36	0.905917
PC(16:0/22:5)	1.227.02	1.006.63	0.02	-0.390.43	0.928249
PC(18:0/18:3)	0.181.13	0.111.77	-0.02	-0.480.44	0.931432
PE(18:1/18:2)	0.5720.26	0.1318.00	-0.01	-0.360.33	0.940025
FFA(20:4)	0.310.60	0.230.72	-0.02	-0.520.49	0.945481
PC(16:0/18:1)	59.6985.82	62.81176.80	0.01	-0.390.41	0.970905
PC(16:0/20:5)	3.1916.00	11.8928.44	0.01	-0.400.41	0.97352
HCER(16:0)	0.200.55	0.140.96	0.00	-0.320.32	0.985975
PC(17:0/18:2)	-0.131.48	-0.371.31	0.00	-0.440.44	0.990453
HCER(24:1)	0.030.19	0.000.21	0.00	-0.480.48	0.998304

Mean (SD) change in lipid concentration before data transformations (ng/mL). ^Lipid concentrations have been log transformed and subsequently mean-scaled. *Below significance threshold required to keep type I error rate at 5%: 0.0010.

8

Mediation of the association between obesity and osteoarthritis by blood pressure, arterial stiffness and subclinical atherosclerosis

Marieke Loef, Rob van der Geest, Hildo Lamb, Renée de Mutsert, Saskia le Cessie, Frits Rosendaal, Margreet Kloppenburg

Rheumatology (Oxford). 2021;60(7):3268-3277

Abstract

Objective We investigated the role of blood pressure, vessel wall stiffness (pulse wave velocity (PWV)) and subclinical atherosclerosis markers (carotid intima-media thickness (cIMT), popliteal vessel wall thickness (pVWT)) as mediators of the association of obesity with osteoarthritis.

Methods We used cross-sectional data from a subset of the population-based NEO study (n=6,334). We classified clinical hand and knee osteoarthritis by the ACR criteria, and structural knee osteoarthritis, effusion and bone marrow lesions on MRI (n=1,285). cIMT was assessed with ultrasonography. pVWT was estimated on knee MRIs (n=1,285), and PWV by abdominal velocity-encoded MRIs (n=2,580), in subpopulations. Associations between body mass index (BMI) and osteoarthritis were assessed with logistic regression analyses, adjusted for age, sex, and education. Blood pressure, cIMT, pVWT and PWV were added to the model to estimate mediation.

Results The population consisted of 55% women, with a mean(SD) age of 56(6) years. Clinical hand osteoarthritis was present in 8%, clinical knee osteoarthritis in 10%, and structural knee osteoarthritis in 12% of participants. BMI was positively associated with all osteoarthritis outcomes. cIMT partially mediated the association of BMI with clinical hand osteoarthritis (10.6 (6.2; 30.5)%), structural knee osteoarthritis (3.1 (1.9; 7.3)%), and effusion (10.8 (6.0; 37.6)%). Diastolic blood pressure (2.1 (1.6; 3.0)%) limitedly mediated the association between BMI and clinical knee osteoarthritis. PWV and pVWT did not mediate the association between BMI and osteoarthritis.

Conclusions cIMT and diastolic blood pressure limitedly mediated the association of BMI with osteoarthritis. This suggests that such mediation is trivial in the middle-aged population.

Introduction

Rheumatic musculoskeletal disorders (RMDs) are among the leading causes of disability in the middle-aged population. One of the most common RMDs is osteoarthritis (OA), which affects over three hundred million people globally. While the prevalence and burden of OA has already surged in the past decade (1), it is expected to increase even further in the coming years due to population ageing and an increasing prevalence of obesity (2). Together with age and sex, obesity is a major risk factor for OA. Increased body weight results in an increase in mechanical stress, which plays a large role in the risk of OA (3–5). However, increased mechanical loading does not fully explain the association between obesity and OA, which is apparent from the association of obesity with non-weightbearing joints such as the hand (6,7).

Obesity is associated with a broad spectrum of systemic effects due to the release of proinflammatory mediators such as adipokines and lipids, resulting in metabolic dysregulation (8). The role of obesity-related metabolic factors in OA has been of increasing interest in OA research, with in particular a focus on the association between cardiovascular disease (CVD) and OA. While some suggest that both disorders might be due to a common pathway of chronic low-grade inflammation, others have suggested a causal relationship between the two. Recent meta-analyses have compiled the evidence on CVD incidence and risk factors in OA patients and showed an increased CVD risk in patients with OA compared with controls (9). An explanation for this association may be an OA-related decrease in physical activity (10,11). In contrast, a reverse causal direction has also been proposed. OA might result from atherosclerotic vascular changes, resulting in a compromised blood flow with detrimental effects on the subchondral bone and on nutrient supply to the cartilage (12). A recent systematic review of the currently available evidence concluded that an association between vascular pathology and risk of hand and knee OA may be present. However, findings varied and different results were obtained for the investigated OA phenotypes (13).

Moreover, to which extent CVD risk factors may actually explain the association between obesity and OA has not been investigated. Therefore, we aimed to assess the potential role of blood pressure, vessel wall stiffness and multiple subclinical atherosclerosis markers as mediators of the association between obesity and OA in a middle-aged population.

Materials and methods

Study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study, designed to investigate pathways that lead to obesity-related diseases and conditions. Detailed description of study design and data collection has been described elsewhere (14). In short, men and women between 45 and 65 years with a self-reported body mass index (BMI) ≥ 27 kg/m^2 living in the greater area of Leiden (The Netherlands) were eligible to participate. This resulted in an oversampling of individuals with overweight or obesity, to ensure an adequate number of responses from individuals with higher BMI. In addition, all inhabitants between 45 and 65 years from one municipality (Leiderdorp) were

invited to participate in the NEO study irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population (15). In total, 6,671 participants were included in the NEO study cohort. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent. The present study is a cross-sectional analysis of baseline measurements. We excluded participants with a missing physical examination (n = 14) and who reported to have concomitant other rheumatic diseases (n = 323).

Questionnaires
Participants completed standardized questionnaires on demographic and medical information, amongst which a history of inflammatory rheumatic diseases and CVD, and pain in hands and knees on most days of the last month. In addition, participants were asked to list any current medication, which was verified during the study visit.

Clinical assessment
Body weight (kg) and total body fat (%) were measured by bioelectrical impedance balance (TBF-310; Tanita Europe BV, Amsterdam, The Netherlands). BMI was calculated from measured body weight and height (kg/m2). Brachial blood pressure was measured three times with five minutes rest between consecutive measurements, in a seated position on the right arm using a validated automatic oscillometric device (OMRON, Model M10-IT, Omron Health Care Inc, IL, USA), from which the mean systolic and diastolic blood pressure were calculated. In participants using antihypertensive mediation, we adjusted for the systematic negative bias introduced by the antihypertensive treatment by adding a constant of 15 mmHg to the measured mean systolic blood pressure and 10 mmHg to the diastolic blood pressure, to account for potential shrinkage bias (16). In addition, physical examination of the hands and knees was performed by trained research nurses, using a standardized scoring form. Of both hands, bony and soft swellings and deformities of the distal interphalangeal, proximal interphalangeal, metacarpophalangeal and first carpometacarpal joints were assessed. Regarding the knees, presence of bony swellings, pain of the bony margins and warmth upon palpation, crepitus and movement restriction were assessed. Hand and knee OA was defined according to the American College of Rheumatology (ACR) clinical classification criteria (17,18).

Magnetic resonance imaging of the knee
A random sample of 1,285 participants without contra-indications (most notably metallic devices, claustrophobia or a body circumference of more than 1.70 m) underwent magnetic resonance imaging (MRI) of the right knee. Imaging was performed on a MR system operating at a 1.5T field strength (Philips, Medical Systems, Best, The Netherlands), using a dedicated knee coil and a standardized scanning protocol as described earlier (19).
All MRI images were analyzed using the validated knee OA scoring system (KOSS) (20) as described previously (19). Structural knee OA was defined when a definite osteophyte and full thickness cartilage loss was present, or one of these features with at least two of the following: subchondral bone marrow lesions (BML), cyst, meniscal subluxation, maceration or degenerative tear, or partial thickness cartilage loss, according to modified criteria by Hunter et al. (21). In addition, BMLs and joint effusion (grade 2 or higher versus smaller or absent) were investigated separately.
Popliteal VWT was assessed on axial fat-suppressed proton density images (repetition time

TR/echo time (TE) 3225/15; echo train length 6, 4 mm slice thickness; 0.8 mm interslice gap) with a 150-160 mm field of view. The VesselMASS software package, developed at our institution (22), was used for semi-automated detection of the luminal and outer boundaries of the vessel wall on five consecutive slices (see figure 1). The popliteal VWT was calculated as the average perpendicular distance between the luminal and outer boundaries measured at 100 positions along the vessel wall circumference, and averaged over the five consecutive slices. The popliteal VWT could not be assessed in 10% of participants due to insufficient quality of the images.

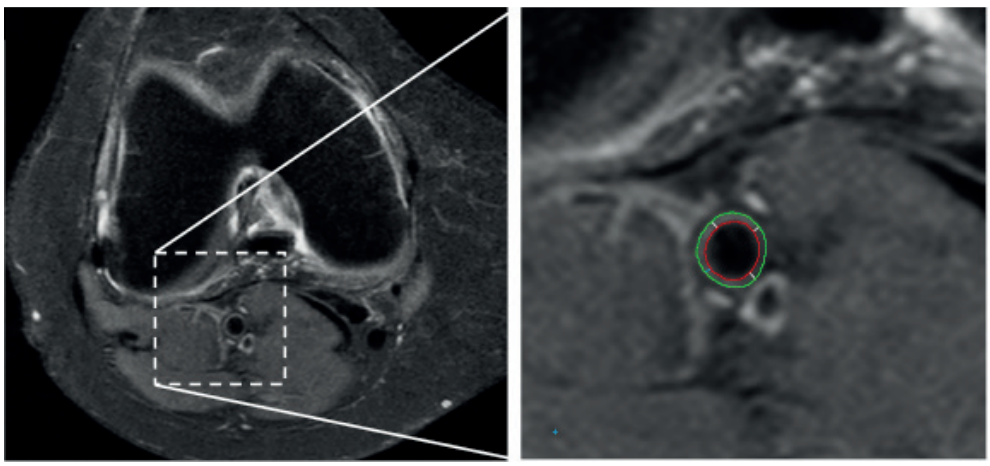

Figure 1. Axial MR image of the knee used for assessment of the popliteal vessel wall thickness.
The right image shows an enlargement of the popliteal artery area. The green line indicates the outer vessel wall boundary, the red line indicates the lumen. At each analysed slice, the vessel wall thickness is calculated as the average distance between the outer and inner vessel boundary at four points (white lines).

Carotid intima-media thickness

Carotid IMT (mm) was measured by ultrasonography of the far wall of the left and right common carotid arteries along a 15 mm long section 10 mm proximal to the bifurcation, with the participant in supine position. The distal common carotid arteries were visualized with a 7.5–10 MHz linear-array transducer (Art.Lab version 2.1, Esaote, Maastricht, The Netherlands) in B-mode setting. A wall track system was used to detect the lumen-intima and media-adventitia boundaries. Carotid IMT data was missing in 1% of participants.

Aortic pulse wave velocity

PWV (m/s) of the aorta was assessed in a random sample of n = 2,580 participants (without overlap with the group receiving a knee MRI) without contraindications for MRI. PWV was determined on a 1.5T field strength whole-body MRI scanner (Philips, Best, the Netherlands) using velocity-encoded MRI. PWV was calculated by the ratio of the aortic path length between the measurements sites and the transit-time of the propagating systolic pulse wave between the measurement sites. Data were analysed using in-house software (MASS and FLOW). Data was missing in 3% of participants due to insufficient coverage or quality of the scans.

Statistical analysis

In the NEO study there is an oversampling of participants with a BMI ≥ 27 kg/m^2. In the present analyses, we aimed to make inferences on associations in the general population. To represent distributions and associations in the general population correctly, adjustment for this oversampling was made by weighting all individuals towards the BMI distribution of participants from the Leiderdorp municipality (n = 1,671) (23), whose BMI distribution was similar to the general Dutch population (15). All results were based on weighted analyses, using probability weights. Consequently, results apply to a population-based study without oversampling.

The clinical hand and knee OA outcome groups were mutually exclusive groups, while overlap between clinical and structural knee OA was allowed (co-occurrence in n = 62). The control group was defined as having no clinical hand or knee OA, nor structural knee OA. We investigated the mediating role of systolic and diastolic blood pressure, carotid IMT, popliteal VWT and aortic PWV by examining the total, direct and indirect effects according to the method by Baron and Kenny (24) as outlined in figure 2. We checked the fulfilment of the four assumptions of the Baron-Kenny framework, by assessing the association between: 1. BMI and OA with logistic regression analyses (total effect C), 2. BMI and mediator with linear regression analyses (indirect effect A), 3. mediator and OA with logistic regression analyses (indirect effect B), and 4. if the association between BMI and OA attenuated after adding the mediator to the model (direct effect C'). Fulfilment of the assumptions was based on the size of the effect estimate in the regression analysis rather than statistical significance. Furthermore, the assumption of no exposure-mediator interaction was checked by adding an interaction term of the independent variable and mediator to the model of the total association. No statistical significance evidence ($p < 0.05$) of interaction was found. For the models fulfilling all assumptions for mediation we calculated the percentage mediation with the Stata package *medeff.* All analyses were adjusted for age, sex and education. Continuous variables (BMI, blood pressure, carotid IMT, popliteal VWT and aortic PWV) were standardized by rescaling them to a mean of zero and a standard deviation of one, to ensure a similar interpretation of the estimated effect. Therefore, the regression coefficient can be interpreted as the association with the dependent variable per standard deviation of the independent variable. Pearson correlation coefficients were calculated to examine the pairwise associations between the different potential mediating variables. We considered 0-0.19 as very weak, 0.2-0.39 as weak, 0.40-0.59 as moderate, 0.6-0.79 as strong and 0.8-1 as very strong correlations.

Several sensitivity analyses were performed. We repeated all analyses substituting BMI for total body fat. Furthermore, we repeated all analyses with exclusion of participants with a history of CVD. Lastly, we compared the percentage mediation calculated by *medeff* with calculation by generalized structural equation modeling with the Stata command *gSEM,* and with calculation according to the Sobel method (25). Stata V14.1 (StataCorp LP, TX, USA) was used for all analyses.

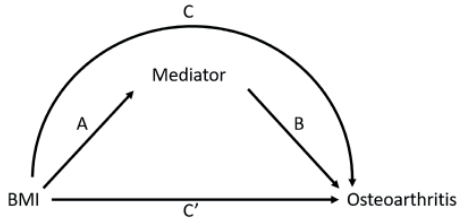

Figure 2. Causal diagram illustrating mediation analysis.
Path C represents the total effect of obesity on osteoarthritis. Path A and B represent the indirect effect via atherosclerosis. Path C' is the direct effect of obesity on osteoarthritis, controlled for atherosclerosis.

Results

Population characteristics

The study population consisted of 6,334 participants with a mean (SD) age of 56 (6) years, of whom 55% were women (table 1). Mean (SD) BMI was 26 (4) kg/m^2. Clinical hand OA was present in 8% of participants, clinical knee OA in 10%. Structural knee OA was defined in 12% of participants, and bone marrow lesions (BML) and effusion in 32% and 12% of participants, respectively. The control group consisting of participants without clinical or structural OA comprised 76% of the study population. Mean (SD) systolic blood pressure was 134 (19) and diastolic blood pressure 85 (12). Mean (SD) carotid IMT was 0.62 (0.09) mm, popliteal VWT was 0.53 (0.05) mm, and aortic PWV was 6.56 (1.30) m/s.

Table 1. Characteristics of the weighted NEO study population

	All n = 6,334
General patient characteristics	
Age (year)	56 (6)
Sex (% women)	55
Education (% high)	46
Body mass index (kg/m^2)	26 (4)
History of cardiovascular disease (%)	6
Antihypertensive medication (% users)	23
Exposure variables	
Systolic blood pressure (mmHg)[a]	134 (19)
Diastolic blood pressure (mmHg)[a]	85 (12)
Carotid intima-media thickness (mm)	0.62 (0.09)
Popliteal artery vessel wall thickness (mm)[b]	0.53 (0.05)
Aortic pulse wave velocity (m/s)[b]	6.56 (1.30)
Osteoarthritis phenotypes	
Clinical hand OA (%)	8
Clinical knee OA (%)	10
Structural knee OA (%)[c]	12
Bone marrow lesions (%)[c]	32
Effusion (%)[c]	12

Results are based on analyses weighted towards the BMI distribution of the general population (n=6,334). Numbers represent mean (SD) unless otherwise specified. Abbreviations: OA = osteoarthritis

[a] Blood pressure was adjusted for antihypertensive medication use when applicable (systolic +15 mmHg, diastolic +10 mmHg).

[b] Popliteal VWT (n=1,095) and PWV (n=2,382) measurements are performed in MRI subpopulations.

[c] Percentage of participants who underwent knee MRI (n=1,285).

Correlation between blood pressure and atherosclerosis measures
Systolic and diastolic blood pressure were moderately (r = 0.40) to weakly (r = 0.34) correlated to aortic PWV, respectively. Carotid IMT and PWV were weakly correlated (r = 0.22); no correlation was present between carotid IMT and popliteal VWT (supplementary table S1).

Mediation of the association between obesity and osteoarthritis
No exposure-mediator interaction was observed when interaction terms between BMI and the potential mediators were added to the logistic regression analyses between BMI and OA. Tables 2 and 3 show the associations of BMI with clinical hand and knee OA, and with structural knee OA, effusion and BML, respectively. In both tables, the second column shows the total effect between BMI and OA (path C). The third column presents the indirect effect between the independent variable (hypertension or atherosclerosis marker) via path A. In the fourth column the direct effect between BMI and OA is given (path C'), as well as the indirect effect of hypertension or the atherosclerosis marker on OA, adjusted for BMI (path B).

Table 2. Preclinical CVD markers as mediators in the association of BMI with clinical OA

Independent variable	Dependent variable			**Mediation** % (95% CI)
	Total effect C	**Indirect effect A**	**Direct effect C` Indirect effect B**	
	Clinical hand OA OR (95% CI)	Mediator β (95% CI)	Clinical hand OA OR (95% CI)	
BMI	1.22 (1.08; 1.37)	0.21 (0.18; 0.25)	1.22 (1.08; 1.38)	NA
Systolic BP			0.98 (0.84; 1.14)	
BMI	1.22 (1.08; 1.37)	0.28 (0.25; 0.32)	1.22 (1.08; 1.38)	NA
Diastolic BP			0.98 (0.84; 1.15)	
BMI	1.21 (1.07; 1.36)	0.23 (0.19; 0.27)	1.19 (1.05; 1.34)	10.6 (6.2; 30.5)
Carotid IMT			1.09 (0.94; 1.25)	
BMI	1.56 (1.17; 2.08)	0.01 (-0.06; 0.09)	1.55 (1.16; 2.07)	NA
Popliteal VWT			1.14 (0.84; 1.55)	
BMI	1.41 (1.15; 1.73)	0.05 (-0.01; 0.11)	1.41 (1.15; 1.73)	NA
Aortic PWV			1.04 (0.81; 1.33)	
	Clinical knee OA OR (95% CI)	Mediator β (95% CI)	Clinical knee OA OR (95% CI)	
BMI	1.46 (1.32; 1.62)	0.21 (0.17; 0.24)	1.46 (1.31; 1.62)	NA
Systolic BP			1.02 (0.90; 1.15)	
BMI	1.46 (1.32; 1.62)	0.27 (0.24; 0.31)	1.45 (1.31; 1.62)	2.1 (1.6; 3.0)
Diastolic BP			1.03 (0.91; 1.15)	
BMI	1.46 (1.32; 1.62)	0.24 (0.20; 0.27)	1.47 (1.33; 1.62)	NA
Carotid IMT			0.97 (0.86; 1.09)	
BMI	1.20 (0.88; 1.64)	0.03 (-0.04; 0.11)	1.21 (0.89; 1.64)	NA
Popliteal VWT			0.95 (0.74; 1.24)	
BMI	1.37 (1.12; 1.67)	0.05 (-0.00; 0.11)	1.37 (1.12; 1.67)	NA
Aortic PWV			0.96 (0.76; 1.21)	

Results are based on analyses weighted towards the BMI distribution of the general population. Due to analyses in subpopulation and control definitions numbers included in the analyses vary; numbers included are provided supplementary figure 1. Continuous variables were standardized (mean 0, SD 1), SD BMI = 4, SD systolic BP = 19, SD diastolic BP = 12, SD carotid IMT = 0.09, SD popliteal VWT = 0.05, SD aortic PWV = 1.30. Analyses were adjusted for age, sex and education. Abbreviations: BMI = body mass index, BP = blood pressure, CI = confidence interval, IMT = intima media thickness, NA = not applicable, OA = osteoarthritis, OR = odds ratio, VWT = vessel wall thickness, PWV = pulse wave velocity, SD = standard deviation.

Clinically defined hand and knee osteoarthritis

After adjusting for age, sex and education, a positive association of BMI with clinically defined hand and knee OA was observed (table 2). Furthermore, BMI was positively associated with systolic and diastolic blood pressure. The association of BMI with clinical hand did not attenuate after adding systolic or diastolic blood pressure to the model. In other words, we observed no mediation of the association between BMI and clinical hand OA by blood pressure. The association between BMI and clinical knee OA attenuated from 1.46 (1.32; 1.62) to 1.45 (1.31; 1.62) upon adding diastolic blood pressure to the model, representing 2.1% (1.6; 3.0) mediation. BMI was positively associated with carotid IMT, and carotid IMT was weakly associated with clinical hand OA with an OR of 1.09 (0.94; 1.25). Carotid IMT mediated the association of BMI with clinical hand OA with 10.6% (6.2; 30.5). No association between carotid IMT and clinical knee OA was observed, hence, no mediation was found. No associations between BMI and popliteal VWT, and between BMI and aortic PWV, were observed. Therefore, mediation of the association between BMI and OA by popliteal VWT and aortic PWV was deemed to be absent.

Structurally defined knee osteoarthritis

BMI was associated with structurally defined knee OA and effusion, but not with BMLs. Systolic blood pressure and diastolic blood pressure were not positively associated with structural knee OA and the mediation assumptions were not fulfilled. In addition, no attenuation of the association between systolic blood pressure and effusion was observed upon addition of the mediator to the model, hence mediation was deemed absent. Carotid IMT attenuated the association between BMI and structural knee OA from 1.58 (1.23; 2.03) to 1.56 (1.19; 2.06), representing 3.1% (1.9; 7.3) mediation. In addition, the association between BMI and effusion was mediated by carotid IMT with 10.8% (6.0; 37.6). Similar to the results described above, BMI was not associated with popliteal VWT in the knee MRI subpopulation.

Sensitivity analyses

We substituted BMI for total body fat and repeated all analyses, which showed similar results (supplementary tables S2 and S3). In addition, repeating the analyses without participants with a history of CVD resulted in similar findings (supplementary tables S4 and S5). We compared the calculation of the percentage mediation by the *medeff* command with *gSEM* and the Sobel method. All three methods yielded similar percentages mediation. However, the observed confidence intervals varied and were generally broader using *gSEM* compared to *medeff* (supplementary table S6).

Table 3. Preclinical CVD markers as mediators in the association of BMI with structural knee OA

Independent variable	Dependent variable			
	Total effect C	**Indirect effect A**	**Direct effect C` Indirect effect B**	**Mediation** % (95% CI)
	Structural knee OA OR (95% CI)	Mediator β (95% CI)	Structural knee OA OR (95% CI)	
BMI	1.58 (1.24; 2.03)	0.29 (0.21; 0.38)	1.67 (1.33; 2.12)	NA
Systolic BP			0.81 (0.64; 1.02)	
BMI	1.58 (1.24; 2.03)	0.35 (0.27; 0.43)	1.65 (1.30; 2.09)	NA
Diastolic BP			0.87 (0.70; 1.09)	
BMI	1.58 (1.23; 2.03)	0.33 (0.25; 0.40)	1.56 (1.19; 2.06)	3.1 (1.9; 7.3)
Carotid IMT			1.03 (0.80; 1.34)	
BMI	1.50 (1.13; 1.99)	0.00 (-0.07; 0.08)	1.50 (1.13; 1.99)	NA
Popliteal VWT			1.09 (0.85; 1.38)	
	Effusion OR (95% CI)	Mediator β (95% CI)	Effusion OR (95% CI)	
BMI	1.46 (1.14; 1.88)	0.30 (0.22; 0.37)	1.48 (1.13; 1.94)	NA
Systolic BP			0.96 (0.74; 1.26)	
BMI	1.46 (1.14; 1.88)	0.34 (0.27; 0.41)	1.54 (1.19; 2.01)	NA
Diastolic BP			0.84 (0.63; 1.11)	
BMI	1.44 (1.12; 1.86)	0.33 (0.26; 0.40)	1.40 (1.06; 1.84)	10.8 (6.0; 37.6)
Carotid IMT			1.12 (0.82; 1.52)	
BMI	1.40 (1.06; 1.84)	0.03 (-0.03; 0.09)	1.38 (1.04; 1.82)	NA
Popliteal VWT			1.29 (1.00; 1.67)	
	BML OR (95% CI)	Mediator β (95% CI)	BML OR (95% CI)	
BMI	1.05 (0.87; 1.27)	NA	NA	NA
Systolic BP				
BMI	1.05 (0.87; 1.27)	NA	NA	NA
Diastolic BP				
BMI	1.06 (0.88; 1.28)	NA	NA	NA
Carotid IMT				
BMI	0.97 (0.79; 1.20)	NA	NA	NA
Popliteal VWT				

Results are based on analyses weighted towards the BMI distribution of the general population. Due to analyses in subpopulation and control definitions numbers included in the analyses vary; numbers included are provided supplementary figure 1. Continuous variables were standardized (mean 0, SD 1), SD BMI = 5, SD systolic BP = 20, SD diastolic BP = 12, SD carotid IMT = 0.09, SD popliteal VWT = 0.05. Analyses were adjusted for age, sex and education. Abbreviations: BMI = body mass index, BML = bone marrow lesion, BP = blood pressure, CI = confidence interval, IMT = intima media thickness, NA = not applicable, OA = osteoarthritis, OR = odds ratio, VWT = vessel wall thickness, SD = standard deviation.

Discussion

In the present population-based study we examined the role of blood pressure, vessel wall stiffness, carotid IMT and popliteal VWT as mediators of the association of BMI with clinically defined hand and knee OA, and structural knee OA. As expected, we observed that BMI was positively associated with all OA outcomes. A small attenuation of the estimated effect of the association between BMI and clinical knee OA was observed when diastolic blood pressure was added to the model. Furthermore, carotid IMT limitedly mediated the associations of BMI with clinical hand OA, structural knee OA, and effusion. No evidence for mediation by PWV or popliteal VWT was observed in any of the associations.

We present novel findings, as the mediating role of preclinical CVD markers in the association between obesity and OA has not previously been investigated. The associations between

these markers and OA (pathway B) have been reported, offering a comparison with the present results. We observed that a small proportion of the association of BMI with OA was mediated by carotid IMT. Although this could represent a chance finding, we observed this for multiple phenotypes: clinical hand OA, structural knee OA, as well as effusion. In addition, this is in line with previous results (26). Furthermore, other atherosclerosis measures have been investigated previously. While a positive association between arterial calcifications and hand OA was shown (27), in knee OA contrasting results have been found (26,28). Also, a positive association of carotid plaques was observed with hand OA, but not knee OA (26,27).

We did not observe an association of BMI with popliteal VWT. Hence, no mediation of the association between BMI and OA by popliteal VWT was found. Since carotid IMT and popliteal VWT are both measures of atherosclerosis, we anticipated similar results. The unexpected discrepancy might be explained by the very limited variation in popliteal VWT in our population. Moreover, in 10% the quality of the knee MRIs was not sufficient to measure the popliteal VWT, and the resulting missingness was not completely at random (MCAR). Rather, we observed that insufficient quality MRIs occurred more often in participants with a higher BMI, implying that the popliteal VWT scores were missing at random (MAR). However, it is unlikely the missingness is related to the popliteal VWT measurements, since we did not take the VWT into account in the decision to discard the MRIs. Therefore, it is doubtful if selection bias has occurred. Moreover, even if the missingness might have distorted the estimated effect, this influence is unlikely as strong to cause the observed null association. However, previous research does suggest an positive association between popliteal VWT and generalized OA (20). In addition, in a population-based cohort without knee disorders, popliteal VWT was negatively associated with cartilage volume, but not BMLs, in cross-sectional and longitudinal analyses (29,30).

Blood pressure played no relevant mediating role in the association of BMI with OA in our population. Current evidence on the association between blood pressure and OA is inconclusive. In the Rotterdam Study, a population-based cohort comparable to the NEO study, no association between hypertension and hand OA was observed after adjustment for BMI (31). In the Framingham study, the association of hypertension with OA was questionable (32). In contrast to this, in a community-based study with only women, hypertension was associated with painful interphalangeal joint OA, even after adjustment for BMI (33). The results from longitudinal studies are equally contradictory (32,34). Similarly, some studies have shown a positive association between hypertension and radiographic knee OA (35–37), while others found no associations (38,39). In addition, we found no mediation by aorta PWV, which is in line with a lack of association of PWV with OA observed previously (40,41).

Our study has notable strengths, among which is the large sample size of the study population. Furthermore, the design of the NEO study, aimed to investigate obesity-related conditions in the middle-aged general population, enabled to study many of the previously investigated preclinical CVD markers. Often, the various measures have been investigated separately, which hinders comparisons. Moreover, in contrast to most studies, we investigated both clinically defined hand and knee OA according to the ACR criteria, as well as the more frequently investigated structural knee OA phenotype. However, our study also has some limitations. The MRIs have been performed in a subpopulation, reducing the number of participants in whom structural knee OA could be measured, and limiting the number of popliteal VWT and

aorta PWV measurements, resulting in a loss of power. To limit the effect this loss of power may have on our conclusions, we focused on the size of the observed effects rather than on statistical significance. Furthermore, perhaps as a result of the relatively healthy and young study population, we observed a narrow distribution of carotid IMT and popliteal VWT, which might have resulted in an underestimation of the mediating effect. In addition, our study has a cross-sectional design, which hinders causal interpretations.

Possible explanations for an association between atherosclerosis and OA have been extensively discussed by Bierma-Zeinstra and Waarsing (42). Due to the long lead-in time of both CVD and OA, as well as the frequent co-occurrence of other morbidities, it is challenging to study the causal direction of the effect. Both directions of the association have been investigated, adding to the controversy on this subject. Moreover, although most studies showed some associations, results of these studies were contradicting with regard to associated joints and type of atherosclerosis measure under investigation, making it difficult to draw a straightforward conclusion (42). Alternatively, CVD and OA may co-occur as a result of common pathophysiological processes, such as obesity-related altered fat metabolism, activation of the innate immune system or changes to the collagen composition that might affect both joints and vascular structure (42,43). Overall, despite the increasing attention for this subject much of the observed association remains incompletely understood. Further research is warranted to draw clear and robust conclusions.

To conclude, in our population mediation of the association between BMI and OA by preclinical CVD measures was questionable. Future research is warranted to further elucidate the association between CVD and OA, which perhaps could be explained by an alternative hypothesis such as shared pathophysiological processes.

References

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Lond Engl. 2018 10;392(10159):1789–858.
2. Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011 Jun 18;377(9783):2115–26.
3. Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. Lancet. 1972 Mar 4;1(7749):519–22.
4. Davis MA, Ettinger WH, Neuhaus JM. Obesity and osteoarthritis of the knee: evidence from the National Health and Nutrition Examination Survey (NHANES I). Semin Arthritis Rheum. 1990 Dec;20(3 Suppl 1):34–41.
5. Visser AW, de Mutsert R, le Cessie S, den Heijer M, Rosendaal FR, Kloppenburg M, et al. The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. Ann Rheum Dis. 2015 Oct;74(10):1842–7.
6. Yusuf E, Nelissen RG, Ioan-Facsinay A, Stojanovic-Susulic V, DeGroot J, van Osch G, et al. Association between weight or body mass index and hand osteoarthritis: a systematic review. Ann Rheum Dis. 2010 Apr;69(4):761–5.
7. Visser AW, Ioan-Facsinay A, de Mutsert R, Widya RL, Loef M, de Roos A, et al. Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. Arthritis Res Ther. 2014 Jan 22;16(1):R19.
8. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011 Jun 1;121(6):2111–7.
9. Mathieu S, Couderc M, Tournadre A, Soubrier M. Cardiovascular profile in osteoarthritis: a meta-analysis of cardiovascular events and risk factors. Joint Bone Spine. 2019 Nov;86(6):679–84.
10. Kendzerska T, Jüni P, King LK, Croxford R, Stanaitis I, Hawker GA. The longitudinal relationship between hand, hip and knee osteoarthritis and cardiovascular events: a population-based cohort study. Osteoarthritis Cartilage. 2017;25(11):1771–80.
11. Hoeven TA, Leening MJG, Bindels PJ, Castaño-Betancourt M, van Meurs JB, Franco OH, et al. Disability and not osteoarthritis predicts cardiovascular disease: a prospective population-based cohort study. Ann Rheum Dis. 2015 Apr;74(4):752–6.
12. Findlay DM. Vascular pathology and osteoarthritis. Rheumatol Oxf Engl. 2007 Dec;46(12):1763–8.
13. Hussain SM, Dawson C, Wang Y, Tonkin AM, Chou L, Wluka AE, et al. Vascular Pathology and Osteoarthritis: A Systematic Review. J Rheumatol. 2020 May 1;47(5):748–60.
14. de Mutsert R, den Heijer M, Rabelink TJ, Smit JWA, Romijn JA, Jukema JW, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol. 2013 Jun;28(6):513–23.
15. Ministerie van VWS. Hoeveel mensen hebben overgewicht? www.rivm.nl/nldemaat. 2013.
16. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat Med. 2005 Oct 15;24(19):2911–35.
17. Altman R, Alarcón G, Appelrouth D, Bloch D, Borenstein D, Brandt K, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum. 1990 Nov;33(11):1601–10.
18. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986 Aug;29(8):1039–49.
19. Visser AW, de Mutsert R, Loef M, le Cessie S, den Heijer M, Bloem JL, et al. The role of fat mass and skeletal muscle mass in knee osteoarthritis is different for men and women: the NEO study. Osteoarthritis Cartilage. 2014 Feb;22(2):197–202.
20. Kornaat PR, Ceulemans RYT, Kroon HM, Riyazi N, Kloppenburg M, Carter WO, et al. MRI assessment of knee osteoarthritis: Knee Osteoarthritis Scoring System (KOSS)--inter-observer and intra-observer reproducibility of a compartment-based scoring system. Skeletal Radiol. 2005 Feb;34(2):95–102.
21. Hunter DJ, Arden N, Conaghan PG, Eckstein F, Gold G, Grainger A, et al. Definition of osteoarthritis on MRI: results of a Delphi exercise. Osteoarthritis Cartilage. 2011 Aug;19(8):963–9.
22. Adame IM, van der Geest RJ, Wasserman BA, Mohamed MA, Reiber JHC, Lelieveldt BPF. Automatic segmentation and plaque characterization in atherosclerotic carotid artery MR images. Magma N Y N. 2004 Apr;16(5):227–34.
23. Lumley, T. Analysis of compex survey samples. http:/www.jstatsoft.org/v09/i08/paper. 2004;
24. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol. 1986 Dec;51(6):1173–82.
25. Sobel ME. Asymptotic Confidence Intervals for Indirect Effects in Structural Equation Models. Sociol Methodol. 1982;13:290–312.
26. Hoeven TA, Kavousi M, Clockaerts S, Kerkhof HJM, van Meurs JB, Franco O, et al. Association of atherosclerosis with presence and progression of osteoarthritis: the Rotterdam Study. Ann Rheum Dis. 2013 May;72(5):646–51.
27. Jonsson H, Helgadottir GP, Aspelund T,

Eiriksdottir G, Sigurdsson S, Ingvarsson T, et al. Hand osteoarthritis in older women is associated with carotid and coronary atherosclerosis: the AGES Reykjavik study. Ann Rheum Dis. 2009 Nov;68(11):1696–700.
28. Gielis WP, Welsing PMJ, van Spil WE, Runhaar J, Weinans H, de Jong PA. A sex-specific association between incident radiographic osteoarthritis of hip or knee and incident peripheral arterial calcifications: 8-year prospective data from Cohort Hip and Cohort Knee (CHECK). Osteoarthritis Cartilage. 2017;25(11):1814–21.
29. Wang Y, Dawson C, Hanna F, Fairley J, Cicuttini FM. Association between popliteal artery wall thickness and knee cartilage volume loss in community-based middle-aged women without clinical knee disease. Maturitas. 2015 Oct;82(2):222–7.
30. Wang Y, Novera D, Wluka AE, Fairley J, Giles GG, O'Sullivan R, et al. Association between popliteal artery wall thickness and knee structure in adults without clinical disease of the knee: a prospective cohort study. Arthritis Rheumatol Hoboken NJ. 2015 Feb;67(2):414–22.
31. Dahaghin S, Bierma-Zeinstra SMA, Koes BW, Hazes JMW, Pols H a. P. Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. Ann Rheum Dis. 2007 Jul;66(7):916–20.
32. Strand MP, Neogi T, Niu J, Felson DT, Haugen IK. Association Between Metabolic Syndrome and Radiographic Hand Osteoarthritis: Data From a Community-Based Longitudinal Cohort Study. Arthritis Care Res. 2018;70(3):469–74.
33. Sanchez-Santos M, Judge A, Gulati M, Spector T, Hart D, Newton J, et al. Association of metabolic syndrome with knee and hand osteoarthritis: A community-based study of women. Semin Arthritis Rheum. 2019 Apr 1;48(5):791–8.
34. Marshall M, Peat G, Nicholls E, Myers HL, Mamas MA, van der Windt DA. Metabolic risk factors and the incidence and progression of radiographic hand osteoarthritis: a population-based cohort study. Scand J Rheumatol. 2019 Jan;48(1):52–63.
35. Yoshimura N, Muraki S, Oka H, Tanaka S, Kawaguchi H, Nakamura K, et al. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. Osteoarthritis Cartilage. 2012 Nov;20(11):1217–26.
36. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. J Rheumatol. 1995 Jun;22(6):1118–23.
37. Monira Hussain S, Wang Y, Cicuttini FM, Simpson JA, Giles GG, Graves S, et al. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. Semin Arthritis Rheum. 2014 Feb;43(4):429–36.
38. Davis MA, Ettinger WH, Neuhaus JM. The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. J Rheumatol. 1988 Dec;15(12):1827–32.
39. Niu J, Clancy M, Aliabadi P, Vasan R, Felson DT. Metabolic Syndrome, Its Components, and Knee Osteoarthritis: The Framingham Osteoarthritis Study. Arthritis Rheumatol Hoboken NJ. 2017 Jun;69(6):1194–203.
40. Saleh AS, Najjar SS, Muller DC, Shetty V, Ferrucci L, Gelber AC, et al. Arterial stiffness and hand osteoarthritis: a novel relationship? Osteoarthritis Cartilage. 2007 Mar;15(3):357–61.
41. Goldsmith GM, Aitken D, Cicuttini FM, Wluka AE, Winzenberg T, Ding CH, et al. Osteoarthritis bone marrow lesions at the knee and large artery characteristics. Osteoarthritis Cartilage. 2014 Jan;22(1):91–4.
42. Bierma-Zeinstra SMA, Waarsing JH. The role of atherosclerosis in osteoarthritis. Best Pract Res Clin Rheumatol. 2017;31(5):613–33.
43. Fernandes GS, Valdes AM. Cardiovascular disease and osteoarthritis: common pathways and patient outcomes. Eur J Clin Invest. 2015 Apr;45(4):405–14.

Part 2

Osteoarthritis disease burden

9

Health-related quality of life in hand osteoarthritis patients from the general population and the outpatient clinic

Marieke Loef, Wendy Damman, Renée de Mutsert, Saskia le Cessie,
Frits Rosendaal, Margreet Kloppenburg

The Journal of Rheumatology 2020;47(9):1409-1415

Abstract

Objective To investigate the association of hand osteoarthritis and concurrent hand and knee osteoarthritis with health-related quality of life (HRQoL) in the general population, and in patients consulting a rheumatology outpatient clinic.

Methods In the population-based NEO study, participants were recruited from the greater area of Leiden. In the HOSTAS study, patients with a rheumatologist's diagnosis of hand osteoarthritis were recruited from a Leiden-based hospital. In both cohorts, hand and knee osteoarthritis were defined by the ACR clinical criteria. In NEO, self-reported hospital-based specialist consultation for OA was recorded. Physical and mental HRQoL was assessed with normalised SF-36 scores. Associations were analysed using linear regression, adjusted for age, sex, education, ethnicity and BMI.

Results Hand osteoarthritis alone and concurrent hand and knee osteoarthritis was present in 8% and 4% of 6,334 NEO participants, and in 57% and 32% of 538 HOSTAS patients. In NEO, hand osteoarthritis alone, and with knee osteoarthritis was associated with lower physical component summary (PCS) scores (mean difference (95% CI)-2.4 (-3.6;-1.3)) and-7.7 (-9.3;-6.2), respectively) compared with no osteoarthritis. Consulting a specialist was associated with worse PCS scores. In the HOSTAS cohort, mean PCS scores were lower than norm values (-3.5 and-7.9 for hand osteoarthritis and combined osteoarthritis, respectively). Mental HRQoL was not clinically relevantly associated in either cohort.

Conclusion Hand osteoarthritis was associated with reduced physical, but not mental HRQoL in the general population and hospital patients. Physical HRQoL was further reduced in hospital care, and with concurrent knee osteoarthritis.

Introduction

The hand is one of the most frequently affected joint sites by osteoarthritis (OA), next to OA of the knee[1,2]. In addition, hand and knee OA frequently co-occur[3]. Hand osteoarthritis may impact the health-related quality of life, which may vary between patient populations. The impact of OA on health-related quality of life (HRQoL) is often studied in patients recruited from secondary or tertiary care. However, these patients may represent a distinct patient group that might differ from the general population. Differences between these populations might be present in symptom severity, disabilities or the co-occurrence of OA in other joints such as the knee. Studies that have investigated the impact of hand OA on physical HRQoL in the general population have shown no or very limited effect[3,4], which is in contrast to findings in patients recruited from the rheumatology clinic[5–7]. A similar difference may be present for the effect of hand OA on mental HRQoL[3–9]. While some studies have shown a high prevalence of mental disorders in hand OA patients[6,9], a recent systematic review did not support that depression and anxiety occurred more often in patients with OA than in individuals without OA[10]. This lends further support to the hypothesis that HRQoL might be affected differently in individuals with hand OA from the general population compared to patients from rheumatology clinics. However, due to a variety of OA definitions and phenotypes used by previous studies, a valid comparison of available findings is hindered, and a direct comparison of the impact of OA on HRQoL in the general population and in patients referred to secondary care is currently lacking.

In the current study, we had the unique opportunity to investigate individuals with hand OA from the general population and a rheumatology outpatient clinic in the same region. We investigated the impact of hand OA on physical and mental HRQoL in the general population, and subsequently compared the impact of hand OA between patients who have, and who have not been referred to a medical specialist. Furthermore, we investigated the added effect of concurrent knee OA on HRQoL.

Patients and methods

NEO study

Study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study, with an oversampling of individuals with overweight or obesity. Detailed description of study design has been described elsewhere[11]. In short, men and women between 45 and 65 years with a self-reported body mass index (BMI) ≥ 27 kg/m^2 living in the greater area of Leiden (The Netherlands) were eligible to participate. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population[12]. In total 6,671 participants were included. We excluded participants with inflammatory rheumatic disease (n=157) or fibromyalgia (n=178), or with missing physical examination (n=14). The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study (NL21981.058.08). All participants gave their written informed consent.

Clinical assessment
Measurement of height (cm) and weight (kg), allowed for calculation of BMI (kg/m^2). In addition, trained research nurses examined the hands and knees, using a standardized scoring form. Of both hands, bony and soft swellings and deformities of distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), carpometacarpal (CMC) and wrist joints were assessed. Regarding the knees, presence of bony swellings, palpable pain and warmth, crepitus and movement restriction were assessed. Hand and knee OA were defined according to the American College of Rheumatology (ACR) clinical classification criteria[13,14] and in patients with an prosthesis or arthrodesis.

Questionnaires
Questionnaires included demographic information, as well as presence of rheumatic diseases other than OA, and whether patients consulted a hospital-based medical specialist for OA (specification of OA type was not available). Education was reported in categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) vs low education (reference). The Australian/Canadian hand OA index (AUSCAN)[15] was used to determine self-reported hand pain and function. Higher scores indicate greater disease burden. Furthermore, the Medical Outcomes Study Short Form-36 (SF-36) was used to assess HRQoL[16]. We calculated separate subscale and summary component scores: physical health (PCS) and mental health (MCS), and standardized scores on a scale of 0 to 100. Age- and sex-specific Dutch population-based norm scores[17,18] were used to derive norm-based scores with a mean of 50 and a SD of 10. Higher SF-36 scores represent better quality of life.

HOSTAS study

Study population
The Hand OSTeoArthritis in Secondary care (HOSTAS) study included consecutive patients from the LUMC rheumatology outpatient clinic between June 2009 and October 2015, based on the rheumatologist's diagnosis of primary hand OA. The LUMC serves both as a secondary and tertiary referral centre for rheumatic diseases. Exclusion criteria included presence of other rheumatic diseases or secondary OA (including inflammatory joint diseases such as rheumatoid arthritis (RA), psoriatic arthritis, spondyloarthritis, and current sarcoidosis; bone diseases such as osteitis deformans and osteochondritis, intraarticular fractures; metabolic diseases associated with joint diseases such as hemochromatosis, Wilson's disease, and ochronosis; endocrine diseases such as acromegaly, major congenital or developmental diseases, bone dysplasias; and major local factors such as hypermobility and severe gout). The study was approved by the LUMC medical ethical committee (NL26201.058.08) and written informed consent was obtained from all participants.

Clinical assessment
Physical examination was performed by trained research nurses. BMI was calculated using measured weight and height (kg/m^2). Physical examination of hands and knee was performed similar as described in the NEO study; the ACR clinical classification criteria for hand and knee OA were applied to define clinical OA phenotypes[13,14]. Also, joints with a prosthesis were regarded as having end-stage OA.

Questionnaires

Demographic data were collected using standardized questionnaires. Education level was grouped into high vs low education, similar to the NEO study methods. The AUSCAN was used to determine hand OA specific disease burden. HRQoL was measured with the Dutch Research and Development translation (version 1) of the SF-36[17]. Similar to the NEO study, we used the scoring algorithm and age- and sex-specific Dutch population-based norm scores from the Dutch SF-36 translation to apply norm-based scoring[18] for the summary component scores and subscales with a mean of 50 and SD of 10.

Statistical analyses

In the NEO study there is an oversampling of individuals with BMI ≥27 kg/m^2. Adjustments were made for the oversampling by weighing all individuals towards the BMI distribution of participants from the Leiderdorp municipality (n=1,671)[19], with a BMI distribution similar to the general Dutch population[12]. All results presented are based on weighted analyses, using probability weights. Consequently, results from the NEO study apply to a population-based study without oversampling. Multivariable linear regression analysis, adjusting for age, sex, education, ethnicity and BMI, was used to study cross-sectional associations of hand and concurrent hand and knee OA with HRQoL in both study populations. We verified absence of multicollinearity, normality and homoskedasticity and using a correlation matrix, Quantile-Quantile plots and residual vs fitted plots, respectively. Data are presented as regression coefficients with 95% confidence intervals (CI).The mean differences in SF-36 scores were compared with the minimal clinically important difference of 2 points to evaluate clinical relevance[20]. All analyses were performed using STATA V14.1 (StataCorp LP, TX, USA).

Results

Study populations

The NEO study population consisted of 6,671 participants. After exclusion of participants with missing physical examination or presence of concomitant other rheumatic diseases, the study population consisted of 6,334 participants, with 55% women and a mean age of 56 years. Eight percent fulfilled only the ACR criteria for hand OA and an additional 4% of participants for both hand and knee OA (table 1). Compared with participants without hand and knee OA, participants with OA were more frequently women, older and less educated. The HOSTAS cohort consisted of 538 hand OA patients with 86% women and a mean age of 61 years. All patients from the HOSTAS cohort were diagnosed with hand OA by the rheumatologist. In 57% of patients only the ACR criteria for hand OA was fulfilled and 171 (32%) were classified with hand and knee OA. In 11% of patients assessment of the ACR clinical criteria was not possible or they did not fulfil the criteria, therefore these patients were not included in the current analyses.

Table 1. Demographics and hand OA specific disease burden

	NEO n = 6,334			HOSTAS n = 538*	
	No hand/knee OA	**Hand OA**	**Hand/knee OA**	**Hand OA**	**Hand/knee OA**
	78%	8%	4%	57%	32%
Demographic					
Age	55 (6)	58 (5)	58 (5)	61 (9)	62 (8)
Sex, % women	52	74	86	86	87
Height, cm	174 (10)	169 (9)	169 (7)	168 (8)	167 (9)
Weight, kg	79 (16)	77 (16)	78 (15)	75 (15)	76 (14)
BMI, kg/m^2	26 (4)	27 (5)	27 (5)	27 (5)	27 (5)
Education level, % high	48	40	36	36	31
Ethnicity, % white	95	94	92	98	96
AUSCAN#					
Total	0 (0-2)	7 (3-15)	13 (8-23)	20 (12-25)	20 (15-27)
Pain	0 (0-0)	3 (0-6)	6 (3-9)	9 (6-12)	10 (7-12)
Function	0 (0-2)	3 (1-10)	7 (3-14)	8 (4-12)	9 (6-12)

Results from the NEO study are based on weighted analyses of the study population. Numbers represent mean (SD) unless otherwise specified, #median (interquartile range). Higher AUSCAN scores reflect higher hand OA specific burden. *11% did not fulfill ACR criteria or were missing data. Abbreviations: OA = osteoarthritis, BMI = body mass index, AUSCAN = Australian/Canadian hand osteoarthritis index

The association of hand OA with health-related quality of life in the general population

The mean SF-36 scores in the NEO study are shown in figure 1. Table 2 shows the mean differences in participants with hand OA compared with participants without hand and knee OA. In participants with only hand OA the PCS was 2.4 points (-3.6;-1.3) lower than in participants without OA. The subscales bodily pain and physical functioning showed greatest differences of -3.4 (-4.6; -2.2) and -2.1 (-3.0; -1.1), while vitality was the least different in participants with hand OA compared to participants without hand OA. Mental HRQoL was not reduced in participants with hand OA compared with participants without OA. Relative to participants without OA, the PCS was-7.7 (-9.3;-6.2), and all physical subscales were reduced below the clinically relevant threshold in participants with concurrent hand and knee OA. The subscales mental health and social functioning were lower with mean differences of-1.7 (-3.1;-0.3) and-1.9 (-3.4;-0.5), respectively. However, these differences were smaller than the minimal clinically important difference of 2 points.

Comparison with hand OA patients referred to secondary care

Of all participants classified with hand OA in the general population, 14% reported to have visited a medical specialist for OA. The participants with hand OA who had not been referred to secondary care reported a median (IQR) AUSCAN total score of 7 (3-13), compared to 14 (4-27) in participants who visited a specialist. Comparison of the HRQoL in participants with hand OA who had been referred to the medical specialist with participants with hand OA who had not reported consulting secondary care for OA showed a lower physical HRQoL with a mean difference in the PCS of -3.9 (-6.7;-1.2). The subscales bodily pain and physical functioning showed the greatest mean differences of -4.9 (-7.6; -2.1) and -4.3 (-7.0; -1.7). In the group classified with concurrent hand and knee OA 38% reported to have visited a specialist for OA. In these participants the greatest difference with participants who had not consulted secondary care was seen in the subscale physical functioning. Mental HRQoL did not differ between participants with hand OA who had, and had not been referred to the medical specialist (table 3).

In the HOSTAS study, no reference group without OA was available. Comparison of the HRQoL in patients with only hand OA from the rheumatology outpatient clinic to the reference group without hand or knee OA in the NEO study showed a mean difference in the PCS of -7.8 (-8.8;-6.8). Similar to the comparisons within the NEO study population, the subscales bodily pain and physical functioning were lowered most (data not shown). The MCS did not differ between patients with hand OA from the HOSTAS study and the NEO study reference group. However, since the mean scores of the reference group in the NEO study were higher than the normative values we deemed this was an unsuitable reference group for the HOSTAS cohort. Therefore, table 4 shows the mean SF-36 scores of the HOSTAS patients compared with the normative value of 50. In patients with only hand OA, the PCS (-3.5), bodily pain (-4.9), vitality (-2.5) and role functioning – physical (-2.2) scales were clinically relevantly lower, but mental HRQOL was not associated with hand OA alone. In patients with concurrent hand and knee OA, all physical HRQoL scales were clinically relevantly lower, as well as the mental HRQoL scale social functioning, with a difference of-2.9.

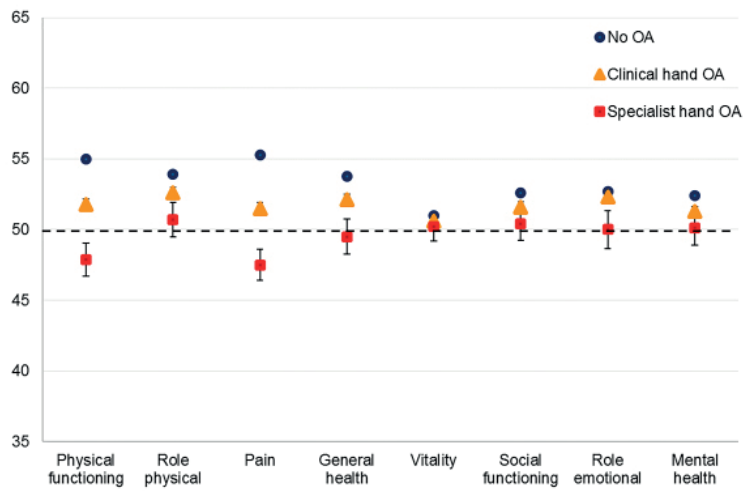

Figure 1. Health-related quality of life in individuals classified with hand OA in the general population.
Results from the NEO study are based on weighted analyses of the study population. The data points reflect mean scores of the SF-36 scales, stratified by hand OA group. SF-36 scores of 50 are the norm, higher/lower values indicate better/worse quality of life. The "No hand/knee OA" group are participants not fulfilling either the ACR criteria for hand or knee OA. The "clinical hand OA" group fulfilled only the ACR criteria for hand OA, and the "specialist hand OA group" is comprised of participant fulfilling the ACR criteria for hand OA, as well as reporting to have visited a hospital-based medical specialist for OA. Error bars represent SEM.

Table 2. Health-related quality of life in the general population

	NEO n = 6,334				
	No hand/knee OA 78%	**Hand OA** 8%	Mean difference (95% CI)	**Hand/knee OA** 4%	Mean difference (95% CI)
Physical component score	**55.1 (7.6)**	**52.0 (8.7)**	**-2.4 (-3.6; -1.3)**	**45.9 (9.7)**	**-7.7 (-9.3; -6.2)**
General health	53.8 (8.1)	52.1 (8.6)	-1.5 (-2.6;-0.3)	49.4 (9.6)	-3.4 (-4.9;-1.8)
Bodily pain	55.3 (8.4)	51.5 (8.9)	-3.4 (-4.6;-2.2)	46.3 (7.6)	-8.1 (-9.4;-6.8)
Vitality	51.0 (8.8)	50.6 (8.6)	-0.6 (-1.6; 0.5)	47.4 (9.3)	-3.2 (-4.8;-1.7)
Physical functioning	55.0 (7.0)	51.8 (8.0)	-2.1 (-3.0;-1.1)	45.3 (9.7)	-7.8 (-9.4;-6.2)
Role functioning- physical	53.9 (7.6)	52.6 (8.8)	-0.9 (-2.0; 0.2)	48.9 (10.5)	-4.1 (-5.8;-2.3)
Mental component score	**51.1 (8.8)**	**51.3 (8.9)**	**0.2 (-1.0; 1.3)**	**51.7 (9.4)**	**0.9 (-0.5; 2.3)**
Mental health	52.4 (7.8)	51.3 (7.8)	-0.8 (-1.7; 0.2)	49.9 (8.6)	-1.7 (-3.1;-0.3)
Social functioning	52.6 (8.0)	51.6 (8.4)	-0.6 (-1.7; 0.4)	49.7 (9.2)	-1.9 (-3.4;-0.5)
Role functioning- emotional	52.7 (8.0)	52.3 (8.6)	-0.2 (-1.3; 0.8)	51.8 (8.9)	-0.5 (-1.7; 0.8)

Results are based on weighted analyses of the study population. Higher SF-36 scores represent a better HRQoL. Numbers represent mean (SD). Results are adjusted for age, sex, BMI, education and ethnicity. Abbreviations: CI = confidence interval, OA = osteoarthritis

Table 3. HRQoL in participants who have, and have not been referred to the medical specialist.

	NEO n = 6,334					
	Hand OA spec$^-$ 6.6%	**Hand OA spec$^+$** 1.0%	Mean difference (95% CI)	**Hand/knee OA spec$^-$** 2.5%	**Hand/knee OA spec$^+$** 1.5%	Mean difference (95% CI)
Physical component score	**52.6 (8.4)**	**48.5 (9.4)**	**-3.9 (-6.7; -1.2)**	**48.5 (9.4)**	**41.7 (8.8)**	**-5.9 (-8.5; -3.4)**
General health	52.5 (8.3)	49.5 (10.0)	-2.6 (-6.2; 0.9)	50.7 (9.7)	47.2 (8.9)	-2.2 (-4.8; 0.5)
Bodily pain	52.2 (8.8)	47.5 (8.9)	-4.9 (-7.6;-2.1)	47.4 (8.1)	44.4 (6.3)	-2.6 (-4.6;-0.5)
Vitality	50.7 (8.7)	50.2 (8.1)	-0.6 (-3.2; 1.9)	47.8 (9.3)	46.8 (9.5)	-0.4 (-3.6; 2.7)
Physical functioning	52.5 (7.6)	47.9 (9.5)	-4.3 (-7.0;-1.7)	48.2 (9.1)	40.7 (9.1)	-7.1 (-9.8;-4.4)
Role functioning- physical	52.9 (8.6)	50.7 (9.9)	-2.1 (-5.0; 0.8)	50.8 (9.8)	45.8 (11.0)	-4.3 (-7.7;-0.8)
Mental component score	**51.3 (8.5**	**50.9 (11.3)**	**-0.6 (-5.0; 3.8)**	**50.9 (9.9)**	**53.0 (8.6)**	**2.0 (-0.7; 4.8)**
Mental health	51.5 (7.4)	50.1 (9.8)	-1.5 (-5.2; 2.1)	50.0 (9.2)	49.6 (7.5)	-0.3 (-2.8; 2.2)
Social functioning	51.8 (8.2)	50.4 (9.6)	-1.4 (-4.8; 2.0)	50.2 (9.4)	48.9 (8.9)	-0.8 (-3.5; 1.9)
Role functioning- emotional	52.6 (8.2)	50.0 (10.8)	-2.5 (-6.3; 1.3)	51.5 (9.2)	52.2 (8.5)	0.6 (-1.9; 3.1)

Results are based on weighted analyses of the study population. Higher SF-36 scores represent a better HRQoL. Numbers represent mean (SD). Results are adjusted for age, sex, BMI, education and ethnicity. Abbreviations: CI = confidence interval, OA = osteoarthritis, spec = referred to specialist

Table 4. HRQoL of patients with hand OA in the rheumatology clinic

	HOSTAS n = 538*			
	Hand OA 57%	**Δ**	**Hand/knee OA** 32%	**Δ**
Physical component score	**46.5 (8.1)**	**-3.5**	**42.1 (7.7)**	**-7.9**
General health	49.2 (6.3)	-0.8	46.5 (6.6)	-3.5
Bodily pain	45.1 (7.7)	-4.9	42.6 (6.7)	-7.4
Vitality	47.5 (8.8)	-2.5	46.0 (8.4)	-4.0
Physical functioning	48.8 (9.2)	-1.2	43.3 (9.3)	-6.7
Role functioning- physical	47.8 (10.2)	-2.2	44.5 (10.4)	-5.5
Mental component score	**51.7 (8.8)**	**1.7**	**51.2 (8.8)**	**1.2**
Mental health	51.0 (8.3)	1.0	49.4 (8.3)	-0.6
Social functioning	50.1 (9.1)	0.1	47.1 (9.5)	-2.9
Role functioning- emotional	51.1 (9.5)	1.1	49.6 (10.3)	-0.4

Higher SF-36 scores represent a better health-related quality of life. Results are adjusted for age, sex, BMI, education and ethnicity. In HOSTAS the delta (Δ) between population scores and norm scores were calculated. *11% did not fulfill ACR criteria or were missing data. Abbreviations: CI = confidence interval, OA = osteoarthritis

The added burden of concurrent knee OA

Concurrent hand and knee OA was associated with a lower physical HRQoL compared to only hand OA, with mean differences (95% CI) in the PCS of-5.3 (-7.2;-3.4) in the NEO cohort and -3.9 (-5.4;-2.4) in the HOSTAS cohort. Table 5 shows that the observed mean differences were above the minimal clinical important difference in both populations, indicating a clinically relevant lower HRQoL in patients with concurrent hand and knee OA compared to patients with only hand OA. Physical functioning showed the greatest mean differences in patients with additional knee OA, with mean differences of -5.7 (-7.5; -3.9) in the NEO cohort and -4.7 (-6.4; -3.0) in the HOSTAS cohort. No differences in mental HRQoL were observed in participants of the NEO study classified with concurrent hand and knee OA compared to participants with only hand OA. In the HOSTAS study social functioning was significantly and clinically relevantly lower in the presence of concurrent hand and knee OA compared to hand OA alone with a mean difference of-3.0 (-4.8;-1.2).

Table 5. The impact of concurrent knee OA on HRQoL compared to hand OA alone

	NEO Mean difference (95% CI)	**HOSTAS** Mean difference (95% CI)
Physical component score	**-5.3 (-7.2; -3.4)**	**-3.9 (-5.4; -2.4)**
General health	-1.9 (-3.8;-0.0)	-2.6 (-3.8;-1.3)
Bodily pain	-4.7 (-6.4;-3.1)	-2.2 (-3.6;-0.9)
Vitality	-2.7 (-4.4;-0.9)	-1.5 (-3.2; 0.1)
Physical functioning	-5.7 (-7.5;-3.9)	-4.7 (-6.4;-3.0)
Role functioning- physical	-3.2 (-5.2;-1.2)	-3.1 (-5.1;-1.1)
Mental component score	**0.7 (-1.0; 2.5)**	**-0.7 (-2.4; 1.0)**
Mental health	-0.9 (-2.5; 0.7)	-1.5 (-3.1; 0.1)
Social functioning	-1.3 (-3.0; 0.4)	-3.0 (-4.8;-1.2)
Role functioning- emotional	-0.2 (-1.8; 1.3)	-1.3 (-3.2; 0.6)

Results from the NEO study are based on weighted analyses of the study population. Higher SF-36 scores represent a better health-related quality of life. Results are adjusted for age, sex, BMI, education and ethnicity. CI = confidence interval, OA = osteoarthritis. Mean SF-36 scores for the OA phenotypes of NEO and HOSTAS can be found in table 2 and 4, respectively.

Discussion

In the current study, we investigated the association of hand OA with HRQoL in the general population and in patients with hand OA referred to the medical specialist. Furthermore, we investigated the association of concurrent knee OA with HRQoL, and compared this between the general population and patients from the rheumatology outpatient clinic. In participants with hand OA in the general population, physical HRQoL was modestly, but clinically relevantly lower than in participants without OA. Moreover, physical HRQoL was lower in patients with hand OA who had consulted a medical specialist. Mental HRQoL was not associated with hand OA alone, neither in the general population nor in patients in secondary care. In both patient groups we observed a lower physical HRQoL in patients with concurrent hand and knee OA, compared with patients with hand OA alone. Furthermore, concurrent hand and knee OA was weakly associated with mental HRQoL; however, besides the effect on social functioning in the HOSTAS cohort, the impact on mental HRQoL was below the minimal clinically important difference threshold in both populations.

Our findings disprove the misconception that hand OA in the general population has no relevant impact on HRQoL. Although hand OA was not significantly associated with a reduced physical HRQoL in a Spanish population-based cohort[3], they used the less extensive SF-12, which might explain the discordance with our findings. In line with our results, hand OA patients in the population-based MUST cohort experienced a reduction in general health[4]. However, in our cohort bodily pain and physical functioning was more strongly associated with hand OA. In the current study, we did not observe an association of hand OA with mental HRQoL. This is supported by other population-based studies[3,4]. Moreover, a systematic review also did not support that depression and anxiety occurred more often in OA patients compared to individuals without OA[10].

Furthermore, we investigated whether HRQoL was associated with hand OA in secondary care patients. We showed that within the population-based NEO study, participants with hand OA who consulted a hospital-based specialist had a lower physical HRQoL than participants classified with hand OA who had not been referred to the medical specialist. In addition, we found that patients with hand OA in the HOSTAS study also experienced a lower physical HRQoL. Previous research in another cohort from our outpatient clinic supports our findings[5], as well as results from a Norwegian study, that showed that hand OA patients from the rheumatology department had a lower physical HRQoL compared to healthy controls. In contrast to our findings, they also observed worse mental health in hand OA patients recruited from their specialized clinic[6]. We did not see an association with lower mental HRQoL in patients referred to the medical specialist in a population-based cohort, nor in patients recruited from the rheumatology outpatient clinic. A number of other studies are in line with our findings, showing no association of hand OA with mental HRQoL in patients from outpatient clinics[5,7].

The additional presence of knee OA was associated with an even lower physical HRQoL in hand OA patients from the general population, as well as in patients from the outpatient clinic. This is supported by previous studies, that all conclude that polyarticular OA has a greater influence on physical HRQoL compared to patients with only hand OA[3,4,6,7]. Furthermore, we observed that the additional presence of knee OA was also associated with a lower score on the social functioning subscale in patients from the rheumatology clinic.

Comparison of HRQoL with other study cohorts should be made with caution due to differences in patient selection, OA definitions, and reference groups. In addition, some studies lacked the use of norm-based scoring, further hampering the comparison. These obstacles highlight the importance of research that compares the general population with patients from specialized care. To our knowledge we are the first to make a comparison between patients from the general population and patients referred to secondary care. Since these cohorts were selected from the same area in the Netherlands, the NEO study population is likely a proper representative for the population of which the patients from our outpatient clinic are sampled. Furthermore, both our cohorts are of substantial size, resulting in well powered analyses and thus allowing robust conclusions.

However, our study also has some limitations. The reported HRQoL of the NEO study participants without OA was higher than the normative value of 50. This may indicate a healthy candidate bias, which is commonly seen in population-based studies. In addition, we cannot exclude that some NEO study participants whom reported to have consulted a hospital-based specialist for OA may have also been included in the HOSTAS study. Unfortunately, we were not able to asses if, or to what extent this may have happened. For this reason, we focussed on within-cohort differences, and in addition compared the mean scores from the HOSTAS cohort to the normative values. Therefore, we deem it unlikely this will have affected our conclusions. Furthermore, in the NEO study no distinction could be made in the type of OA that was the indication for specialist consultation, which may have led to misclassification. Also, the intra- and interobserver agreement for the scoring of OA signs on physical examination of the hands and knees was not assessed. However, since these scores were obtained by trained research nurses in a standardized way, we do not expect that this will have affected our results. Lastly, the cross-sectional study design does not allow exploration of how the effect of OA on HRQoL develops over time and hinders causal interpretations. Future research is needed to investigate the association between OA progression and the effect this may have on HRQoL.

In conclusion, hand OA is associated with a clinically relevant lower physical, but not mental HRQoL in both the general population as in patients referred to secondary care. In patients in secondary care HRQoL was lower compared to patients with hand OA from the general population. In addition, co-occurrence of knee OA was associated with an even lower physical HRQoL than hand OA alone. The burden of hand and knee OA on the quality of life in the general population as well as in hospital care should be carefully considered in the management of patient care.

References

1 Oliveria SA, Felson DT, Reed JI, Cirillo PA, Walker AM. Incidence of symptomatic hand, hip, and knee osteoarthritis among patients in a health maintenance organization. Arthritis Rheum 1995; 38: 1134–41.

2 van Saase JL, van Romunde LK, Cats A, Vandenbroucke JP, Valkenburg HA. Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheum Dis 1989; 48: 271–80.

3 Carmona L, Ballina J, Gabriel R, Laffon A, EPISER Study Group. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. Ann Rheum Dis 2001; 60: 1040–5.

4 Lombnæs GØ, Magnusson K, Østerås N, Nordsletten L, Risberg MA, Hagen KB. Distribution of osteoarthritis in a Norwegian population-based cohort: associations to risk factor profiles and health-related quality of life. Rheumatol Int 2017; 37: 1541–50.

5 Kwok WY, Vliet Vlieland TPM, Rosendaal FR, Huizinga TWJ, Kloppenburg M. Limitations in daily activities are the major determinant of reduced health-related quality of life in patients with hand osteoarthritis. Ann Rheum Dis 2011; 70: 334–6.

6 Slatkowsky-Christensen B, Mowinckel P, Loge JH, Kvien TK. Health-related quality of life in women with symptomatic hand osteoarthritis: a comparison with rheumatoid arthritis patients, healthy controls, and normative data. Arthritis Rheum 2007; 57: 1404–9.

7 Moe RH, Grotle M, Kjeken I, Hagen KB, Kvien TK, Uhlig T. Disease impact of hand OA compared with hip, knee and generalized disease in specialist rheumatology health care. Rheumatol Oxf Engl 2013; 52: 189–96.

8 Cuperus N, Vliet Vlieland TPM, Mahler EAM, Kersten CC, Hoogeboom TJ, van den Ende CHM. The clinical burden of generalized osteoarthritis represented by self-reported health-related quality of life and activity limitations: a cross-sectional study. Rheumatol Int 2015; 35: 871–7.

9 Axford J, Butt A, Heron C, et al. Prevalence of anxiety and depression in osteoarthritis: use of the Hospital Anxiety and Depression Scale as a screening tool. Clin Rheumatol 2010; 29: 1277–83.

10 Stubbs B, Aluko Y, Myint PK, Smith TO. Prevalence of depressive symptoms and anxiety in osteoarthritis: a systematic review and meta-analysis. Age Ageing 2016; 45: 228–35.

11 de Mutsert R, den Heijer M, Rabelink TJ, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol 2013; 28: 513–23.

12 Ministerie van VWS. Hoeveel mensen hebben overgewicht? www.rivm.nl/nldemaat; 2013.

13 Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29: 1039–49.

14 Altman R, Alarcón G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990; 33: 1601–10.

15 Bellamy N, Campbell J, Haraoui B, et al. Dimensionality and clinical importance of pain and disability in hand osteoarthritis: Development of the Australian/Canadian (AUSCAN) Osteoarthritis Hand Index. Osteoarthritis Cartilage 2002; 10: 855–62.

16 Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992; 30: 473–83.

17 Zee KI van der, Sanderman R. Het meten van de algemene gezondheidstoestand met de RAND-36: een handleiding. Groningen: Noordelijk Centrum voor Gezondheidsvraagstukken, Rijksuniversiteit Groningen, 1993.

18 Aaronson NK, Muller M, Cohen PD, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol 1998; 51: 1055–68.

19 Lumley, T. Analysis of compex survey samples. http:/www.jstatsoft.org/v09/i08/paper 2004.

20 Angst F, Aeschlimann A, Stucki G. Smallest detectable and minimal clinically important differences of rehabilitation intervention with their implications for required sample sizes using WOMAC and SF-36 quality of life measurement instruments in patients with osteoarthritis of the lower extremities. Arthritis Rheum 2001; 45: 384–91.

10

The association of clinical and structural knee osteoarthritis with physical activity in the middle-aged population: The NEO study

Sietse Terpstra, Jeroen van der Velde, Renee de Mutsert, Dieuwke Schiphof, Monique Reijnierse, Frits Rosendaal , Lotte van de Stadt, Margreet Kloppenburg, Marieke Loef

Osteoarthritis Cartilage. 2021;29(11):1507-1514

Abstract

Objective To investigate if knee osteoarthritis (OA) is associated with lower physical activity in the general middle-aged Dutch population, and if physical activity is associated with patient-reported outcomes in knee OA.

Design Clinical knee OA was defined in the Netherlands Epidemiology of Obesity population using the ACR criteria, and structural knee OA on MRI. We assessed knee pain and function with the Knee Injury and Osteoarthritis Score (KOOS), health-related quality of life (HRQoL) with the Short Form-36, and physical activity (in Metabolic Equivalent of Task (MET) hours) with the Short Questionnaire to Assess Health-enhancing physical activity. We analysed the associations of knee OA with physical activity, and of physical activity with knee pain, function, and HRQoL in knee OA with linear regression adjusted for potential confounders.

Results Clinical knee OA was present in 14% of 6,212 participants, (mean age 56 years, mean BMI 27 kg/m^2, 55% women, 24% having any comorbidity) and structural knee OA in 12%. Clinical knee OA was associated with 9.60 (95% CI 3.70;15.50) MET hours per week more physical activity, versus no clinical knee OA. Structural knee OA was associated with 3.97 (-7.82;15.76) MET hours per week more physical activity, versus no structural knee OA. In clinical knee OA, physical activity was not associated with knee pain, function or HRQoL.

Conclusions Knee OA was not associated with lower physical activity, and in knee OA physical activity was not associated with patient-reported outcomes. Future research should indicate the optimal treatment advice regarding physical activity for individual knee OA patients.

Introduction

Rheumatic musculoskeletal disorders (RMDs) are among the leading causes of disability in the middle-aged population. One of the most common RMDs is osteoarthritis (OA), which affected approximately 300 million people globally in 2017, and its prevalence is expected to keep rising (1).

Currently, no disease-modifying treatment is available for OA, which often leads to chronic use of analgesics to suppress symptoms, until eventually joint replacement surgery is performed in end-stage disease (2). As pain, reduced quality of life and functional complaints are among the most prevalent knee OA symptoms and can impede physical activity (1), insight in lifestyle factors that reduce pain, increase functional performance and perhaps even slow down progression is highly warranted. Physical activity is such a modifiable lifestyle factor that has shown to be associated with better disease outcomes. In elderly individuals with knee OA, lack of physical activity has been shown to be associated with depressive symptoms, poorer functional performance, cardiovascular events, and increased mortality (3-6). This underscores the vital need for estimates of the level of physical activity in individuals with knee OA, and of its impact on quality of life and patient-reported outcomes.

Previous studies have reported low adherence to physical activity guidelines in individuals with knee OA (7-9), which might be caused by pain and psychological distress caused by OA (8). Furthermore, physical activity has shown to be an effective intervention for weight loss, which in turn diminishes the risk and complaints of knee OA (9). However, some studies found no clear difference in physical activity between individuals with and without knee OA (10-13). Most available studies concerning physical activity in individuals with knee OA studied relatively old populations in countries where a sedentary lifestyle is common (14). Large-scale measurement of physical activity across general worldwide populations revealed differences in physical activity between countries (15-16). This might be caused by cultural and lifestyle differences. For example, in some countries such as the Netherlands, Denmark and Germany, walking and cycling are regularly used as ways of transportation, instead of transport by car (17).

Furthermore, data on individuals having early stages of knee OA were not found, while lifestyle interventions in knee OA preferably take place in an early disease stage (18). The median age of knee OA diagnosis was 55 years of age in the general population of the United States of America (19). Because of this, information on physical activity status in the middle-aged OA population is warranted. This is vital in order to assess the potential of physical activity as a target for intervention in early stages of knee OA.

Therefore, we investigated if knee OA is associated with lower physical activity compared with no knee OA in the general middle-aged Dutch population. Furthermore, as lack of physical activity has shown to be associated with wide-ranging adverse health outcomes (3-6), we investigated the association of physical activity with patient-reported outcomes such as knee pain, function and health-related quality of life (HRQoL) in individuals with knee OA.

Materials and methods

Study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study, with an oversampling of overweight or obese individuals. Detailed study design and data collection have been described elsewhere (20). In short, men and women between 45 and 65 years with a self-reported body mass index (BMI) ≥ 27 kg/m^2 living in the greater area of Leiden (the Netherlands) were eligible to participate. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population (21). The collection of data started in September 2008 and was completed at the end of September 2012. In total, 6,671 participants were included in the NEO study. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent.

Questionnaires

Participants completed a general questionnaire to report demographic, lifestyle and clinical information, including a medical history on inflammatory rheumatic diseases, fibromyalgia and general comorbid diseases. We investigated the following general comorbid diseases: cardiovascular disease, chronic pulmonary disease, liver disease, diabetes, kidney disease and cancer.

Physical activity was measured with the validated Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) (22), which includes questions on activities regarding a normal week in recent months. The SQUASH consists of three main queries: days per week, average time per day, and intensity. Items were converted to age-specific metabolic equivalent tasks (METs), derived from Ainsworths's compendium of physical activity (23), in hours per week based on reported frequency and duration of the activities (24). SQUASH items were combined to calculate a total physical activity level in MET hours per week. In addition, we combined the SQUASH items sports, walking, gardening, cycling and household activities in order to assess the category of "leisure time" physical activity in concordance with the SQUASH guideline.

Knee specific symptoms were measured with the Knee Injury and Osteoarthritis Outcome Score (KOOS) (25, 26). The KOOS consists of five subscales: pain, symptoms, function in activities of daily living (ADL), sport and recreation function and knee-related quality of life. All patients scored the KOOS for their right knee considering the previous week. Items were scored from 0 (no problems) to 4 (extreme problems) on a 5-point Likert scale. Subscale scores were calculated according to the KOOS user's guide (27) as the sum of the items included, and subsequently transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems. A KOOS subscale score was considered valid when at least 50% of the items were completed. If more than 50% of data from a subscale was missing, the participant was excluded from analyses of that subscale (27). In the current analyses we included the KOOS subscales pain and ADL function.

HRQoL was measured with the Short Form (SF)-36 (28). The physical health summary component score (PCS) and mental health summary component score (MCS) were calculated. Age- and sex-specific Dutch population-based norm scores (29) were used to derive norm-based scores with a mean of 50 and a SD of 10. Higher SF-36 scores represent better quality of life.

Accelerometer

Physical activity was measured for the duration of four consecutive days by an accelerometer in a random subset (n = 955) of the study population. An accelerometer was combined with 2 ECG electrodes (ActiHeart, CamNtech Ltd, UK), which was placed on the chest of the participants at the level of the third intercostal space. This combined heart rate monitor and accelerometer simultaneously measures heart rate and uniaxial (vertical when standing up) acceleration of the torso. Using a branched equation algorithm the acceleration and heart rate information was translated into calibrated estimates of physical activity energy expenditure (30, 31). Participants with a valid wear time <24 hours were excluded from the analyses. To allow comparison with the SQUASH, we converted the data from the accelerometer (kJ/kg/day) to MET hours per week by dividing by 4.2 and subsequently multiplying by 7 (27).

Clinical assessment

Body mass index (BMI) was calculated from measured body weight and height (kg/m^2). In addition, extensive physical examination of the knees was performed by trained research nurses, with a standardized scoring form. The presence of bony swellings, palpable pain and warmth, crepitus and movement restriction were assessed.

Clinical and structural knee OA definitions

We used self-reported knee pain on most days of the last week, in combination with the physical assessment of the knee, to define a clinical knee OA phenotype according to the American College of Rheumatology (ACR) clinical classification criteria (32).

Structural knee OA in the right knee was defined on magnetic resonance imaging (MRI), in a random sample of 1,285 participants without contra-indications (most notably metallic devices, claustrophobia or a body circumference of more than 1.70 m). Imaging was performed on a MR system operating at a 1.5T field strength (Philips, Medical Systems, Best, the Netherlands), using a dedicated knee coil and a standardized scanning protocol as described earlier (33). All MRI images were analysed using the validated semi-quantitative knee OA scoring system (KOSS) (34) as described previously (33). Structural knee OA was defined according to the modified criteria by Hunter et al. (35), when a definite osteophyte and full thickness cartilage loss was present, or one of these features with at least two of the following: subchondral bone marrow lesions, cyst, meniscal subluxation, maceration or degenerative tear, or partial thickness cartilage loss.

Statistical analyses

For individuals from the city of Leiden and its surroundings (n=4541), oversampling was done of individuals with BMI ≥ 27 kg/m2. In order to correctly represent associations in the general Dutch population, individuals from the general population from Leiderdorp without any oversampling were included (n=1671), as the BMI distribution of this municipality is representative for the general Dutch population (21). Due to weighting of the BMI of our study to the general Leiderdorp population, our results will apply to a Dutch population-based study without oversampling of participants with BMI ≥27 kg/m2 (36).

We performed a cross-sectional analysis of baseline measurements. We excluded participants who reported to have inflammatory rheumatic disease or fibromyalgia, participants with missing physical examination of the knees, or missing physical activity data. Population characteristics were summarized as mean (standard deviation (SD)), median (25th, 75th percentiles) or as percentages.

Linear regression analyses were performed to investigate the association of clinical and structural knee OA (independent variables) with physical activity (dependent variable, MET hours per week), compared with respectively no clinical and no structural knee OA. Additionally, in order to assess the effect of the individual potential confounding factors on the association, we added these factors to the model in a stepwise manner. In the subgroup of participants with clinical knee OA, linear regression analyses were performed for each patient-reported outcome measure to investigate the association between physical activity (independent variable) and knee specific outcomes measured by the KOOS, and the PCS and MCS of the SF-36 (dependent variables). In order to account for possible biases commonly observed in self-reported physical activity measures (for example social desirability bias) (37), we performed a sensitivity analysis to assess the association between clinical knee OA and physical activity measured by an accelerometer. All analyses were adjusted for age, sex, BMI, education, ethnicity and comorbidities, as these factors were assessed as likely associated with both knee OA and physical activity based on previous literature (38, 39). Stata V14.1 (StataCorp LP, TX, USA) was used for all analyses.

Results

Study population

The NEO study population consisted of 6,671 participants. After exclusion of participants with concomitant other rheumatic diseases (n = 323), with missing physical examination (n = 14), or missing SQUASH data (n = 120) the study population for the current analyses consisted of 6,212 participants (figure 1). The percentage missing of all included variables can be found in supplementary table S1. Demographic characteristics of the population stratified by clinical knee OA classification status are shown in table 1.

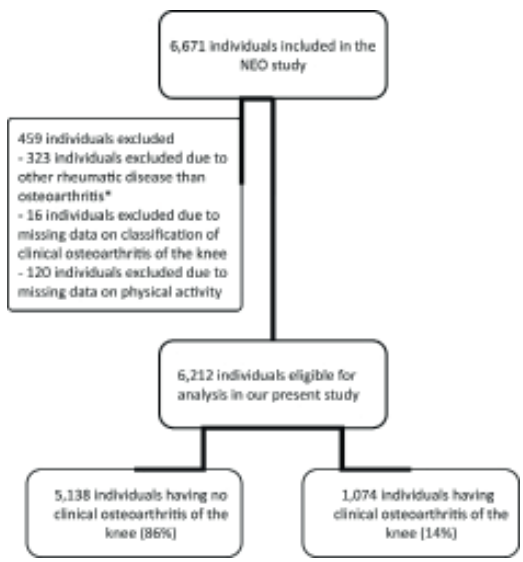

Figure 1. Flowchart of the individuals included in the present study
*Rheumatic diseases excluded were: rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, psoriatic arthritis and fibromyalgia. OA = osteoarthritis, n = number

Table 1. Characteristics of the weighted NEO study population

	All n = 6,212	No clinical knee OA 86% (n = 5.138)	Clinical knee OA 14% (n = 1.074)
General patient characteristics			
Age (year)	55.7 (6.0)	55.4 (6.1)	57.5 (5.0)
Sex (% women)	55	54	67
BMI (kg/m^2)	26.3 (4.4)	26.1 (4.3)	27.6 (5.1)
Education (% high)	46	48	38
Ethnicity (% white)	95	95	94
Comorbidities (% present)	24	23	32
Patient reported outcomeS			
KOOS pain^	100 (94;100)	100 (97;100)	83 (64;94)
KOOS ADL function^	100 (96;100)	100 (99;100)	88 (70;97)
SF-36 physical component scale	53.8 (8.4)	54.8 (7.8)	47.7 (9.5)
SF-36 mental component scale	51.2 (8.9)	51.1 (8.8)	51.6 (9.6)
Physical activity			
Total^ (MET hours per week)	118.8 (76.8;155.0)	118.4 (76.6;154.4)	123.5 (77.8;157.2)
Leisure time^ (MET hours per week)	30.0 (15.8;49.5)	29.0 (15.5;49.0)	33.2 (18.5;50.8)

Results are based on analyses weighted towards the BMI distribution of the general population. KOOS scale range 0-100, higher scores are better. SF-36 norm-based scores with mean of 50, SD of 0, higher scores are better. Numbers represent mean (SD) unless otherwise specified. ^median (25th, 75th percentiles). Abbreviations: ADL = activities of daily living, BMI = body mass index, KOOS = Knee Injury and Osteoarthritis Outcome Score, n = number, OA = osteoarthritis, SF = short form, MET = metabolic equivalent of task

Clinical knee OA was present in 14% of participants. Compared with participants without clinical knee OA, those with clinical knee OA were slightly older (mean (SD) 57.5 (5.0) vs 55.4 (6.1) years), were more often female (67% vs 54%), had a somewhat higher BMI (27.6 (5.1) vs 26.1 (4.3) kg/m^2), and were less often highly educated (38% vs 48%). Participants having clinical knee OA also had a worse KOOS pain (median (IQR) 83 (64;94) vs 100 (97;100)) and ADL function (88 (70;97) vs 100 (99;100)), as well as physical HRQoL (mean (SD) 47.7 (9.5) vs 54.8 (7.8)) compared with participants without clinical knee OA (table 1). The random subsample having knee MRI (n=1.205) was comparable to the total study population in terms of age, sex, BMI and patient-reported outcome measures. Structural knee OA was present in 12% of participants who underwent MRI of the knee. Of the participants having structural knee OA, 15% was also classified with clinical knee OA.

Association of knee OA with self-reported physical activity

Clinical knee OA was positively associated with physical activity, having a crude effect of on average 4.77 (-1.22; 10.76) more MET hours. However, structural knee OA was negatively associated with physical activity, having a crude effect of -2.95 (-15.15; 9.24) MET hours compared with no structural knee OA. Adjusted for age, sex, BMI, education, ethnicity and comorbidities, there was a positive association between physical activity and clinical knee OA (table 2). In comparison with participants without clinical knee OA, those with clinical knee OA had on average 9.60 (95% CI 3.70;15.50) MET hours per week more total physical activity. There was a weak positive association between physical activity and structural knee OA, as participants with structural knee OA had on average 3.97 (-7.82; 15.76) MET hours per week more total physical activity than participants without structural knee OA. The stepwise addition of individual potential confounders to the regression model is shown in supplementary figure S1 and S2. The association of knee OA with leisure time physical activity was less evident. However, the association suggested that participants with knee OA were more physically active than participants without knee OA during leisure time (table 2).

Association of self-reported physical activity with patient reported outcomes

In the subpopulation of individuals with knee OA, physical activity was not associated with knee pain, function or HRQoL (table 3).

Table 2. Association of clinical and structural knee OA with physical activity

	Clinical knee OA (n = 1.074 (14%)) **Beta (95% CI)†**		Structural knee OA (n = 163 (12%)) **Beta (95% CI)†**	
	Crude	Adjusted*	Crude	Adjusted*
Total	4.77 (-1.22; 10.76)	9.60 (3.70; 15.50)	-2.95 (-15.15; 9.24)	3.97 (-7.82; 15.76)
Leisure time	3.38 (-0.12; 6.88)	2.55 (-1.21; 6.32)	3.65 (-2.96; 10.25)	3.45 (-3.09; 9.98)

Results are based on analyses weighted towards the BMI distribution of the general population. †The beta can be interpreted as the mean difference in MET hours physical activity between individuals with, and without knee OA. *Analyses were adjusted for age, sex, BMI, education, ethnicity and comorbidities. Abbreviations: CI= confidence interval, MET = metabolic equivalent of task, OA = osteoarthritis

Table 3. Association of physical activity with disease burden in participants with clinical knee OA

	Physical activity	
	Total Beta (95% CI)†	**Leisure** Beta (95% CI)†
KOOS pain	-0.0041 (-0.026; 0.018)	-0.0073 (-0.048; 0.034)
KOOS ADL function	-0.0073 (-0.029; 0.014)	-0.0059 (-0.043; 0.031)
SF-36 PCS	-0.0012 (-0.017; 0.014)	0.010 (-0.018; 0.038)
SF-36 MCS	-0.0032 (-0.015; 0.0088)	-0.015 (-0.042; 0.012)

Results are based on analyses weighted towards the BMI distribution of the general population. † The beta can be interpreted as the mean difference in outcome score per MET hour of physical activity. Analyses were adjusted for age, sex, BMI, education, ethnicity and comorbidities. KOOS scale range 0-100, higher scores are better. SF-36 norm-based scores with mean of 50, SD of 0, higher scores are better. Abbreviations: ADL = activities of daily living, CI= confidence interval, KOOS = Knee Injury and Osteoarthritis Outcome Score, MCS = mental component score, MET = metabolic equivalent of task, n = number, OA = osteoarthritis, PCS = physical component scale, SF = short form

Sensitivity analysis

To account for possible information bias commonly associated with self-reported physical activity measures, we additionally investigated the association between clinical knee OA and physical activity measured by an accelerometer. Of the 955 participants having an accelerometer, 831 were eligible for inclusion in our sensitivity analysis (figure 2). In this analysis, we observed 2.78 (-5.73; 11.29) MET hours per week more physical activity in participants with clinical knee OA, than in individuals without clinical knee OA. Correlation between physical activity measured by the SQUASH questionnaire, and physical activity measured by an accelerometer was low (r = 0.239).

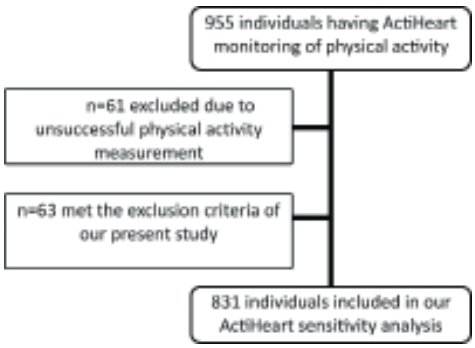

Figure 2. Flowchart of the individuals included in the ActiHeart sensitivity analyses

Discussion

We aimed to assess the potential of physical activity as a target for lifestyle intervention in middle-aged individuals. Therefore, this study investigated if knee OA was associated with lower physical activity compared with no knee OA in a large middle-aged Dutch population, and investigated the association of physical activity with patient reported outcomes in individuals with clinical knee OA.
We did not find an association of knee OA with lower physical activity compared with no knee OA, as we observed that clinical knee OA was associated with somewhat higher physical activity compared with no clinical knee OA. Also, structural knee OA was not associated with lower physical activity, compared with no structural knee OA.

Our study did not show less physical activity in individuals with knee OA compared with those without knee OA, which was in line with most comparable studies. One of these studies investigated a group of 4,125 participants aged above 50 years from the Netherlands (10), using the SQUASH questionnaire. No major differences were found in the amount of physical activity between individuals having knee OA and individuals not having knee OA, being respectively 15.3, 12.3, 18.1 and 17.8 hours per week for individuals in primary care, secondary care, post total-joint arthroplasty care, and the general population. A second study investigated 2,551 individuals aged 65-85 from Sweden, the United Kingdom, Germany, Italy, Spain and the Netherlands (11). The study recorded physical activity using an accelerometer (Activity Monitor) as well as the self-reported Longitudinal Aging Study Amsterdam (LASA) physical activity questionnaire. In individuals having knee OA, on average 18.6 minutes per day less physical activity was found compared with individuals having no knee OA. This finding is in contrast with our study. However, in the Swedish and Dutch subpopulations no lower physical activity was found in individuals having knee OA compared with individuals having no knee OA. This lack of difference is in line with our findings in our Dutch population. A study from the United States of America compared individuals 491 individuals aged 50-79 with symptomatic knee OA with 449 individuals without symptomatic knee OA using an accelerometer (12). Levels of physical activity were found to be similarly low in both groups. Time of moderate to vigorous physical activity was found to be comparable between individuals with symptomatic knee OA and the general population without symptomatic knee

OA. Another study from the United States of America compared the physical activity status of 486 individuals having symptomatic knee OA with a control group of 1455 individuals having no symptomatic knee OA (13). Physical activity was measured using an accelerometer. The odds of walking at least 10 minutes per day were found to be similar for individuals with symptomatic knee OA relative to the general population.

We found 9.60 (3.70; 15.50) more MET hours per week of physical activity in individuals with clinical knee OA, compared with individuals with no clinical knee OA. This is approximately equivalent to walking 30 minutes per day for most days of the week, or performing one hour per week of vigorous sports (20). The finding contrasts with previous studies, which might be due to several reasons. First of all, these previous study populations consisted of mostly elderly individuals (10-13), as opposed to our middle-aged study population. Since ageing has been associated with decreased physical activity (40), it is likely that our relatively young study population is more active in general than these older study populations. This is underscored by a relatively strong positive effect of adjustment for age in our study on the association between knee OA and physical activity (supplementary figure S 1.1, S1.2). Related to this, it is also likely that our study population consisted of individuals with knee OA in an early disease stage, as knee OA is a progressive disease (41). Probably, middle-aged individuals are not restricted in their activities, or even well-motivated to address their complaints by a targeted increase in physical activity or by physical therapy. Indeed, the encouragement to be physically active is usually part of the treatment of early-stage knee OA in the Netherlands (42). Another factor that might explain this contrast we found, is a difference in the method of physical activity measurement. For example, one study used a different physical activity questionnaire than our study (LASA) (11). Also, three out of four studies solely investigated physical activity measured by an accelerometer, and used a different accelerometer than our study (10, 12, 13).

Our usage of an accelerometer in our sensitivity analysis solves the problem of social desirability bias. This analysis showed an association in line with the aforementioned association between structural knee OA and self-reported physical activity. The association found by sensitivity analysis is also in line with the association between clinical knee OA and physical activity mentioned previously, although somewhat weaker. Therefore, all adjusted analyses of knee OA suggest a positive association of knee OA with physical activity.

As a second aim, this study aimed to investigate the association of physical activity with patient reported outcomes in individuals with clinical knee OA. We did not observe any association between physical activity and knee pain, knee function or HRQoL in these individuals. Our lack of association between physical activity and knee pain is in line with a recent cross-sectional Korean study including 9,196 participants with structural knee OA (43). This study did not find an association between physical activity levels and knee pain status. The lack of association between physical activity and physical functioning we found contrasts with a systematic review comprising eight cross-sectional studies (44). The review investigated factors associated with a low level of physical activity in patients with OA of the hip or knee. Studies of relatively young as well as relatively old individuals were included. The review concluded that lower physical function is associated with lower physical activity for individuals having knee OA. However, limited evidence was found for this conclusion. One Turkish study cross-sectionally investigated the association between physical activity and HRQoL in 55 individuals over 65 years of age having structural knee OA (45). Mean physical functioning

score of the SF-36 scale was found to be higher in the physically active group than in the physically inactive group (42.0 vs. 33.7), which is in contrast with the lack of association we found. The mental component score however was comparable between physically active and physically inactive individuals (48.6 vs. 47.7), which is in line with our results. The difference in physical component score between this study and our study could be due to the older age or smaller sample size in the Turkish study, or due to cultural differences (15).

Our study has several strengths. One of the key strengths of our study is the large study population. Other strengths are the facts that we included both clinical and structural knee OA in our analyses, and performed a sensitivity analysis. In addition, we investigated patient-reported outcome measures. Moreover, the middle-aged population we investigated likely gives a reflection of an early disease stage in which interventions should be started when indicated.
Notably, there are also some limitations to our study. We used a cross-sectional design, which hinders causal inference and leaves the potential of reverse causation. Currently, 10-year follow-up measurements are being performed in the NEO study population, which will give us more insight in the causality of our findings. Another limitation of our study is the subjective nature of the physical activity data we used. Self-reported physical activity is likely to be less accurate than objectively measured activity as it is for example prone to forms of response bias, such as social desirability bias (37). This might cause overestimation of the physical activity reported. The possibility of this bias is supported by our sensitivity analysis, which showed that the association of knee OA and physical activity was somewhat weaker using objectively measured physical activity instead of self-reported physical activity, although it points in the same positive direction. It is further supported by the weak but positive correlation between self-reported physical activity and physical activity measured by an accelerometer. Another limitation of our study is that MRI of the knee was solely performed in the right knee due to logistical reasons. This means that patients having solely structural left knee OA were missed, leading to underestimation of the presence of structural knee OA in our cohort. However, previous literature has shown that structural knee OA is mostly bilateral, and that the right and left knee have a comparable disease course (46, 47).

In conclusion, knee OA was not associated with lower physical activity in this middle-aged Dutch population. Future research should indicate what the optimal treatment advice is regarding physical activity for individual knee OA patients, and we should not discourage individuals with knee OA to be physically active.

References

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Lond Engl. 2018 10;392(10159):1789–858.
2. Kloppenburg M, Berenbaum F. Osteoarthritis year in review 2019: epidemiology and therapy. Osteoarthritis Cartilage. 2020;28(3):242–8.
3. Kinikli GI, Kilinc H, Callaghan MJ, Atilla B, Tokgozoglu AM. Can depression, functional performance and kinesiophobia predict lower physical activity levels in patients with knee osteoarthritis? Osteoarthritis Cartilage. 2018 Apr 1;26:S241–2.
4. Hawker GA, Croxford R, Bierman AS, Harvey PJ, Ravi B, Stanaitis I, et al. All-cause mortality and serious cardiovascular events in people with hip and knee osteoarthritis: a population based cohort study. PloS One. 2014;9(3):e91286.
5. Kendzerska T, Jüni P, King LK, Croxford R, Stanaitis I, Hawker GA. The longitudinal relationship between hand, hip and knee osteoarthritis and cardiovascular events: a population-based cohort study. Osteoarthritis Cartilage. 2017;25(11):1771–80.
6. Corsi M, Alvarez C, Callahan LF, Cleveland RJ, Golightly YM, Jordan JM, et al. Contributions of symptomatic osteoarthritis and physical function to incident cardiovascular disease. BMC Musculoskelet Disord. 2018 Nov 10;19(1):393.
7. Dunlop DD, Song J, Semanik PA, Chang RW, Sharma L, Bathon JM, et al. Objective physical activity measurement in the osteoarthritis initiative: Are guidelines being met? Arthritis Rheum. 2011 Nov;63(11):3372–82.
8. Murphy LB, Hootman JM, Boring MA, Carlson SA, Qin J, Barbour KE, et al. Leisure Time Physical Activity Among U.S. Adults With Arthritis, 2008-2015. Am J Prev Med. 2017 Sep;53(3):345–54.
9. Zheng, H., & Chen, C. (2015). Body mass index and risk of knee osteoarthritis: systematic review and meta-analysis of prospective studies. BMJ Open, 5(12), e007568. doi: 10.1136/bmjopen-2014-007568
10. Pelle T, Claassen AAOM, Meessen JMTA, Peter WF, Vlieland TPMV, Bevers K, et al. Comparison of physical activity among different subsets of patients with knee or hip osteoarthritis and the general population. Rheumatology International. 2020;40(3):383–92.
11. Herbolsheimer F, Schaap LA, Edwards MH, Maggi S, Otero Á, Timmermans EJ, et al. Physical Activity Patterns Among Older Adults with and Without Knee Osteoarthritis in Six European Countries. Arthritis Care Res. 2016;68(2):228–36.
12. Thoma, L. M., Dunlop, D., Song, J., Lee, J., Tudor-Locke, C., Aguiar, E. J., Master, H., Christiansen, M. B., & White, D. K. (2018). Are Older Adults With Symptomatic Knee Osteoarthritis Less Active Than the General Population? Analysis From the Osteoarthritis Initiative and the National Health and Nutrition Examination Survey. Arthritis care & research, 70(10), 1448–1454. https://doi.org/10.1002/acr.23511
13. Thoma L, Tudor-Locke C, Aguiar E, Master H, Christiansen M, White D. Comparison of Objectively Measured Physical Activity Among People with Symptomatic Knee Osteoarthritis with the General US Population [abstract]. Arthritis Rheumatol. 2016; 68 (suppl 10).
14. Wallis JA, Webster KE, Levinger P, Taylor NF. What proportion of people with hip and knee osteoarthritis meet physical activity guidelines? A systematic review and meta-analysis. Osteoarthritis Cartilage. 2013 Nov;21(11):1648–59.
15. Althoff T, Sosič R, Hicks JL, King AC, Delp SL, Leskovec J. Large-scale physical activity data reveal worldwide activity inequality. Nature. 2017 Jul 20;547(7663):336-339. doi: 10.1038/nature23018. Epub 2017 Jul 10. PMID: 28693034; PMCID: PMC5774986.
16. Gelius P, Tcymbal A, Abu-Omar K, Mendes R, Tribuzi Morais S, Whiting S, Breda J. Status and contents of physical activity recommendations in European Union countries: a systematic comparative analysis. BMJ Open. 2020 Feb 20;10(2):e034045. doi: 10.1136/bmjopen-2019-034045. PMID: 32086356; PMCID: PMC7044960.
17. Physical activity and health in Europe – Evidence for action. [cited 25 January 2021]. Available from: https://www.euro.who.int/__data/assets/pdf_file/0011/87545/E89490.pdf
18. Chen W-H, Tsai W-C, Wang H-T, Wang C-H, Tseng Y-T. Can early rehabilitation after osteoarthritis reduce knee and hip arthroplasty risk?: A national representative cohort study. Medicine (Baltimore). 2019 May;98(21):e15723.
19. Losina E, Weinstein AM, Reichmann WM, et al. Lifetime risk and age at diagnosis of symptomatic knee osteoarthritis in the US. Arthritis Care Res (Hoboken). 2013;65(5):703-711. doi:10.1002/acr.21898
20. de Mutsert R, den Heijer M, Rabelink TJ, Smit JWA, Romijn JA, Jukema JW, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol. 2013 Jun;28(6):513–23.
21. Ministerie van VWS. Hoeveel mensen hebben overgewicht? (Dutch) [cited 3 November 2020]. Available from: www.rivm.nl/nldemaat. 2013.
22. Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003 Dec;56(12):1163–9.
23. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR, Montoye HJ, Sallis JF, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc. 1993 Jan;25(1):71–80.
24. Roos EM, Roos HP, Lohmander LS, Ekdahl C,

Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. J Orthop Sports Phys Ther. 1998 Aug;28(2):88–96.
25. de Groot IB, Favejee MM, Reijman M, Verhaar JAN, Terwee CB. The Dutch version of the Knee Injury and Osteoarthritis Outcome Score: a validation study. Health Qual Life Outcomes. 2008 Feb 26;6:16.
26. The 2012 User's Guide to Knee injury and Osteoarthritis Outcome Score KOOS. [cited 3 November 2020]. Available from: www.koos.nu. 2012.
27. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992 Jun;30(6):473–83.
28. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol. 1998;51:1055–68.
29. Brage S, Brage N, Franks PW, Ekelund U, Wong M-Y, Andersen LB, et al. Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. J Appl Physiol Bethesda Md 1985. 2004 Jan;96(1):343–51.
30. Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. Eur J Clin Nutr. 2005 Apr;59(4):561–70.
31. Unit Conversions- Compendium of Physical Activities [Internet]. Sites.google.com. [cited 2 November 2020]. Available from: https://sites.google.com/site/compendiumofphysicalactivities/help/unit-conversions. 2020;
32. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986 Aug;29(8):1039–49.
33. Visser AW, de Mutsert R, Loef M, le Cessie S, den Heijer M, Bloem JL, et al. The role of fat mass and skeletal muscle mass in knee osteoarthritis is different for men and women: the NEO study. Osteoarthritis Cartilage. 2014 Feb;22(2):197–202.
34. Kornaat PR, Ceulemans RYT, Kroon HM, Riyazi N, Kloppenburg M, Carter WO, et al. MRI assessment of knee osteoarthritis: Knee Osteoarthritis Scoring System (KOSS)--inter-observer and intra-observer reproducibility of a compartment-based scoring system. Skeletal Radiol. 2005 Feb;34(2):95–102.
35. Hunter DJ, Arden N, Conaghan PG, Eckstein F, Gold G, Grainger A, et al. Definition of osteoarthritis on MRI: results of a Delphi exercise. Osteoarthritis Cartilage. 2011 Aug;19(8):963–9.
36. Lumley, T. Analysis of compex survey samples. [cited 3 November 2020]. Available from: http:/www.jstatsoft.org/v09/i08/paper. 2004;
37. Furnham A. Response Bias, Social Desirability And Dissimulation". Personality And Individual Differences, vol 7, no. 3, 1986, pp. 385-400. Elsevier BV, doi:10.1016/0191-8869(86)90014-0.
38. Iqbal MN, Haidri FR, Motiani B, Mannan A. Frequency of factors associated with knee osteoarthritis. J Pak Med Assoc. 2011 Aug;61(8):786-9. PMID: 22356003
39. Veenhof C, Huisman PA, Barten JA, Takken T, Pisters MF. Factors associated with physical activity in patients with osteoarthritis of the hip or knee: a systematic review. Osteoarthritis Cartilage. 2012 Jan;20(1):6-12. doi: 10.1016/j.joca.2011.10.006. Epub 2011 Oct 19. PMID: 22044842.
40. Chmelo E, Nicklas B, Davis C, Miller GD, Legault C, Messier S. Physical activity and physical function in older adults with knee osteoarthritis. J Phys Act Health. 2013;10(6):777-783. doi:10.1123/jpah.10.6.777
41. Santos Castañeda J, Roman-Blas R, Largo R, Gabriel Herrero-Beaumont G, Osteoarthritis: a progressive disease with changing phenotypes, Rheumatology, Volume 53, Issue 1, January 2014, Pages 1–3, https://doi.org/10.1093/rheumatology/ket247
42. Richtlijnen.nhg.org. 2020. Niet-Traumatische Knieklachten. [cited 27 November 2020] Available from: https://richtlijnen.nhg.org/standaarden/niet-traumatische-knieklachten. 2020;
43. Shim H-Y, Park M, Kim H-J, Kyung H-S, Shin J-Y. Physical activity status by pain severity in patients with knee osteoarthritis: a nationwide study in Korea. BMC Musculoskelet Disord. 2018 Oct 20;19(1):380.
44. Veenhof C, Huisman PA, Barten JA, Takken T, Pisters MF. Factors associated with physical activity in patients with osteoarthritis of the hip or knee: a systematic review. Osteoarthritis Cartilage. 2012 Jan;20(1):6–12.
45. Mesci E, Icagasioglu A, Mesci N, Turgut ST. Relation of physical activity level with quality of life, sleep and depression in patients with knee osteoarthritis. North Clin Istanb. 2015;2(3):215.
46. Metcalfe, A.J., Andersson, M.L., Goodfellow, R. et al. Is knee osteoarthritis a symmetrical disease? Analysis of a 12 year prospective cohort study. BMC Musculoskelet Disord 13, 153 (2012). https://doi.org/10.1186/1471-2474-13-153
47. Ho-Pham, L. T., Lai, T. Q., Mai, L. D., Doan, M. C., Pham, H. N., & Nguyen, T. V. (2014). Prevalence of Radiographic Osteoarthritis of the Knee and Its Relationship to Self-Reported Pain. PLoS ONE, 9(4). https://doi.org/10.1371/journal.pone.0094563

11

Percentile curves for the Knee injury and Osteoarthritis Outcome Score in the middle-aged Dutch population

Marieke Loef, Féline Kroon, Stephan Böhringer, Ewa Roos, Frits Rosendaal, Margreet Kloppenburg

Osteoarthritis and Cartilage 2020;28:1046-54

Abstract

Objective To improve the interpretation of the Knee injury and Osteoarthritis Outcome Score (KOOS) in individual patients, we explored associations with age, sex, BMI, history of knee injury and presence of clinical knee osteoarthritis, and developed percentile curves.

Methods We used cross-sectional data of middle-aged individuals from the population-based Netherlands Epidemiology of Obesity (NEO) study. Clinical knee osteoarthritis was defined using the ACR classification criteria. KOOS scores were handled according to the manual (zero= extreme problems, 100= no problems). Patient characteristics associated with KOOS were explored using ordered logistic regression, and sex and body mass index (BMI)-specific percentile curves were developed using quantile regression with fractional polynomials. The curves were applied as a benchmark for comparison of KOOS scores of participants with knee osteoarthritis and comorbidities.

Results The population consisted of 6,643 participants (56% women, mean (SD) age 56(6) years). Population-based KOOS subscale scores (median;IQR) near optimum: pain (100;94-100), symptoms (96;86-100), ADL function (100;96-100), sport/ recreation function (100;80-100), quality of life (100;75-100). Worse KOOS scores were observed in women and in participants with higher BMI. Clinical knee osteoarthritis was defined in 15% of participants, and was, in comparison to other patient characteristics, associated with the highest odds of worse KOOS scores. Furthermore, presence of any comorbidity and cardiovascular disease specifically, was associated with worse KOOS scores, particularly in women.

Conclusions In the middle-aged Dutch population KOOS scores were generally good, but worse in women and with higher BMI. These percentile curves may be used as benchmarks in research and clinical practice.

Introduction

Knee complaints, such as pain and functional disability, are among the most reported complaints of the musculoskeletal system in the general practitioner's office[1]. The prevalence of knee complaints in the Dutch population is estimated to be 32.1 per 1000 persons per year. Besides injury, knee osteoarthritis (OA) is an important cause of knee complaints, especially in the elderly. Knee OA is one of the most common chronic joint disorders, with a prevalence in the Dutch general practice of 37.9 per 1000 patient years; occurring more often in women and increasing with age[2].

To assess the patient's burden due to knee complaints the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire was developed[3], which evaluates short-term and long-term knee symptoms, function and quality of life (QOL)[4]. KOOS is a widely used patient-reported outcome measure, which underwent extensive metric testing and is considered valid, reliable and responsive across groups with knee injury and knee OA[5]. The interpretation of the KOOS depends on relevant benchmarks. This is illustrated by previous studies on different knee-specific questionnaires in the general population, that show that a suboptimal score may be unrelated to musculoskeletal pathology[6–8]. Previous studies have developed reference values in population-based samples[9–11], but these studies have important limitations. Either the study populations were small, or only age-specific mean and median scores were reported[9], or they did not take into account the effect of body mass index (BMI)[9,11]. Importantly, none of these studies explored how knee OA or other relevant knee-related factors or comorbidities affect KOOS scores.

Therefore, we aimed to develop percentile curves in a large population-based cohort of Dutch middle-aged individuals. We explored possible association of factors such as age, sex, BMI, history of knee injury, presence of clinical knee OA and comorbidities with KOOS scores. Furthermore, we illustrate the use of the percentile curves as a benchmark for comparison of KOOS scores of individual patients and specific patient groups.

Methods

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study. Detailed description of study design and data collection has been provided elsewhere[12]. Briefly, men and women between 45 and 65 years with a self-reported BMI ≥ 27 kg/m^2 living in the greater area of Leiden (The Netherlands) were eligible to participate. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population[13]. In total, 6,671 participants were included in the NEO study. The present study is a cross-sectional analysis of baseline measurements. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study and all participants gave written informed consent. We excluded participants with missing physical examination (n = 14) or missing all KOOS subscales (n = 14).

Questionnaires

Prior to the study visit, participants completed questionnaires on demographic and clinical information; including self-reported presence of inflammatory rheumatic disease, history

of leg fractures and knee surgery, and presence of comorbidities (cardiovascular disease, liver disease, diabetes, renal disease, cancer and chronic pulmonary disease). In addition, participants completed the KOOS[3,14]. The KOOS consists of five subscales: pain (nine items), symptoms (seven items), function in activities of daily living (ADL) (17 items), sport and recreation function (five items) and knee-related QOL (four items). All patients scored the KOOS for their right knee and items were scored considering the previous week from 0 (no problems) to 4 (extreme problems), on a 5-point Likert scale. Subscale scores were calculated according to the KOOS user's guide[15] as the sum of the items included, and subsequently transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems. A KOOS subscale score was considered valid when at least 50% of the items were completed. If more than 50% of data from a subscale was missing, the participant was excluded from analyses of that subscale[15]. The symptom scale was missing in 0.6% of participants, the pain scale in 0.8%, the ADL scale in 0.6%, the sport and recreational function scale in 2.1% and the quality-of-life scale was missing in 0.8% of participants.

Clinical assessment

BMI was calculated from measured body weight and height (kg/m^2). Physical examination of the knees was performed by trained research nurses, using a standardized scoring form. Of both knees, presence of bony swellings, palpable pain and warmth, crepitus and movement restriction were assessed. Clinical knee OA was defined according to the American College of Rheumatology (ACR) classification criteria[16].

Statistical analysis

In the NEO study participants were recruited in two phases. At first participants with a BMI ≥ 27 kg/m^2 were oversampled. Secondly, a reference population was recruited with a BMI distribution similar to the Dutch general population. In this study we aimed to make inferences on the associations in the general population, and the over-representation of overweight and obese participants may induce bias due to the skewed BMI distribution. To represent distributions and associations in the general population correctly, adjustment for this oversampling was made by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality (n = 1,671)[17], whose BMI distribution was similar to the general Dutch population[13]. All results were based on weighted analyses, using the Stata command *pweight,* that denotes the inverse of the probability that the participant is included because of the sampling design. Consequently, results can be interpreted as corresponding to a population-based study without oversampling. Ordinal logistic regression analyses were performed to explore determinants associated with worse KOOS subscale scores, stratified by sex. KOOS scores were categorized into three categories with cut-offs (provided in supplementary file A) chosen such that the first category contains participants with a maximum score (no complaints), and the two remaining categories were approximately equal in size. Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CI), representing the OR of being in the lowest compared with the middle or highest KOOS category for a unit change in the determinant. Age and BMI were used as continuous variables, and standardized to a mean of zero and standard deviation (SD) of one prior to the analysis. Because the proportional odds assumption could not formally be tested in combination with the weight factor, we performed a multinomial logistic regression analyses as a sensitivity analysis (supplementary table A3). We explored which of the general patient characteristics influenced KOOS scores most to aid decisions about relevant subgroups for development

of the KOOS percentile curves. Subsequently, we developed sex and BMI specific percentile curves for all KOOS subscales, to facilitate the interpretation of KOOS scores in patients of a particular sex and BMI. For development of the curves, BMI was included as a continuous variable. Participants with a BMI below the 1st or above the 99th percentile were excluded due to a low number of observations leading to unreliable estimations at those points. We used quantile regression with fractional polynomials[18] to derive the percentile curves as this method is suited for data that do not meet the usual regression assumptions of normality, linearity, and constant variance[19–21]. The 50th, 25th, 10th, 5th and 2.5th centiles were estimated. Powers for the fractional polynomial models were taken from a predefined set (S= {-2, -1, -0.5, 0, 0.5, 1, 2, 3}). More complicated functions were only accepted if they resulted in a substantially improved fit, aiming to improve the feasibility in practical use of the percentile curves. Goodness of fit of the curve was inspected visually. The 95% CIs of the curves are provided in supplementary file C. Subsequently, KOOS scores of participants with clinical knee OA, and with comorbidities were compared to the percentile curves developed in the whole population. Lastly, we investigated whether there are specific items from each KOOS subscale that drive a low score. Stata V14.1 (StataCorp LP, TX, USA) was used for all analyses.

Results

Patient characteristics

After exclusion of participants with missing physical examination (n = 14) or missing all KOOS subscales (n = 14), the study population consisted of 6,643 participants with a mean (SD) age of 56 (6) years and a mean BMI of 26 (5) kg/m^2. About half of the population consisted of men (44%). As shown in table 1, general patient characteristics varied only slightly between sexes. Clinical knee OA was more common in women (18.3%) than in men (10.4%), while men more often had a history of knee surgery (20.8% in men vs. 13.8% in women) and a history of leg fractures (9.3% in men vs. 6.2% in women). The number and frequency of any comorbidity was equal between the sexes, while cardiovascular disease occurred more often in men (7.6%) compared to women (4.1%). KOOS subscale scores (median; IQR) were high: pain (100; 94-100), symptoms (96; 86-100), ADL function (100; 96-100), sport and recreation function (100; 80-100), QOL (100; 75-100).

Patient characteristics associated with worse KOOS scores

Female sex was associated with an increased odds of being in a worse KOOS score category (compared to no complaints) on all subscales, with odds ratios (95% CI) ranging from 1.39 (1.22; 1.58) for the symptoms scale, to 1.63 (1.41; 1.88) for the pain subscale. Therefore, further analyses were stratified by sex. BMI was also associated with worse KOOS scores, with ORs of 1.08 (0.97; 1.21) in men and 1.46 (1.32; 1.61) in women on the KOOS subscale pain for each standard deviation increase in BMI. For each standard deviation increase in age, we observed ORs of 0.86 (0.77; 0.97) in men and 1.01 (0.90; 1.12) in women on the subscale pain.

Table 1. Characteristics of the weighted study population (n = 6,643)

	Men		Women	
	44%		56%	
General patient characteristics				
Age, year	56 (6)		55 (6)	
Ethnicity, % Caucasian	95		95	
Education, % high	48		44	
BMI, kg/m^2	26.9 (3.7)		25.9 (4.9)	
Clinical knee OA, %	10.4		18.3	
Inflammatory rheumatic disease, %	4.5		3.7	
History of knee surgery, %	20.8		13.8	
Knee prosthesis for OA, %	0.2		0.3	
Knee prosthesis other, %	0.4		1.3	
Arthroscopy, %	10.2		8.1	
Meniscus operation, %	11.8		7.6	
Knee surgery other, %	3.6		2.8	
History of leg fracture, %	9.3		6.2	
Any comorbidities, %	24.6		25.2	
Cardiovascular disease, %	7.7		4.2	
KOOS subscales				
Pain	95 (12)	100 (97-100)†	92 (15)	100 (92-100)†
Symptoms	92 (12)	100 (89-100)†	90 (14)	96 (86-100)†
ADL function	96 (11)	100 (97-100)†	93 (14)	100 (93-100)†
Sport and recreation function	88 (22)	100 (85-100)†	82 (28)	100 (75-100)†
Quality of life	88 (18)	100 (75-100)†	84 (21)	94 (75-100)†

Results are based on analyses weighted towards the BMI distribution of the general population (n = 6,643). Numbers represent mean (SD) unless otherwise specified, † = median (IQR). KOOS sub scores are transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems.

Table 2 shows that among the patient characteristics that were investigated, clinical knee OA was associated with the highest odds of worse KOOS scores in all subscales. The largest ORs were found for the subscale pain in men 13.79 (9.61; 19.79) and for the subscale QOL in women 9.45 (7.06; 12.65). The symptom subscale was least affected by clinical knee OA (4.84 (3.48; 6.74) in men and 5.31 (4.05; 6.95) in women). Also, inflammatory rheumatic diseases were positively associated with worse KOOS scores. In men the associations attenuated in the multivariable analyses, in women the OR varied between 2.07 (1.05; 4.11) for QoL and 2.85 (1.52; 5.33) for ADL function. A history of knee surgery was associated with approximately two to four times higher odds of worse KOOS scores compare to no history of knee surgery. A history of leg fractures was mostly associated with worse ADL (1.60 (1.03; 2.46)) and sport and recreation scores (1.66 (1.13; 2.46)) in women. Furthermore, each additional comorbidity increased the odds of worse KOOS scores, which was most evident for the sport and recreation scale with an OR of 1.31 (1.09; 1.57) in men, and for the ADL function subscale with an OR of 1.34 (1.13; 1.59) in women.

Table 2. Factors associated with Knee injury and Osteoarthritis Outcome Score, stratified by sex

	Univariable OR (95% CI)		Multivariable OR (95% CI)	
	Men	**Women**	**Men**	**Women**
Pain				
Age	0.95 (0.86; 1.06)	1.20 (1.09; 1.32)	0.86 (0.77; 0.97)	1.01 (0.90; 1.12)
BMI	1.22 (1.10; 1.35)	1.65 (1.51; 1.79)	1.08 (0.97; 1.21)	1.46 (1.32; 1.61)
Education, high vs other	0.77 (0.62; 0.96)	0.69 (0.57; 0.83)	0.93 (0.73; 1.19)	0.87 (0.69; 1.08)
Clinical knee osteoarthritis	18.10 (12.81; 25.58)	11.13 (8.56; 14.47)	13.79 (9.61; 19.79)	8.51 (6.49; 11.17)
History of knee surgery	4.33 (3.33; 5.62)	3.97 (3.04; 5.19)	3.18 (2.37; 4.25)	2.67 (1.95; 3.66)
History of leg fracture	1.09 (0.78; 1.53)	1.34 (0.93; 1.95)	0.93 (0.64; 1.36)	1.33 (0.86; 2.06)
Inflammatory rheumatic disease	1.53 (1.07; 2.18)	2.69 (1.53; 4.71)	1.17 (0.77; 1.79)	2.27 (1.24; 4.13)
Number of comorbidities	1.34 (1.14; 1.57)	1.49 (1.28; 1.74)	1.26 (1.05; 1.50)	1.20 (1.01; 1.43)
Symptoms				
Age	0.83 (0.75; 0.91)	1.05 (0.96; 1.15)	0.78 (0.71; 0.86)	0.90 (0.81; 0.99)
BMI	1.29 (1.19; 1.41)	1.57 (1.44; 1.70)	1.19 (1.08; 1.31)	1.37 (1.25; 1.51)
Education, high vs other	0.67 (0.56; 0.81)	0.67 (0.55; 0.80)	0.73 (0.60; 0.90)	0.76 (0.62; 0.94)
Clinical knee osteoarthritis	6.27 (4.54; 8.65)	6.45 (4.99; 8.33)	4.84 (3.48; 6.74)	5.31 (4.05; 6.95)
History of knee surgery	2.80 (2.22; 3.55)	3.09 (2.29; 4.17)	2.16 (1.68; 2.79)	2.16 (1.56; 2.99)
History of leg fracture	0.97 (0.72; 1.31)	1.12 (0.76; 1.64)	0.87 (0.63; 1.20)	1.07 (0.71; 1.62)
Inflammatory rheumatic disease	1.49 (1.01; 2.18)	2.51 (1.51; 4.15)	1.29 (0.83; 1.99)	2.18 (1.26; 3.77)
Number of comorbidities	1.16 (0.99; 1.37)	1.33 (1.14; 1.55)	1.11 (0.93; 1.32)	1.10 (0.93; 1.29)
ADL function				
Age	1.10 (0.99; 1.22)	1.25 (1.14; 1.38)	1.05 (0.94; 1.18)	1.05 (0.94; 1.17)
BMI	1.34 (1.21; 1.47)	1.84 (1.68; 2.01)	1.23 (1.11; 1.36)	1.63 (1.48; 1.81)
Education, high vs other	0.63 (0.50; 0.78)	0.61 (0.51; 0.74)	0.74 (0.59; 0.94)	0.77 (0.62; 0.96)
Clinical knee osteoarthritis	11.72 (8.38; 16.38)	11.08 (8.48; 14.47)	8.35 (5.83; 11.96)	8.53 (6.45; 11.28)
History of knee surgery	3.77 (2.94; 4.84)	3.65 (2.81; 4.73)	2.69 (2.03; 3.57)	2.49 (1.83; 3.40)
History of leg fracture	1.37 (0.97; 1.92)	1.50 (1.04; 2.17)	1.30 (0.89; 1.91)	1.60 (1.03; 2.46)
Inflammatory rheumatic disease	1.81 (1.23; 2.66)	3.01 (1.79; 5.07)	1.32 (0.87; 2.00)	2.82 (1.56; 5.12)
Number of comorbidities	1.40 (1.18; 1.67)	1.66 (1.43; 1.92)	1.20 (0.99; 1.46)	1.34 (1.13; 1.59)
Sport and recreation function				
Age	0.95 (0.86; 1.05)	1.27 (1.16; 1.40)	0.86 (0.77; 0.97)	1.08 (0.97; 1.20)
BMI	1.24 (1.13; 1.36)	1.80 (1.65; 1.96)	1.11 (1.01; 1.23)	1.62 (1.47; 1.79)
Education, high vs other	0.73 (0.60; 0.90)	0.73 (0.60; 0.88)	0.86 (0.69; 1.08)	0.96 (0.78; 1.19)
Clinical knee osteoarthritis	11.93 (8.66; 16.45)	11.44 (8.61; 15.22)	8.83 (6.33; 12.31)	8.33 (6.14; 11.30)
History of knee surgery	4.36 (3.34; 5.69)	4.86 (3.61; 6.53)	3.30 (2.48; 4.39)	3.54 (2.56; 4.90)
History of leg fracture	1.28 (0.92; 1.78)	1.60 (1.12; 2.29)	1.22 (0.85; 1.74)	1.66 (1.13; 2.46)
Inflammatory rheumatic disease	1.69 (1.12; 2.55)	3.37 (2.06; 5.53)	1.36 (0.88; 2.12)	2.85 (1.52; 5.33)
Number of comorbidities	1.39 (1.17; 1.64)	1.60 (1.36; 1.87)	1.31 (1.09; 1.57)	1.28 (1.07; 1.52)
Quality of life				
Age	0.92 (0.83; 1.02)	1.15 (1.05; 1.26)	0.85 (0.76; 0.95)	0.93 (0.84; 1.04)
BMI	1.25 (1.14; 1.37	1.61 (1.47; 1.75)	1.15 (1.04; 1.27)	1.40 (1.27; 1.55)
Education, high vs other	0.85 (0.69; 1.04)	0.72 (0.59; 0.86)	1.05 (0.84; 1.31)	0.87 (0.71; 1.08)
Clinical knee osteoarthritis	14.24 (10.04; 20.20)	12.15 (9.22; 16.00)	10.73 (7.48; 15.38)	9.45 (7.06; 12.65)
History of knee surgery	4.44 (3.40; 5.80)	5.21 (3.92; 6.92)	3.39 (2.54; 4.51)	3.92 (2.85; 5.40)
History of leg fracture	1.21 (0.89; 1.65)	1.19 (0.80; 1.77)	1.10 (0.79; 1.52)	1.11 (0.74; 1.66)
Inflammatory rheumatic disease	1.58 (1.13; 2.21)	2.56 (1.45; 4.50)	1.30 (0.85; 1.98)	2.07 (1.05; 4.11)
Number of comorbidities	1.23 (1.04; 1.44)	1.55 (1.33; 1.80)	1.13 (0.95; 1.34)	1.32 (1.12; 1.57)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 6,643). Age and BMI were standardized (mean 0, SD 1), leading to odds per SD increase of the variable. Inflammatory rheumatic disease: rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis or spondyloarthritis. Comorbidities: cardiovascular disease, liver disease, diabetes, renal disease, cancer and chronic pulmonary disease. Abbreviations: CI= confidence interval, KOOS= Knee injury and Osteoarthritis Outcome Score, OR= odds ratio, SD = standard deviation.

Percentile values

The observed KOOS subscale scores for the 50th, 25th, 10th, 5th and 2.5th percentile are presented in table 3. In figure 1 the KOOS percentile curves were plotted for the five KOOS subscales. The curves were derived using first-degree polynomials, as the fit of the curves did not markedly improve using higher degree fractional polynomials. Since the 50th percentile curves of the subscales sport and recreation function and quality of life in men were constant at the maximum value of 100, these were fitted with linear functions. Evident from table 3 and from figure 1 is that KOOS scores were worse in women than men and that KOOS scores were worse with higher BMI in all KOOS subscales in both men and women. Intercepts, regression coefficients and fractional polynomial powers are provided in supplementary file B, along with an example calculation.

Table 3. Observed sex and BMI specific percentile values of the Knee injury and Osteoarthritis Outcome Score

		Percentiles									
		Men					**Women**				
KOOS subscale	**BMI**	**50th**	**25th**	**10th**	**5th**	**2.5th**	**50th**	**25th**	**10th**	**5th**	**2.5th**
Pain											
	≤25	100.0	97.2	86.1	75.0	66.7	100.0	97.2	86.1	75.0	55.6
	>25- ≤30	100.0	97.2	80.6	69.4	58.3	100.0	88.9	66.7	55.6	44.4
	>30	100.0	91.7	71.4	52.8	41.7	97.2	77.8	55.6	38.9	30.6
Symptoms											
	≤25	100.0	92.9	82.1	71.4	64.3	96.4	89.3	75.0	71.4	60.7
	>25- ≤30	96.4	85.7	71.4	67.9	57.1	92.9	82.1	67.9	60.7	50.0
	>30	96.4	78.6	71.4	57.1	46.4	89.3	75.0	57.1	46.4	39.3
ADL function											
	≤25	100.0	98.5	89.7	76.5	70.6	100.0	98.5	88.2	79.4	66.2
	>25- ≤30	100.0	97.1	83.8	70.6	61.8	100.0	89.7	69.1	54.4	45.6
	>30	100.0	92.6	73.5	55.9	43.8	96.3	76.5	52.9	41.2	32.3
Sport and recreation											
	≤25	100.0	90.0	65.0	45.0	25.0	100.0	85.0	55.0	30.0	20.0
	>25- ≤30	100.0	85.0	55.0	35.0	25.0	95.0	65.0	30.0	15.0	5.0
	>30	100.0	75.0	35.0	15.0	5.0	80.0	35.0	10.0	0.0	0.0
Quality of life											
	≤25	100.0	83.3	62.5	56.3	43.8	100.0	75.0	62.5	50.0	37.5
	>25- ≤30	100.0	75.0	62.5	43.8	37.5	87.5	68.8	50.0	37.5	31.3
	>30	100.0	75.0	50.0	37.5	25.0	81.3	56.3	37.5	31.3	18.8

Results are based on analyses weighted towards the BMI distribution of the general population (n = 6,643). Abbreviations: ADL= activities of daily living, BMI= body mass index, KOOS= Knee injury and Osteoarthritis Outcome Score

Use of the percentile curves in practice: an example

Patient X consults her orthopaedic surgeon with longstanding knee complaints to see whether there is an indication for a total knee replacement. She is obese, with a BMI of 33 kg/m^2. Patient X completes the KOOS questionnaire. She suffers from pain in her knee on a daily basis, and experiences severe pain when she goes up and down stairs and when pivoting on her knee. She reports moderate pain in her knee when bending her knee fully and when walking on a flat surface, or when she has to stand for prolonged periods of time. She has mild pain when sitting. Her responses add up to a KOOS pain subscale score of 50. To get a better grasp of what a pain score of 50 means in comparison to the general population, the score was plotted on the percentile curves (see figure 1). This showed that the pain score of this particular patient is below the 10th percentile, indicating that less than 10% of the general population has a pain score this severe.

KOOS scores in specific population groups

In figure 2A, the KOOS pain subscale scores of participants with clinical knee OA were plotted over de percentile curves, which demonstrates that the KOOS scores in these participants were lower than in the reference population. The median KOOS subscale pain score in participants with clinical knee OA lay between the 25th and 5th percentile in men and between the 50th and 10th percentile in women.

In women with comorbidities, median KOOS pain scores were between the 50th and 25th percentile, and worse scores were observed in individuals with a higher BMI (figure 2B). In contrast, in men with any comorbidity or cardiovascular disease, median KOOS pain scores were at the 50th percentile, with exception of men with an extremely high BMI (above 37 kg/m2), who had worse KOOS pain scores.

Items driving low KOOS subscale scores

We investigated which items were most often reported to be at least mildly affected in patients in the worst KOOS subscale score category (category cut-offs can be found in supplementary file A) and drove worse KOOS subscale scores. In participants in the worst category of the KOOS pain subscale scores, 94% of participants reported a higher frequency of knee pain and the item “going up or down stairs” was scored positive in 91% of participants of the worst score category. Most frequent reported symptoms were feeling grinding or hearing a clicking noise when the knee moves (65%), and restrictions in movement, in particular inability to fully bend the knee (65%). A low score on the ADL function scale resulted mostly from difficulties with heavy domestic duties, which was scored positive in 92% of participants in the worst category of ADL function scores, followed by getting in and out of a car (89%) rising from sitting (88%) and ascending stairs (87%). In patients in the category with the worst sport and recreation function subscale scores all items were relevant (90-96% reported at least mild difficulty). Similarly, in patients within the worst QOL subscale scores, at least mild difficulty was reported for all items with high frequency (87-99%). Results were similar between men and women (data not shown).

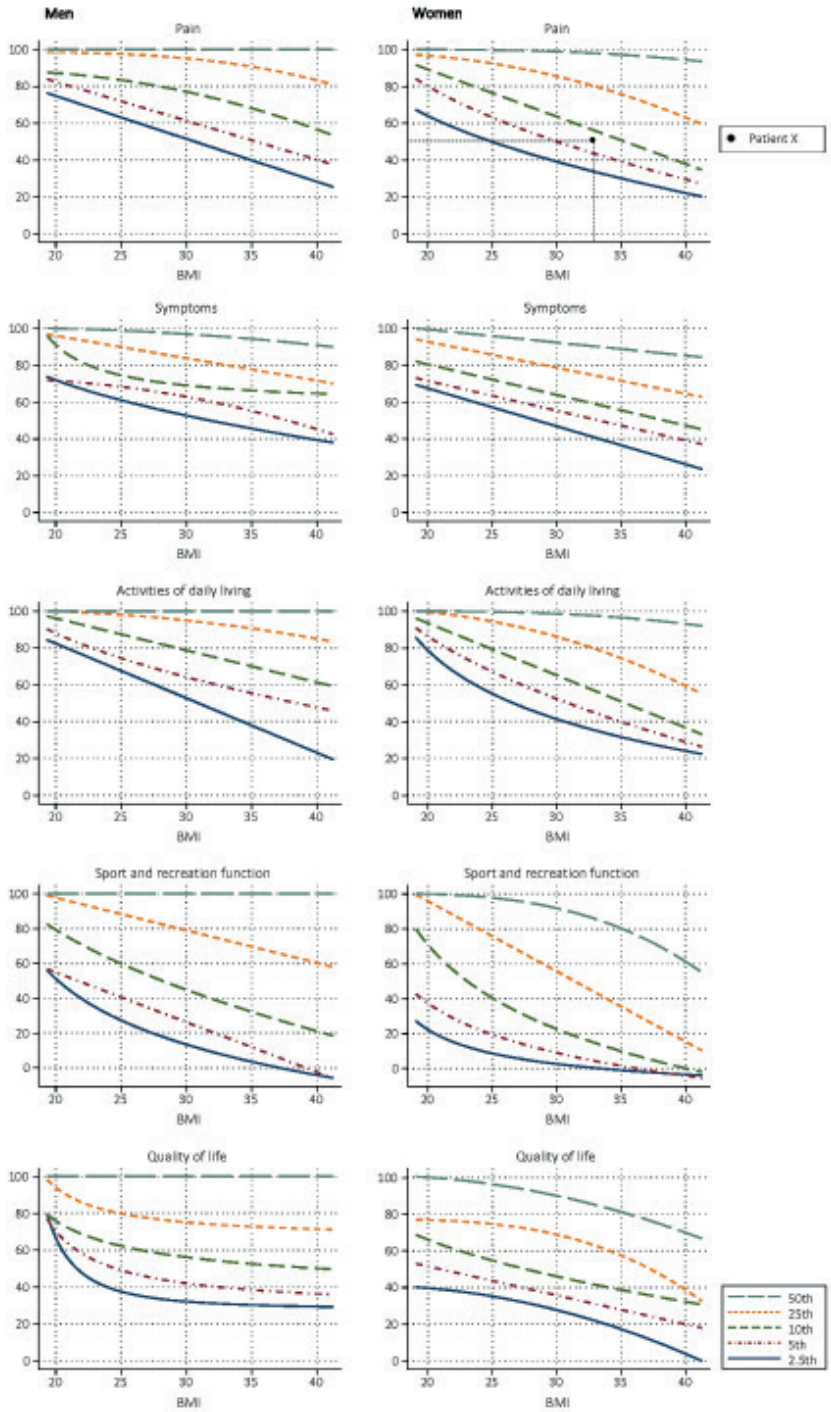

Figure 1. Sex- and BMI- specific percentile curves for the five Knee injury and Osteoarthritis Outcome Score subscales. Results are based on analyses weighted towards the BMI distribution of the general population (n = 6,438). Participants with a BMI below the 1st or above the 99th percentile were excluded (n = 205). Patient X is included for illustrative purposes; see text for a more detailed explanation.

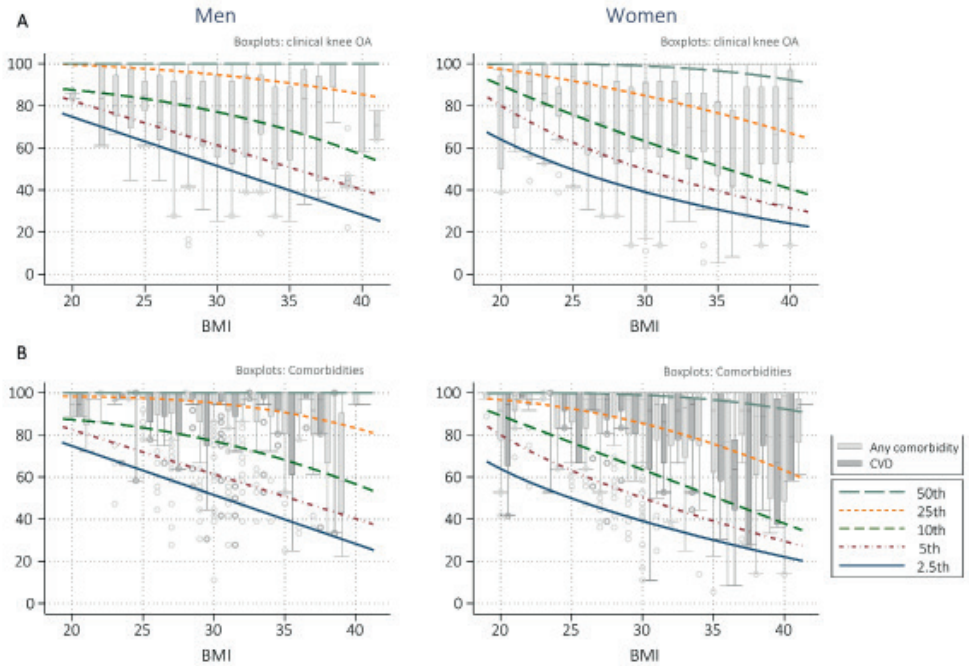

Figure 2. Percentile curves of the Knee injury and Osteoarthritis Outcome Score subscale pain compared to scores of participants classified with knee OA [A] and presence of any comorbidity and cardiovascular disease (CVD) [B]. Results are based on analyses weighted towards the BMI distribution of the general population (n = 6,438). Participants with a BMI below the 1st or above the 99th percentile were excluded (n = 205).

Discussion

We developed percentile curves for the five KOOS subscales in a large middle-aged population-based cohort. We showed that sex and BMI were strongly associated with KOOS scores, while age was not consistently associated with the KOOS scores. Therefore, the percentile curves are sex- and BMI-specific. In addition, we illustrated possible applications of the curves, and investigated how the scores of specific subgroups related to the curves. As expected, we observed that median KOOS scores of participants with knee OA were well below the 50th percentile of the general population. In addition, we observed that in women, but not in men, with comorbidities the median KOOS scores were worse compared to the general population, especially in women with a higher BMI.

In the current study, women scored worse on all KOOS subscales, which is in line with previous research[9–11]. Furthermore, our results show that a higher BMI was associated with worse KOOS scores. The association of BMI with KOOS scores has only been briefly touched upon by a limited number of other studies[10,11]. Marot et al. did not find relevant differences in KOOS scores with higher BMI, however they compared KOOS scores in participants between 16 and 97 years with a BMI ≥ 25 kg/m^2 to participants with a BMI < 25[10]. Williamson et al. investigated age-related effects on KOOS and additionally compared the effects of BMI and age. Compared to age, they observed a stronger effect of BMI on the subscale sport and

recreation function, and a smaller effect of BMI on the QoL subscale. Unfortunately, results regarding the effect of BMI on the other subscales were not mentioned[11]. Our results indicate that increasing BMI may play an important role in the interpretation of KOOS scores, and the limited number of studies available underscore the necessity to further explore the role of BMI on pain, function and QOL.

In our population between 45 and 65 years of age, we found no associations of age with KOOS subscale scores in the multivariable analyses. Previous population-based studies have included populations with participants between 18 and 84 years[9], 16 and 97 years[10] and between 18 and 64 years[11], and found varying results for the effect of age on the different KOOS subscales. Discrepancies with, and between, these studies may be explained by treating age as a continuous or categorical variable, or the different age ranges investigated. Of note, the population of interest should be kept in mind when interpreting these results. One of the major patient groups in which the KOOS is used are middle-aged patients with osteoarthritis. Previous studies have used study populations which for a considerable part consisted of participants who are not part of the target population. Our study is the first to focus on the effect of age on KOOS scores in the middle-aged population.

Furthermore, we have illustrated possible applications of the percentile curves. The curves may be used to determine how the KOOS scores of individual patients relate to the reference population, but could also be used to track changes in scores following for example physical therapy or knee surgery. In addition, the curves may be used to get more insight in how the scores of specific patient groups relate to the scores in the general population. We plotted the scores participants with knee OA on the percentile curves. As expected, in both sexes KOOS scores of participants with knee OA were below the 50th percentile curves. In men, median scores were around the 10th percentile and in women around the 25th percentile, which constitute clinically relevant reductions[3]. Furthermore, we investigated the association of other comorbidities with KOOS scores. The presence of any comorbidity was associated with worse KOOS scores, most notably in women. On the percentile curves, median scores of women with comorbidities were between the 50th and 25th percentile, while median scores of men were above or just below the 50th percentile. This demonstrates that these curves can be used to visualize to what extent scores of specific patient groups deviate from the general population. Our results further imply that while knee OA was strongly associated with worse KOOS scores compared to the general population, it is important to realize that a lot of different factors, such as presence of comorbidities or a history of knee surgery, may influence these results.

To our knowledge, we are the first to develop and apply KOOS percentile curves to investigate knee OA disease burden in a population-based study sample of considerable size. Another strength of our study is that we have accounted for the non-normal distribution of the KOOS subscales scores by using non-parametric tests. Previous studies have used parametric statistical methods, which might be less suited for the investigation of KOOS percentile values, as KOOS scores are very skewed towards high scores in population-based studies. To overcome this problem, we used quantile regression with fractional polynomials to develop the percentile curves. Furthermore, while tables provided by previous studies may give detailed information, we deemed that curves, similar to the growth curves extensively used in paediatrics, facilitate the interpretation and use of these benchmarks. The rather

narrow age range in our study might be seen as a limitation. However, as discussed above, we believe that the age range of our population is representative for patients most at risk for developing symptomatic knee OA, and may therefore be the most relevant age group to investigate. A further limitation is that individuals willing to participate may be more mobile and healthier, which could have led to a healthy-candidate bias. In addition, the history of other musculoskeletal conditions, among which inflammatory rheumatic diseases, and comorbidities was obtained by questionnaire, which could be subject to recall bias and misclassification.

To conclude, we have developed sex- and BMI-specific percentile curves for the five KOOS subscales. As we have shown, these curves can be used to help interpretation of KOOS scores of individual patients, as well as to assess the deviation of KOOS scores of specific patient groups from the general population. These charts may be used as benchmarks to improve interpretation of KOOS scores in research and daily clinical care.

References

1 van der Linden M, Westert G, de Bakker D, Schellevis F. Tweede nationale studie naar ziekten en verrichtingen in de huisartspraktijk. Klachten en aandoeningen in de bevolking en in de huisartspraktijk. Niv Bilthoven RIVM 2005.
2 Nielen M, Boersma-van Dam M, Schermer T. Incidentie en prevalentie van gezondheidsproblemen in de Nederlandse huisartsenpraktijk in 2017. Uit: NIVEL Zorgregistraties eerste lijn. Feb 2019. https://www.nivel.nl/nl/zorgregistraties-eerste-lijn/incidenties-en-prevalenties.
3 Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998; 28: 88–96.
4 Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 2003; 1: 64.
5 Collins NJ, Prinsen C a. C, Christensen R, Bartels EM, Terwee CB, Roos EM. Knee Injury and Osteoarthritis Outcome Score (KOOS): systematic review and meta-analysis of measurement properties. Osteoarthritis Cartilage 2016; 24: 1317–29.
6 Bremner-Smith AT, Ewings P, Weale AE. Knee scores in a 'normal' elderly population. The Knee 2004; 11: 279–82.
7 Demirdjian AM, Petrie SG, Guanche CA, Thomas KA. The outcomes of two knee scoring questionnaires in a normal population. Am J Sports Med 1998; 26: 46–51.
8 Jinks C, Jordan K, Croft P. Measuring the population impact of knee pain and disability with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Pain 2002; 100: 55–64.
9 Paradowski PT, Bergman S, Sundén-Lundius A, Lohmander LS, Roos EM. Knee complaints vary with age and gender in the adult population. Population-based reference data for the Knee injury and Osteoarthritis Outcome Score (KOOS). BMC Musculoskelet Disord 2006; 7: 38.
10 Marot V, Murgier J, Carrozzo A, et al. Determination of normal KOOS and WOMAC values in a healthy population. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 2018; published online Sept 24. DOI:10.1007/s00167-018-5153-6.
11 Williamson T, Sikka R, Tompkins M, Nelson BJ. Use of the Knee Injury and Osteoarthritis Outcome Score in a Healthy United States Population. Am J Sports Med 2016; 44: 440–6.
12 de Mutsert R, den Heijer M, Rabelink TJ, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol 2013; 28: 513–23.
13 Ministerie van VWS. Hoeveel mensen hebben overgewicht? www.rivm.nl/nldemaat; 2013.
14 de Groot IB, Favejee MM, Reijman M, Verhaar JAN, Terwee CB. The Dutch version of the Knee Injury and Osteoarthritis Outcome Score: a validation study. Health Qual Life Outcomes 2008; 6: 16.
15 The 2012 User's Guide to Knee injury and Osteoarthritis Outcome Score KOOS. www.koos.nu. 2012.
16 Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29: 1039–49.
17 Lumley, T. Analysis of compex survey samples. http:/www.jstatsoft.org/v09/i08/paper 2004.
18 Royston P, Wright EM. A Method for Estimating Age-Specific Reference Intervals (`Normal Ranges') Based on Fractional Polynomials and Exponential Transformation. J R Stat Soc Ser A Stat Soc 1998; 161: 79–101.
19 Wei Y, Pere A, Koenker R, He X. Quantile regression methods for reference growth charts. Stat Med 2006; 25: 1369–82.
20 Lassere M, Houssein D, Scott D, Edmonds J. Reference curves of radiographic damage in patients with rheumatoid arthritis: application of quantile regression and fractional polynomials. J Rheumatol 1997; 24: 1288–94.
21 Kroon FPB, Ramiro S, Royston P, Le Cessie S, Rosendaal FR, Kloppenburg M. Reference curves for the Australian/Canadian Hand Osteoarthritis Index in the middle-aged Dutch population. Rheumatol Oxf Engl 2017; 56: 745–52.

12

Application of the Knee Injury and Osteoarthritis Outcome Score percentile curves for longitudinal data of patients undergoing total knee arthroplasty: the LOAS study

Marieke Loef, Maaike Gademan, Daisy Latijnhouwers, Herman Kroon, Herman Kaptijn, Willem Marijnissen, Rob Nelissen, Thea Vliet Vlieland, Margreet Kloppenburg, on behalf of the LOAS study group

Journal of Arthroplasty. 2021;36(8):2779-2787

Abstract

Background We aimed to investigate the application of the Knee Injury and Osteoarthritis Outcome Score (KOOS) percentile curves, using pre- and postoperative data of patients with knee osteoarthritis (OA) undergoing total knee arthroplasty (TKA).

Methods We used Longitudinal Leiden Orthopaedics Outcomes of Osteo-Arthritis study (LOAS) data of patients between 45 and 65 years and undergoing primary TKA. KOOS scores (0-100) were obtained preoperatively and 6, 12 and 24 months after TKA. Preoperative knee radiographs were assessed according to Kellgren-Lawrence (KL) in a subset (37%) of patients. Comorbidities were self-reported using a standardized questionnaire. The median (interquartile range) population-level KOOS scores were plotted on previously developed population-based KOOS percentile curves. Additionally, we assessed the application of the curves on patient-level, and investigated differences in scores between patients with preoperative KL-scores ≤2 and ≥3, and presence (versus absence) of comorbidities.

Results The study population consisted of 853 patients (62% women, mean age 59 years, BMI 30 kg/m^2) with knee OA undergoing primary TKA. Preoperatively, median KOOS scores of all subscales were at or below the 2.5th percentile. Scores increased to approximately the 25th percentile 12 months postoperatively. Greater improvements were observed in pain, and less improvements in sport and recreational function and quality of life. Patients with higher preoperative KL scores and without comorbidities showed greater improvements.

Conclusion The KOOS percentile curves provided visual insights in knee complaints of patients relative to the general population. Furthermore, the KOOS percentile curves give insight in how preoperative patient characteristics are correlated with postoperative results.

Introduction

Patient-reported outcome measures (PROMs) have been vastly incorporated in clinical research, and are nowadays increasingly used in daily clinical practice [1]. PROMs enable capturing the patients' health status in a standardized way, and support a more comprehensive understanding of outcomes and effectiveness. There are various ways in which PROMs can be used. Individual, patient-level PROM data can be routinely used to aid shared-decision making and patient-centred care by facilitating patient-clinician and multidisciplinary communication [2,3]. In addition, PROMs can be used for monitoring of disease progression and treatment effects. On population-level, PROMs can be used to identify patient groups that benefit most from treatment, assess treatment (cost-)effectiveness, or compare performance of health organizations [4–6]. However, the interpretation of PROMs can be difficult if benchmarks are lacking and if there is uncertainty about which level of change in score is clinically meaningful.

Knee complaints, such as pain and functional disability, are estimated to occur in 32.1 per 1000 persons per year in the Dutch population. Knee osteoarthritis (OA) is one of the most important causes of knee complaints [7]. Due to the absence of disease modifying drugs for OA, knee OA is treated symptomatically until progression to end-stage disease warrants a total knee arthroplasty (TKA). In the Netherlands, the annual number of TKAs has tripled in the last decade to over 25 thousand TKAs in 2018 [8]. The Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire is a condition-specific PROM developed to investigate the patients' burden due to knee complaints [9]. The KOOS consists of five subscales, measuring different knee-specific domains: symptoms, pain, activities of daily living (ADL) function, sport and recreational function, and quality of life (QOL). The items of these domains are transformed to subscale scores ranging from 0 to 100. By itself, these scores can be difficult to interpret, as suboptimal scores may be unrelated to musculoskeletal pathology [10–12]. Therefore, we have previously developed KOOS percentile curves in a middle-aged population-based cohort of Dutch men and women [13], to provide a benchmark for comparison of patient scores with the general population. Alternative ways to show PROMs data can optimise the interpretation of PROMs both by clinicians and patients, to support patient-clinical communication and making well-informed shared treatment decisions.

The aim of the present study was to investigate the application of the KOOS percentile curves to compare the pre- and postoperative KOOS scores of patients with knee OA undergoing primary TKA, with the distribution of KOOS scores in the Dutch general population. Furthermore, we compared KOOS scores between specific patient groups to gain insight in possible differences in treatment benefit.

Materials and methods

Study population

The Longitudinal Leiden Orthopaedics Outcomes of Osteo-Arthritis (LOAS) study (Trial ID NTR3348) started in June 2012, and is an ongoing, observational, multicentre, longitudinal cohort study designed to determine long-term outcomes of TKA and total hip arthroplasty. Patient recruitment has been described previously [14]. Briefly, patients were eligible if they had a diagnosis of OA, an age of 18 years or older, were listed for total hip arthroplasty or TKA, and were fluent in the Dutch language. Patients were recruited consecutively from eight hospitals in The Netherlands: Leiden University Medical Center (LUMC), Alrijne Hospital

Leiderdorp, Alrijne Hospital Leiden, Groene Hart Hospital Gouda, LangeLand Hospital Zoetermeer, Reinier de Graaf Groep Delft, Albert Schweitzer Hospital Dordrecht, Waterland Hospital Purmerend [15]. Informed consent was obtained according to the declaration of Helsinki. The Medical Ethical Committee of the LUMC approved the design of the study. The current analyses are comprised of patients who have been included from June 2012 until June 2017, who were between 45 and 65 years of age, and undergoing primary TKA. Supplementary figure S1 presents a flowchart of included and excluded patient numbers.

Demographic data and comorbidities
Patient characteristics including age, sex, bodyweight (kg) and height (m) were collected by questionnaire and verified with data from the Landelijke Registratie Orthopedische Implantaten (LROI). Weight and height were used to calculate body mass index (BMI) (kg/m^2). A comorbidity questionnaire provided by the Dutch Central Bureau of Statistics (CBS) was used to determine the presence of comorbidities in the past year [16].

Patient reported outcomes
Patients completed the KOOS [9,17] preoperatively and at 6, 12 and 24 months after surgery. Patient numbers at each timepoint are presented in supplementary table S2. The KOOS is a knee-specific instrument consisting of five subscales: pain (nine items), symptoms (seven items), ADL function (17 items), sport and recreation function (five items) and knee-related QOL (four items). Items were scored considering the previous week from 0 (no problems) to 4 (extreme problems), on a 5-point Likert scale. Subscale scores were calculated according to the KOOS user's guide [18] as the sum of the items included, and subsequently transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems.
Patient treatment satisfaction was assessed at 6 and 12 months postoperatively using the Friends and Family Test phrasing [19], asking patients if they would recommend the surgery to friends or family members if they would have the same complaints.
The Short Form Health Survey (SF-12) was used to measure patients' health-related quality of life. This questionnaire consists of 12 questions covering 8 different dimensions (General Health, Physical Functioning, Role Physical, Role Emotional, Bodily Pain, Vitality, Social Functioning, and Mental Health). We calculated summary scores for the physical component (PCS), and mental component (MCS). The MCS and PCS scores range from 0 (worst QOL) to 100 (best QOL) [20]. Average scores of the United States population were used to derive norm-based scores with a mean of 50 and a standard deviation of 10.

Radiographic knee osteoarthritis severity
Weight-bearing anteroposterior knee radiographs of the affected knee were obtained in all patients prior to surgery as part of routine care. The radiographs were retrieved from five of the local hospitals (Leiden University Medical Center (LUMC), Alrijne Hospital Leiderdorp, Alrijne Hospital Leiden, Groene Hart Hospital Gouda, LangeLand Hospital Zoetermeer, Albert Schweitzer Hospital Dordrecht), and therefore available in a subset (37%) of patients. The radiographs were centrally scored by an experienced musculoskeletal radiologist. The Kellgren and Lawrence (KL) grading system was used to assess radiographic OA severity on a 0-4 scale (grade 0: no OA; grade 1: doubtful OA; grade 2: minimal OA; grade 3: moderate OA and grade 4: severe OA) [21]. Ten percent of radiographs was scored twice for assessment of an intraobserver reliability, which was 98% (97-99%) [14]. A comparison of baseline

characteristics between patients with and without radiographs showed no differences (supplementary table S1).

Reference population

The reference population consisted of middle-aged individuals included in the population-based Netherlands Epidemiology of Obesity (NEO) study. The NEO study is a prospective cohort study that included Dutch men and women between 45 and 65 years of age living in the greater are of Leiden (The Netherlands) between 2008 and 2012. Detailed study design and data collection have been described elsewhere (20). Briefly, individuals with a self-reported body mass index (BMI) ≥ 27 kg/m^2 were eligible to participate resulting in an oversampling of overweight or obese individuals. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference BMI distribution comparable to the general Dutch population (21). To correctly represent associations in the general population, adjustments were made for the oversampling of individuals with BMI ≥27 kg/m^2 (36). This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality (n=1671), whose BMI distribution was similar to the BMI distribution in the general Dutch population (21). Consequently, results apply to a population-based study without oversampling of participants with BMI ≥27 kg/m^2. The Medical Ethical Committee of the LUMC approved the design of the study and all participants gave written informed consent.

Population-based outcomes

Participant characteristics and KOOS scores were collected cross-sectionally at baseline. The NEO study reference population consisted of 6,643 participants. Mean age of the population was 56 years, with a mean BMI of 26 kg/m^2, and 56% were women. Clinical knee OA was defined using the American College of Rheumatology classification criteria, 15% of the population was classified with clinical knee OA. KOOS scores were handled according to the KOOS user's manual similar to the LOAS study. The majority of this middle-aged general population showed a lack of pain and other knee-related problems, with KOOS subscale scores (median; IQR) of: pain (100; 94-100), symptoms (96; 86-100), ADL function (100; 96-100), sport and recreation function (100; 80-100), and quality of life (100; 75-100). Among investigated patient characteristics we showed that sex and BMI were most strongly associated with KOOS scores. Hence, sex and BMI-specific percentile curves were developed using quantile regression with fractional polynomials [13]. The curves can be interpreted as follows: the 50th percentile is equal to the median. A score at the 25th percentile means that 25% of the scores in the (reference) population are at or below this score and 75% of the population has a higher score. A similar interpretation applies to the other percentiles.

Statistical analysis

Patient characteristics, radiographic knee OA severity and presence of comorbidities were analysed using descriptive statistics. In previous analyses by our group KOOS scores were influenced by sex and BMI, therefore reference curves have been developed stratified for these variables. Therefore, we provided the LOAS patient characteristics stratified by sex. We plotted the preoperative and postoperative KOOS scores of patients with knee OA included in the LOAS cohort on the KOOS percentile curves for comparison of patient scores with the Dutch general population, as well as to visualize the score trajectories following TKA. We assessed the application of the reference curves on both patient-level and population-level.

To get more insight in the differences of TKA treatment effect, KOOS scores of patients with preoperatively low (≤2) and high (≥3) KL scores were compared, as well as KOOS scores of patients with at least one comorbidity of any kind, and without comorbidities. Stata V16.0 (StataCorp LP, College Station, TX, USA) was used for all analyses.

Data availability

The data underlying this article were provided by the LOAS study group by permission. The data will be shared on reasonable request to the corresponding author, with permission of the LOAS study group.

Results

Patient characteristics

The study population consisted of 853 patients, with a mean age of 59.1 years, a mean BMI of 30 kg/m^2, and predominantly women (62%). Overall, 75% of the LOAS population had one or more comorbidities; patients reported more often non-musculoskeletal (68%) than musculoskeletal (23%) comorbidities. Mean MCS and PCS scores were 54 and 31, respectively. While 74% of the population had moderate to severe radiographic OA, a subset had no (4%), doubtful (6%) or minimal (17%) radiographic OA (table 1).

Table 1. Baseline patient characteristics of the LOAS study, stratified by sex

	Overall	**Men**	**Women**
	853	321 (36%)	532 (64%)
Patient characteristics			
Age, year	59.1 (4.7)	59.6 (4.4)	58.8 (4.9)
BMI, kg/m^2	30.4 (5.0)	29.8 (4.4)	30.8 (5.3)
Any comorbidities, n (%)	636 (75)	230 (72)	406 (76)
Kellgren & Lawrence score*			
0, n (%)	13 (4)	3 (3)	10 (5)
1, n (%)	18 (6)	5 (5)	13 (6)
2, n (%)	52 (17)	16 (15)	36 (17)
3, n (%)	163 (52)	59 (55)	104 (50)
4, n (%)	68 (22)	24 (22)	44 (21)
SF-12^			
MCS	54.1 (10.2)	54.4 (10.2)	53.9 (10.2)
PCS	31.1 (8.7)	33.2 (8.7)	29.7 (8.5)

Numbers represent mean (SD) unless otherwise specified. KOOS subscale scores are transformed to a 0–100 scale, with zero representing extreme knee problems and 100 representing no knee problems. *Knee radiographs were scored in a random subset of n = 314 (37%) patients. ^SF-12 scores were missing in 101 patients. Abbreviations: ADL = activities daily living, BMI = body mass index, KOOS = Knee Injury and Osteoarthritis Outcome Score, n = number, SD = standard deviation.

Treatment satisfaction

At 12 months postoperatively, 92% of patients (90% of men, 92% of women) replied that they would recommend the surgery to friends or family if they would have the same complaints, reflecting treatment satisfaction in the great majority of patients.

Knee-specific outcomes up to 2 years after total knee arthroplasty
Preoperatively, KOOS scores were very poor across all subscales, and were lower in women compared to men. All subscale scores increased to a great extent 6 months after surgery, and showed further improvement between 6 and 12 months. Twelve months after surgery, KOOS scores stabilized. With exception of sport and recreational function, which remained lower in women, postoperative KOOS subscale scores were similar between men and women (supplementary table S2).

Using the KOOS percentile curves for population-level comparison of patients' KOOS scores with the general population
For comparison of population-level patient KOOS scores with the Dutch general population, the median (interquartile range) preoperative and 12-month postoperative KOOS scores of all five subscales were plotted on the KOOS percentile curves (figures 1, 2 and 3). By example, pain scores of all postoperative timepoints are shown in supplementary figure S2. All subscale scores showed notable inter-patient variability, as can be seen from the wide range of the boxplots and accompanying error bars. Visual comparison of the graphs in figure 1 and 2 showed that prior to TKA, median KOOS pain scores were worse in women compared to men. In comparison to the general population, preoperative median KOOS pain scores were below the 2.5th percentile (solid blue line) in both men and women. At 12 months postoperatively, median pain scores were around the 25th percentile (dotted yellow line) in men, and between the 25th and 50th percentile (striped navy line) in women. Pre-operatively, median scores of the other subscales varied from below the 2.5th percentile, to around the 5th percentile (dotted maroon line) in patients with a higher BMI. Median symptom and ADL function scores increased to around the 25th percentile postoperatively in both men (figure 1) and women (figure 2). Similarly, postoperative QOL scores were around the 25th percentile in men (figure 3). In women, somewhat higher postoperative scores were observed, approaching the 50th percentile in women with a higher BMI. A flooring effect was observed preoperatively in the sport and recreational function scores (figure 3). Postoperatively, sport and recreation scores increased; however, they remained around the 10th percentile (striped green line) of the general population.

Applying the KOOS percentile curves for follow-up of patient-level KOOS scores after total knee arthroplasty
To show the use of the KOOS percentile curves on a patient-level, for illustrative purposes five randomly selected men and women with knee OA were selected, and the preoperative and postoperative KOOS pain scores were plotted alongside the distribution in the general population (supplementary figure S3). A clear inter-patient variability in preoperative pain status, as well as at postoperative time points was observed. Despite that all depicted patients start with a preoperative KOOS pain score at or below the 2.5th percentile of the general population, some improve to (almost) the 50th percentile already at 6 months postoperatively, while others improve more gradually or to a lesser extent.

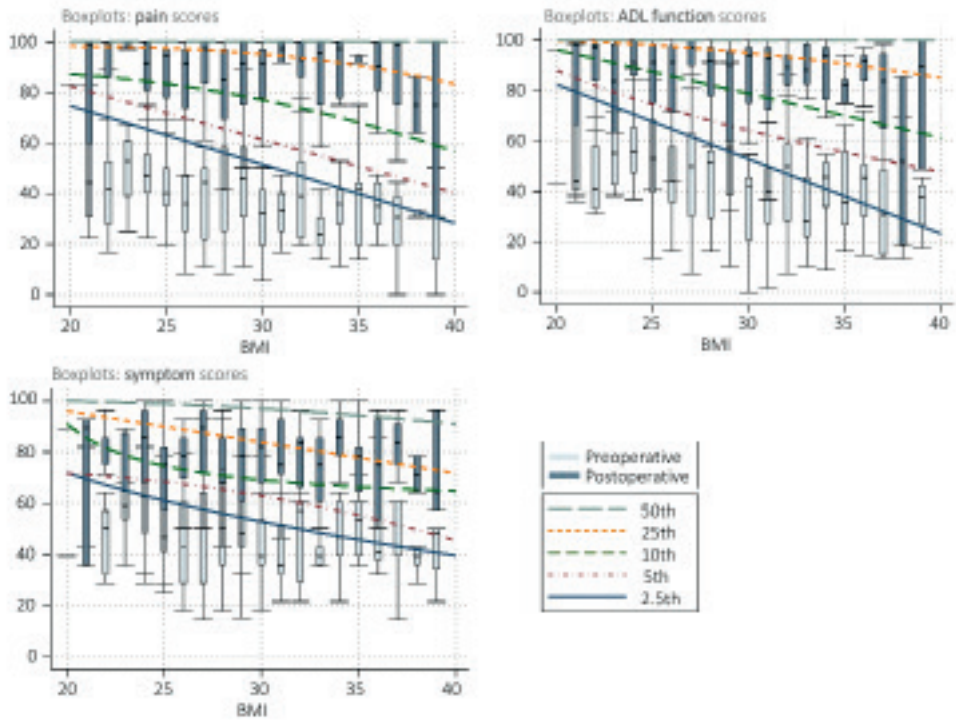

Figure 1. Comparison of the preoperative and 12 months postoperative KOOS pain, symptom and ADL scores in men undergoing primary total knee arthroplasty, with KOOS scores in the general population. The KOOS scores (Y-axis) are given over BMI (X-axis). The preoperative KOOS scores are represented by the light grey boxplots, and the postoperative scores are given in dark grey boxplots. The boxplots represent the median (horizontal line) and interquartile range. The KOOS score distribution of the general population is depicted with the coloured percentile lines.

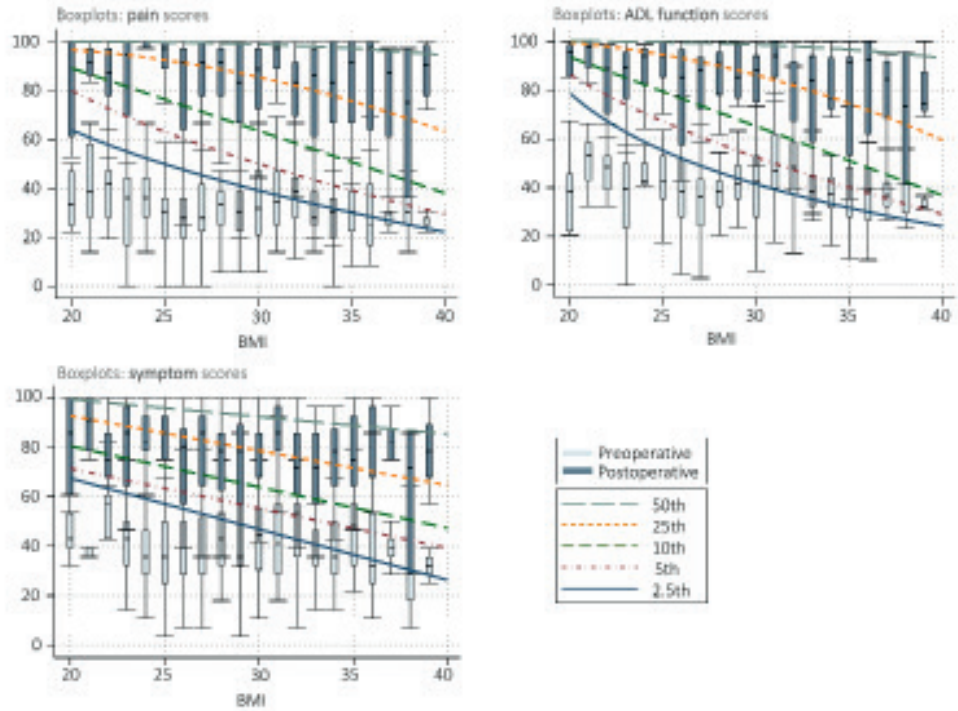

Figure 2. Comparison of the preoperative and 12 months postoperative KOOS pain, symptom and ADL scores in women undergoing primary total knee arthroplasty, with KOOS scores in the general population. The KOOS scores (Y-axis) are given over BMI (X-axis). The preoperative KOOS scores are represented by the light grey boxplots, and the postoperative scores are given in dark grey boxplots. The boxplots represent the median (horizontal line) and interquartile range. The KOOS score distribution of the general population is depicted with the coloured percentile lines.

Comparison of KOOS score trajectories between specific patient groups

KOOS pain scores from patients with preoperative KL scores below or equal to 2 points were compared with scores from patients with preoperative KL scores of 3 points or higher (figure 4). In men, patient numbers were too low to give conclusive results. In women, median preoperative pain scores did not differ with respect to KL score. Postoperatively, pain scores improved to a greater extent in women with preoperative moderate to severe radiographic OA compared with women with preoperative no to mild radiographic OA.

Figure 5 shows the KOOS pain scores of patients included in the LOAS study without any comorbidity and with at least one comorbidity. Preoperatively, across both sexes median pain scores were below the 2.5th percentile, with no differences between study group patients with and without comorbidities. At 12 months postoperatively, in both groups of LOAS patients median pain scores were about the 25th percentile in men. In women, median pain scores were at, or just below the 50th percentile in patients without comorbidities, while median pain scores were about the 25th percentile in women who had at least one comorbidity.

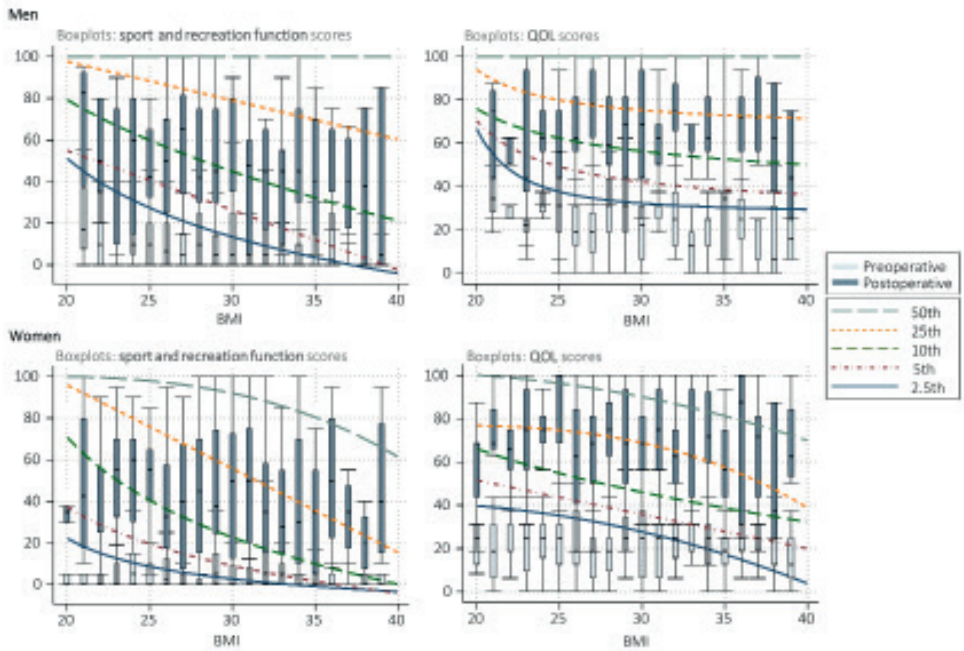

Figure 3. Comparison of the preoperative and 12 months postoperative KOOS sport and recreation, and QOL subscale scores in patients undergoing primary total knee arthroplasty, with KOOS scores in the general population. The KOOS scores (Y-axis) are given over BMI (X-axis). The preoperative KOOS scores are represented by the light grey boxplots, and the postoperative scores are given in dark grey boxplots. The boxplots represent the median (horizontal line) and interquartile range. The KOOS score distribution of the general population is depicted with the coloured percentile lines.

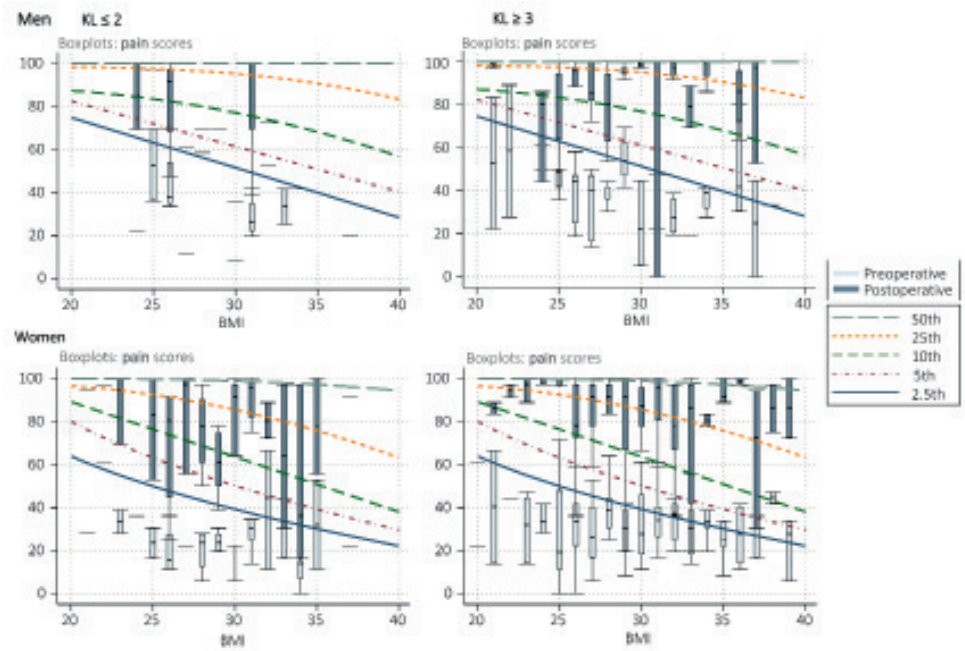

Figure 4. Comparison of the preoperative and 12 months postoperative KOOS pain scores in patients with low versus high preoperative radiographic OA severity. Preoperative and 12 months postoperative KOOS pain scores were stratified by Kellgren-Lawrence (KL) scores, comparing patients with a preoperative KL score ≤ 2 with patients with a preoperative KL score ≥ 3. The KOOS scores (Y-axis) are given over BMI (X-axis). The preoperative KOOS scores are represented by the light grey boxplots, and the postoperative scores are given in dark grey boxplots. The boxplots represent the median (horizontal line) and interquartile range. The KOOS score distribution of the general population is depicted with the coloured percentile lines.

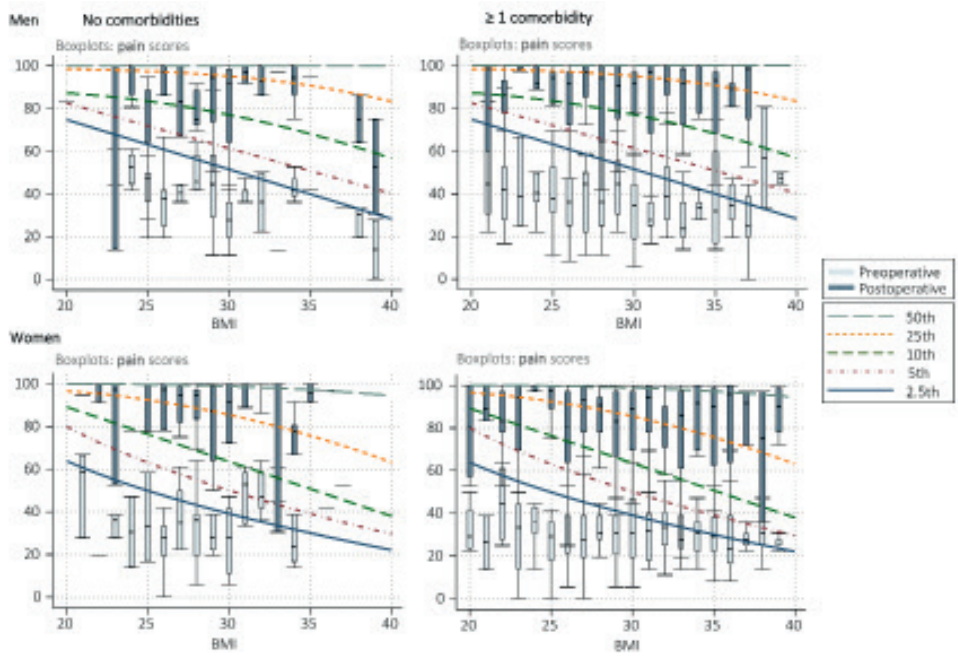

Figure 5. Comparison of the preoperative and 12 months postoperative KOOS pain scores in patients with no comorbidities versus patients with at least one comorbidity. The KOOS scores (Y-axis) are given over BMI (X-axis). The preoperative KOOS scores are represented by the light grey boxplots, and the postoperative scores are given in dark grey boxplots. The boxplots represent the median (horizontal line) and interquartile range. The KOOS score distribution of the general population is depicted with the coloured percentile lines.

Discussion

The increasing routine use of PROMs to evaluate conservative as well as surgical interventions necessitates the development of methodology to optimize the interpretation and evaluation of PROMS across several follow-up moments. Therefore, we aimed to compare the KOOS scores of a large prospective cohort of TKA patients with the KOOS scores of the general population, to show the use of the previously developed population-based percentile curves of the KOOS questionnaire [13] as method to aid the interpretation of the KOOS subscale scores. Applying preoperative and up to two years postoperative KOOS scores of patients with knee OA undergoing TKA to the general population-based percentile curves, allowed to visualize the pre- and postoperative knee-specific health status of these patients relative to the general population. Furthermore, we have shown that the KOOS percentile curves can give insight in the correlation of specific preoperative patient characteristics with postoperative results. We observed that absence of moderate to severe preoperative radiographic OA, as well as the presence of comorbidities, was associated with less improvement after surgery.

Implementing alternative ways to show PROMs data can support patient-clinician communication about the patients' symptoms and quality of life. Moreover, it may aid managing patients' expectations, making treatment decisions, and improve patient autonomy [3]. In the Netherlands, the number of TKAs has strongly risen in the last decade. The annual

number of TKAs has more than tripled, with a little over 7 thousand TKAs in 2007 to more than 25 thousand TKAs in 2018 [8]. A systematic review has reported that 10-34% of patients are not satisfied after knee replacement surgery [22]. In our patient population, at one year postoperatively, only 8% of patients responded that they would not recommend the surgery to friends or family if they would have the same complaints, which can be interpreted as dissatisfaction with the treatment result. Data on potential underlying factors related to this specific query were not collected in the current study, which prohibited in-depth insight in explanations for (dis)satisfaction after surgery. In addition, we showed that although most patients show great improvements far beyond the minimal important change [23] in all KOOS subscales after surgery, in the majority of patients KOOS scores do not normalize to the median score of the general population. We observed worse preoperative KOOS scores compared to previous studies investigating KOOS scores in TKA populations [24,25]. For example, Lyman et al. observed in a TKA population with a mean age of 74 years mean preoperative KOOS pain and ADL function scores of 51 and 55, respectively (versus 34 and 44 in our population). One year postoperative, KOOS scores were more similar [24]. Vestergaard et al. observed better preoperative KOOS pain and sport and recreation scores compared with ours. Scores on the other KOOS subscales were similar to the scores we observed [25]. The observed differences may be explained by differences in lifestyle and physical activities associated with age, since our population was notably younger than the populations included in previous studies. Our results give insight in the expected postoperative improvements in knee pain, symptoms and function. Therefore, they are important to communicate with patients, as part of the shared decision making process during the preoperative consultation, to manage their expectations, as this may reduce treatment dissatisfaction [26].

Visualizing differences in treatment benefit in different patient groups may help making a well-informed patient-centred (conservative or surgical) treatment decision. In line with others investigating patients undergoing TKA [27,28], we observed a high frequency of comorbidities in our study population. Similarly, we observed less improvement postoperatively in patients having at least one comorbidity compared to patients without comorbidities. Furthermore, we observed greater improvements in patients with preoperatively more severe radiographic OA compared to patient with no to minimal radiographic OA, which is in line with previous findings in the LOAS study [29,30], and with others [31,32]. However, not all previous studies are in agreement [33,34], which could be explained by the inclusion of a limited patient number [33], including only patients with mild radiographic OA in contrast to also including patients with no radiographic OA [33,34], as well as other differences in patient characteristics such as higher age.

Our study has notable strengths. The LOAS study has a multicentre design allowing the inclusion of a diverse patient population from both academic and non-academic hospitals with a low threshold for inclusion, reflecting a real-life care situation and improves the generalizability of the study results. However, as only Dutch hospitals were included, extrapolating our data to other countries, with likely differences in health-care access or insurance, should be done cautiously. In addition, the prospective longitudinal design resulted in a structured data collection at standardized timepoints. Furthermore, the present analyses show a variety of applications for the KOOS percentile curves, which are easy to implement in research and clinical care.

However, our study is also limited in several ways. The age range of the population in which the percentile curves were developed was restricted to persons between 45 and 65 years of age. This makes the percentile curves less ideal for the use of end-stage OA or TKA data, as a considerable number of these patients will be older than 65 years. Restriction of the LOAS population to the required age range resulted in a loss of data from almost two thirds of LOAS patients. However, the age range between 45 and 65 years is well suited for other patient populations, for example to track conservative treatment response in patients with an earlier stage of OA. In addition, the percentile curves may be extrapolated to a broader age range. However, no data is available on accuracy and reliability of extrapolation at this moment. Another limitation is the healthy attendant bias that is inherent to the population-based design in which the percentile curves were developed. This form of selection bias may lead to overly optimistic results. To which extent this might play a role depends on the patient group under investigation, as the patient group may also be subject to a degree of selection. In addition, we observed that a minority of operated patients had no to minimal preoperative radiographic OA. Many factors influence the decision to perform TKA, which may go beyond OA-related health status [35]. Unfortunately, we did not obtain data on which factors drove the orthopaedic surgeon's decision to perform TKA. Furthermore, we did not have data within 3 months after surgery, which could have given information on performance in the time window shortly after surgery when no improvement or even worsening of complaints could be anticipated. In addition, we did not obtain lateral knee X-rays, which might have resulted in underreporting of predominantly patellofemoral knee OA. However, we used one of the most commonly reported radiographic OA scoring methods, the KL grading, which does not include lateral view X-rays. Therefore, current results are well comparable to previous OA literature. Lastly, in our subgroup analyses the patient numbers were rather small, especially for men, which hampered conclusiveness. However, despite the smaller patient number, our results were in line with previous findings [29].

In conclusion, our study showed that the previously developed KOOS percentile curves can be used in research and clinical care to examine the pre- and postoperative knee-related health status of patients with knee OA undergoing TKA. The percentile curves may aid patient-clinician communication, improve management of treatment expectations and support shared-decision making.

References

[1] Rolfson O, Wissig S, van Maasakkers L, Stowell C, Ackerman I, Ayers D, et al. Defining an International Standard Set of Outcome Measures for Patients With Hip or Knee Osteoarthritis: Consensus of the International Consortium for Health Outcomes Measurement Hip and Knee Osteoarthritis Working Group. Arthritis Care Res 2016;68:1631–9. https://doi.org/10.1002/acr.22868.

[2] Santana M-J, Feeny D. Framework to assess the effects of using patient-reported outcome measures in chronic care management. Qual Life Res 2014;23:1505–13. https://doi.org/10.1007/s11136-013-0596-1.

[3] Yang LY, Manhas DS, Howard AF, Olson RA. Patient-reported outcome use in oncology: a systematic review of the impact on patient-clinician communication. Support Care Cancer 2018;26:41–60. https://doi.org/10.1007/s00520-017-3865-7.

[4] Appleby J, Poteliakhoff E, Shah K, Devlin N. Using patient-reported outcome measures to estimate cost-effectiveness of hip replacements in English hospitals. J R Soc Med 2013;106:323–31. https://doi.org/10.1177/0141076813489678.

[5] Devlin NJ, Appleby J. Getting the most out of PROMs: putting health outcomes at the heart of NHS decision-making. London: King's Fund; 2010.

[6] Smith PC, Street AD. On the uses of routine patient-reported health outcome data. Health Econ 2013;22:119–31. https://doi.org/10.1002/hec.2793.

[7] Nielen M, Boersma-van Dam M, Schermer T. Incidentie en prevalentie van gezondheidsproblemen in de Nederlandse huisartsenpraktijk in 2017. Uit: NIVEL Zorgregistraties eerste lijn. Feb 2019 n.d. https://www.nivel.nl/nl/zorgregistraties-eerste-lijn/incidenties-en-prevalenties.

[8] LROI- Registered procedures 2007-2018 n.d. https://www.lroi-rapportage.nl/knee-numbers-registered-procedures-2007-2018 (accessed March 31, 2020).

[9] Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998;28:88–96. https://doi.org/10.2519/jospt.1998.28.2.88.

[10] Bremner-Smith AT, Ewings P, Weale AE. Knee scores in a "normal" elderly population. The Knee 2004;11:279–82. https://doi.org/10.1016/j.knee.2003.06.001.

[11] Demirdjian AM, Petrie SG, Guanche CA, Thomas KA. The outcomes of two knee scoring questionnaires in a normal population. Am J Sports Med 1998;26:46–51. https://doi.org/10.1177/03635465980260012401.

[12] Jinks C, Jordan K, Croft P. Measuring the population impact of knee pain and disability with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Pain 2002;100:55–64.

[13] Loef M, Kroon FPB, Böhringer S, Roos EM, Rosendaal FR, Kloppenburg M. Percentile curves for the knee injury and osteoarthritis outcome score in the middle-aged Dutch population. Osteoarthritis Cartilage 2020. https://doi.org/10.1016/j.joca.2020.03.014.

[14] Leichtenberg CS, Meesters JJL, Kroon HM, Verdegaal SHM, Tilbury C, Dekker J, et al. No associations between self-reported knee joint instability and radiographic features in knee osteoarthritis patients prior to Total Knee Arthroplasty: A cross-sectional analysis of the Longitudinal Leiden Orthopaedics Outcomes of Osteo-Arthritis study (LOAS) data. The Knee 2017;24:816–23. https://doi.org/10.1016/j.knee.2017.04.001.

[15] Tilbury C, Leichtenberg CS, Kaptein BL, Koster LA, Verdegaal SHM, Onstenk R, et al. Feasibility of Collecting Multiple Patient-Reported Outcome Measures Alongside the Dutch Arthroplasty Register. J Patient Exp 2019:2374373519853166. https://doi.org/10.1177/2374373519853166.

[16] Centraal Bureau voor de Statistiek (CBS). Zelfgerapporteerde medische consumptie, gezondheid en leefstijl n.d.

[17] de Groot IB, Favejee MM, Reijman M, Verhaar JAN, Terwee CB. The Dutch version of the Knee Injury and Osteoarthritis Outcome Score: a validation study. Health Qual Life Outcomes 2008;6:16. https://doi.org/10.1186/1477-7525-6-16.

[18] The 2012 User's Guide to Knee injury and Osteoarthritis Outcome Score KOOS. WwwKoosNu 2012.

[19] Wilberforce M, Poll S, Langham H, Worden A, Challis D. Measuring the patient experience in community mental health services for older people: A study of the Net Promoter Score using the Friends and Family Test in England. Int J Geriatr Psychiatry 2019;34:31–7. https://doi.org/10.1002/gps.4978.

[20] Gandhi SK, Salmon JW, Zhao SZ, Lambert BL, Gore PR, Conrad K. Psychometric evaluation of the 12-item short-form health survey (SF-12) in osteoarthritis and rheumatoid arthritis clinical trials. Clin Ther 2001;23:1080–98. https://doi.org/10.1016/s0149-2918(01)80093-x.

[21] Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. Ann Rheum Dis 1957;16:494–502. https://doi.org/10.1136/ard.16.4.494.

[22] Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. BMJ Open 2012;2:e000435. https://doi.org/10.1136/bmjopen-2011-000435.

[23] Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 2003;1:64. https://doi.org/10.1186/1477-7525-1-64.

[24] Lyman S, Lee Y-Y, McLawhorn AS, Islam W,

MacLean CH. What Are the Minimal and Substantial Improvements in the HOOS and KOOS and JR Versions After Total Joint Replacement? Clin Orthop Relat Res 2018;476:2432–41. https://doi.org/10.1097/CORR.0000000000000456.
[25] Vestergaard V, Colon Iban YE, Kappel A, Melnic CM, Bedair H, Huddleston JI, et al. Do Knee Osteoarthritis Patterns Affect Patient-Reported Outcomes in Total Knee Arthroplasty? Results From an International Multicenter Prospective Study With 3-Year Follow-Up. J Arthroplasty 2021;36:507–13. https://doi.org/10.1016/j.arth.2020.08.033.
[26] Scott CEH, Howie CR, MacDonald D, Biant LC. Predicting dissatisfaction following total knee replacement: a prospective study of 1217 patients. J Bone Joint Surg Br 2010;92:1253–8. https://doi.org/10.1302/0301-620X.92B9.24394.
[27] Peter WF, Dekker J, Tilbury C, Tordoir RL, Verdegaal SHM, Onstenk R, et al. The association between comorbidities and pain, physical function and quality of life following hip and knee arthroplasty. Rheumatol Int 2015;35:1233–41. https://doi.org/10.1007/s00296-015-3211-7.
[28] Hawker GA, Badley EM, Borkhoff CM, Croxford R, Davis AM, Dunn S, et al. Which patients are most likely to benefit from total joint arthroplasty? Arthritis Rheum 2013;65:1243–52. https://doi.org/10.1002/art.37901.
[29] van de Water RB, Leichtenberg CS, Nelissen RGHH, Kroon HM, Kaptijn HH, Onstenk R, et al. Preoperative Radiographic Osteoarthritis Severity Modifies the Effect of Preoperative Pain on Pain/Function After Total Knee Arthroplasty: Results at 1 and 2 Years Postoperatively. J Bone Joint Surg Am 2019;101:879–87. https://doi.org/10.2106/JBJS.18.00642.
[30] Keurentjes JC, Fiocco M, So-Osman C, Onstenk R, Koopman-Van Gemert AWMM, Pöll RG, et al. Patients with severe radiographic osteoarthritis have a better prognosis in physical functioning after hip and knee replacement: a cohort-study. PloS One 2013;8:e59500. https://doi.org/10.1371/journal.pone.0059500.
[31] Kahn TL, Soheili A, Schwarzkopf R. Outcomes of total knee arthroplasty in relation to preoperative patient-reported and radiographic measures: data from the osteoarthritis initiative. Geriatr Orthop Surg Rehabil 2013;4:117–26. https://doi.org/10.1177/2151458514520634.
[32] Valdes AM, Doherty SA, Zhang W, Muir KR, Maciewicz RA, Doherty M. Inverse relationship between preoperative radiographic severity and postoperative pain in patients with osteoarthritis who have undergone total joint arthroplasty. Semin Arthritis Rheum 2012;41:568–75. https://doi.org/10.1016/j.semarthrit.2011.07.002.
[33] Perry KI, Strasser NL, Harmsen WS, Pagnano MW, Trousdale RT. Minimal Preoperative Degenerative Arthritis May Not Predict Poor TKA Outcome. Orthopedics 2015;38:e681-684. https://doi.org/10.3928/01477447-20150804-54.
[34] Meding JB, Ritter MA, Faris PM, Keating EM, Harris W. Does the preoperative radiographic degree of osteoarthritis correlate to results in primary total knee arthroplasty? J Arthroplasty 2001;16:13–6. https://doi.org/10.1054/arth.2001.16501.
[35] Gossec L, Paternotte S, Maillefert JF, Combescure C, Conaghan PG, Davis AM, et al. The role of pain and functional impairment in the decision to recommend total joint replacement in hip and knee osteoarthritis: an international cross-sectional study of 1909 patients. Report of the OARSI-OMERACT Task Force on total joint replacement. Osteoarthritis Cartilage 2011;19:147–54. https://doi.org/10.1016/j.joca.2010.10.025.

13

Summary and general discussion

Introduction

Osteoarthritis (OA) is one of the most common rheumatic musculoskeletal disorders (RMDs), affecting approximately 300 million people globally in 2017[1]. Moreover, as a result of its chronic disease course and rising incidence due to aging of the population and increasing prevalence of obesity, its prevalence is expected to increase even further. OA is a condition with a high disease burden, causing pain, stiffness, functional disability and a reduced quality of life. OA is a disease of the whole joint, characterised by cartilage degradation, subchondral bone remodeling, osteophyte formation, degradation of ligaments and synovial inflammation[2,3]. Although OA can affect any joint, the hands and knees are among the most often affected joint locations[2]. Currently, treatment options are limited to symptom reduction, leaving a high unmet need for disease modifying drugs for the treatment of OA.

Despite major advances in our knowledge over the last years, the pathophysiology of OA remains incompletely understood. An important player in OA development is obesity, a risk factor that has been recognised decades ago[4,5]. Until recently, the effect of obesity on OA was solely ascribed to the increased mechanical loading that is associated with increased body weight pressing on the joints[6]. However, results from studies investigating the association of obesity with non-weightbearing joints such as the hand, have pointed toward an additional systemic effect of obesity[7–9], possibly via the release of soluble pro-inflammatory cytokines and mediators such as adipokines and lipids[10,11]. Evidence on these mechanisms from human clinical studies is still limited. Therefore, the first part of this thesis has focused on investigating several obesity-related systemic factors and their association with hand and knee OA. Increased knowledge on the pathophysiology of OA may reveal potential treatment targets, and may bring us a step closer to the development of a treatment to slow down or stop disease progression. Another essential consideration in treatment development, and particularly in clinical trials, is the inclusion of relevant endpoints. Therefore, in the second part of this thesis, we aimed to further our understanding of several patient reported outcomes (PROMs), to provide insight in the patients' experience of hand and knee OA disease burden.

The results described in this thesis are based on research in several study populations, which all offer different perspectives, and come with different strengths and limitations. The main differences between the study populations can be found in the involvement of either participants from the general population or patients, differences in patient selection inherent to the study design, or differences in which OA joint location was the primary focus of the investigation.

In this chapter we summarize and discuss the main findings that we have presented in this thesis. Finally, we consider future perspectives and raise some questions that remain to be answered.

Part 1 – Lipids, inflammation and other metabolic factors in OA

Increasing our knowledge on the role of obesity and inflammation-related systemic factors in OA may aid our understanding of OA aetiology, and contribute to the discovery of new targets for the treatment of OA. In the first part of this thesis, we focused specifically on the association of lipids and OA, and if metabolic dysregulation can explain the well-known association of obesity with hand and knee OA.

Fatty acids and OA

We commence part 1 with a summary of the current evidence on the role of fatty acids in the development and progression of OA. In chapter 2 we review the available pre-clinical *in vitro* and animal studies, as well as clinical studies involving humans, to provide a comprehensive overview of the literature. From these studies, we have learned that fatty acids appear to have effects on both symptoms and structural abnormalities associated with OA. Interestingly, the different fatty acid types seem to have distinct effects. Animal *in vitro* studies have shown that stimulation of chondrocytes with omega-3 polyunsaturated fatty acids (PUFAs) reduces the expression of inflammatory markers, proteinases involved in cartilage degradation, and markers of oxidative stress. Additionally, omega-3 PUFAs seemed to delay interleukin (IL)-α induced cell death. Conversely, stimulation with omega-6 PUFAs increased makers of cartilage degradation and inflammation, and saturated fatty acids (SFAs) increased makers of oxidative stress. Additionally, animal intervention studies have suggested that the ratio of omega-6 to omega-3 PUFAs is also relevant, as a decrease in this ratio might reduce the expression of inflammatory markers and OA-related structural damage. In line, supplementation of the diet with omega-3 PUFAs reduced OA-associated damage, in comparison with diets high in omega-6 PUFAs or SFAs. Furthermore, omega-6 PUFA- and SFA-enriched diets increased the expression of inflammatory markers and induced chondrocyte apoptosis. However, the results from these animal intervention studies are difficult to put into a human perspective. Furthermore, negative results were infrequently encountered, which may indicate publication bias. In humans, not many studies have been performed. The few available findings from studies in humans were mainly in line with the findings described by *in vitro* and animal studies, showing an adverse effect of total fatty acid levels and SFAs with structural OA-related abnormalities, while omega-3 PUFAs were associated with less damage and improvement in symptoms. However, more research is needed to draw firm conclusions. Moreover, studies were limited to patients with knee and hip OA. Since systemic factors may play a role in hand OA in particular, as mechanical factors due to obesity are here less likely, it would be interesting to investigate the association of fatty acids in patients with hand OA.

Therefore, we aimed to further our knowledge on the association of plasma fatty acids with hand and knee OA in chapter 3. We used the population-based Netherlands Epidemiology of Obesity (NEO) study, and defined a clinical hand and knee OA phenotype according to the American College of Rheumatology (ACR) classification criteria, as well as a structural knee OA phenotype in a subset of participants who underwent magnetic resonance imaging (MRI) of the knee. We investigated different fatty acid classes: SFA, monounsaturated fatty acids (MUFA), omega-3 PUFAs and omega-6 PUFAs, while taking into account possible confounding due to age, education, ethnicity and total body fat percentage. Because of previous sex-differences observed in the association between systemic factors and OA in the NEO study, we stratified our analyses by sex. We found that in men the plasma concentrations of SFAs, total PUFAs, as well as omega-3 PUFAs were positively associated with clinical hand OA. Both SFAs as PUFAs were associated with an increased odds of structural, but not clinical knee OA. Remarkably, none of these associations were observed in women. Although we encounter sex differences in the association of systemic factors and OA more often, its explanation is not that straightforward. Possibly, the different associations may be explained by differences in relative contribution of systemic and mechanic effects of obesity in men or women. Alternatively, an explanation could be found in sex differences in lipid metabolism. Additionally, we observed that none of the fatty acid classes were associated with hand and knee pain. The positive

associations of SFA, as well as of PUFA concentrations with clinical hand OA and structural knee OA in men were striking. A positive relation between SFA and structural knee OA has been shown previously. However, our results are in contrast to previous research suggesting an opposing effect of SFA and omega-3 PUFAs. Since the association of plasma fatty acid levels with clinical hand OA has not been described before, we were not able to make a direct comparison with previous human studies, warranting verification of our results by future research. Additionally, it would be insightful to look beyond the broad fatty acid classes, at the levels of individual lipids. Lipidomic profiling studies may offer a good opportunity to gather data for this purpose. Furthermore, downstream bioactive oxylipins may provide a clearer view of the association between lipids and OA.

Reproducibility of lipid measurements

Lipidomics is a subset of metabolomics that involve the identification and quantification of molecular lipid species. Since lipids are highly dynamic molecules that are constantly changing with differing physiological and pathological conditions[12], lipidomics may be valuable for identification of candidate biomarkers. Biomarkers can be used as indicators of normal or pathogenic biological processes to help understand the pathogenesis of diseases, as well as to measure disease presence or predict disease progression. In observational studies measurements are often taken on a single day, assuming that these measurements are representative of the metabolic status of an individual. However, fluctuations may occur due to sampling techniques, assay variation or biological variability. Only few studies have investigated the reliability of repeated measurements of lipid metabolite concentrations[13–17]. Moreover, while some studies have compared reproducibility in serum and plasma[13,17], to our knowledge the reproducibility of lipid measurements in erythrocytes has not been investigated before. Erythrocytes have a particularly long half-life (approximately 120 days) and may therefore be a good representation of long-term exposure, which may be relevant when studying chronic diseases. To this regard, we investigated in chapter 4 the biological reproducibility of a large lipid platform that has the potential of measuring over a 1000 individual higher order lipid species, the Lipidyzer™. We assessed within person variation by using measurements from the placebo group of the Hand Osteoarthritis Prednisolone Efficacy (HOPE) study (a controlled setting without interventions) at multiple timepoints, while keeping other factors such as the blood sampling procedure, sample processing and storage constant. We analysed lipid profiles in plasma samples, as well as in erythrocyte samples, which we hypothesised might show less variability due to their long half-life. We observed that in plasma a larger array of lipids was present than in erythrocytes. In addition, the abundance of individual lipids and lipid classes showed remarkable variation between the sample types. Furthermore, we observed that biological reproducibility was good for the majority of lipids, and overall reproducibility was better in plasma compared to erythrocytes. However, notable differences were observed at individual- and lipid class-level that may favour the use of a particular sample type. Additionally, we provided a standardised method to pre-process the Lipidyzer™ data to guide future research with this platform.

Lipid profiling of OA severity

In our research in the NEO study we lacked insight in individual lipid species, as well as in more downstream metabolites. To overcome these limitations, we used in a subsequent project the Lipidyzer™ platform for the quantitative measurement of a large number of individual higher order lipids, as well as an in-house developed platform for the measurement of oxylipins. In chapter 5, we investigated the association of the plasma lipid profile with hand and knee

OA severity in the Applied Public-Private Research enabling OsteoArthritis Clinical Headway (APPROACH) study cohort, which is a collaborative effort between five European hospitals. Patients were included in the APPROACH cohort based on the presence of clinical knee OA and a high probability of structural or pain progression. We measured radiographic knee and hand OA severity with the Kellgren-Lawrence grading system. Furthermore, knee and hand OA disease burden were measured with the Knee Injury and Outcome Score (KOOS) subscales pain and daily function, and Functional Index for Hand OsteoArthritis (FIHOA) and a numeric rating scale (NRS) for hand pain, respectively. We used elastic net linear regularised regression to investigate the association of the lipid profile with knee and hand OA severity. Regularised regression allows to simultaneously perform automatic predictor selection and shrinkage, while also dealing with the high correlations among the lipid variables. While keeping clinical variables as age, sex, BMI and the use of lipid lowering medication constant, we observed that the lipid profile explained between 3 to 8% of the variation in radiographic OA severity in the population, depending on the specific model parameters. The lipid profile and the abovementioned clinical characteristics together explained 28% and 51% of the variation in radiographic knee and hand OA, respectively. While the lipid profile explained 12% of the variation in hand pain and 7% of the variation in hand function, we did not observe an association between the lipid profile and knee pain and function. In this study, we were limited to baseline data, and refrained from causal inferences. Follow-up measurements in the APPROACH study are still ongoing. It would be interesting to extend on this research by investigating whether the lipid profile predicts structural or pain progression in patients with knee and hand OA. Furthermore, the lipid profile could be used in deep phenotyping, which may reveal distinct phenotypes in which lipid-driven inflammation plays an important role in OA pathophysiology.

Lipidomics and anti-inflammatory treatment

In addition to the use of lipids as biomarkers of disease severity, lipidomics may be used to predict the individual response to pharmacological treatments. At the moment, there is a high unmet need for disease modifying drugs for the treatment of OA. The role of inflammation in hand OA and its association with pain[18,19] has piqued an interest for targeting inflammation in therapeutic research. The HOPE study is a blinded, randomised placebo-controlled trial, that showed a significant and clinically relevant decrease in pain in patients with hand OA treated with prednisolone[20]. However, not all patients responded to treatment. The variability in treatment response is a hurdle encountered in many treatments, that may be overcome by beforehand selecting patients who are most likely to benefit from treatment. We hypothesised that the patients' lipid profile may be predictive of prednisolone treatment response, which we explored in chapter 6. We observed that lipidomics improved the discriminative accuracy of the prediction, when compared to using commonly measured patient outcomes alone. However, it should be noted that our sample size in this study was small, thereby making the models susceptible to overfitting. Furthermore, no comparable data was available, prohibiting external validation. Also, our results may not be directly generalizable to other patient populations, or to different anti-inflammatory treatments. Despite these limitations, our results suggest that lipidomics is a promising field for biomarker discovery for the prediction of anti-inflammatory treatment response, which may further the development of personalised treatment.

Subsequently, we investigated in chapter 7 the effect of prednisolone on the patient's lipid profile. We investigated differences in change in lipid concentration after six weeks between

patients treated with prednisolone or with placebo. In order to obtain relevant and reliable results, we only analysed lipids that were well reproducible in the absence of anti-inflammatory treatment. By comparing the change in lipid concentration from baseline to week 6 between prednisolone-treated patients and placebo-treated patients, we observed that the far majority of associated lipids were glycerophospholipids. Taking multiple testing into account, we identified a significant change in the concentration of three phosphocholines, which were all comprised of a stearic acid, combined with either arachidonic acid, linolenic acid or docosahexaenoic acid. In addition, we observed that the decrease in the concentration of these lipids was associated with a decrease in AUSCAN pain and synovial thickening. These results suggest that the pain-reducing effect of prednisolone treatment could be explained by an effect on lipid levels. Potentially, these lipids are involved in the inflammatory or pain processes in patients with inflammatory hand OA. Although of an exploratory nature, our results imply that lipids could present an interesting target for future treatment development.

Metabolic consequences

Lipid dysregulation is involved in many metabolic conditions, such as cardiovascular disease (CVD). CVD is, similar to OA, a chronic condition with a long preclinical disease phase. Important markers of CVD are increased blood pressure, vessel wall stiffness and atherosclerosis. Associations between these CVD markers and OA have been shown previously, although findings are not conclusive. It has been hypothesised that atherosclerotic vascular changes result in a compromised blood flow to the joint, leading to detrimental effects on the subchondral bone and on nutrient supply to the cartilage, subsequently resulting in OA[21]. A recent systematic review concluded that an association between vascular pathology and the risk of hand and knee OA may be present. However, findings varied between studies, and different results were obtained for different OA phenotypes[22]. Moreover, to which extent CVD markers may explain the well-known association between obesity and OA has not been investigated. Therefore, in chapter 8 we assessed whether the previously established association between obesity and OA is mediated by blood pressure, arterial stiffness and subclinical atherosclerosis. While previous studies focussed mostly on systemic markers of subclinical atherosclerosis, measured as the intima-media thickness of the carotid arteries, we additionally measured the vessel wall thickness of the popliteal artery of the same knee as in which we measured structural knee OA. We hypothesised that when atherosclerotic vascular changes lead to OA, this would be most evident in arteries in close proximity to the affected joint. We used data from the NEO study, which was designed to investigate obesity-related conditions in the middle-age general population. This enabled us to study many of the previously investigated preclinical CVD markers, as well as the less often studied vessel wall thickness of the popliteal artery. We observed that blood pressure played no relevant mediating role in the association between body mass index (BMI) and OA in this population. In line with previous studies, we found no association between vessel wall stiffness and OA. We observed that carotid intima-media thickness limitedly mediated the associations of BMI with clinical hand OA, structural knee OA, and effusion. However, we found no evidence for mediation by popliteal vessel wall thickness in any of the associations. Perhaps the unexpected discrepancy in the associations of the two atherosclerosis measures, carotid intima-media thickness and popliteal vessel wall thickness, is explained by the limited variation in popliteal vessel wall thickness in our population. In addition, popliteal vessel wall thickness was measured in a subpopulation, and some data were not missing completely at random. We concluded in chapter 7 that in our investigated population mediation of the

association between BMI and OA by preclinical CVD measures was trivial. However, it should be noted that our study had a cross-sectional design, which impedes the assessment of temporal relations and hampers causal inference.

In conclusion

Taking all the results from our research described in the first part of this thesis together, we can conclude we have broadened and deepened our understanding of the association between lipids and hand and knee OA. By summarizing the available literature on the association between fatty acids and OA, we identified the major gaps in our knowledge. Notably, there was a lack of results from human (observational) studies. The research findings described in the subsequent chapters may not have closed the knowledge gaps, but hopefully has made them somewhat smaller. Previous research has focussed mainly on fatty acid classes, showing potential beneficial effects of omega-3 fatty acids. However, we found an increased odds for clinical hand OA in men for increasing levels of all fatty acids, without an indication of different effects of the fatty acid classes. In addition, we did not observe an association between fatty acids and knee OA, or any associations in women. As the relation between lipids and OA may not be as straightforward as one that can be observed by looking at the effects of entire fatty acids classes, we further focussed our research on a large array of individual lipid species. We showed which lipids, measured by an extensive, standardised and commercially available lipidomics platform, were available and reliably reproducible in two different sample types, plasma and erythrocytes. These results may guide the design of future lipidomics research. Using this lipidomics platform, we observed that the patients' lipid profile explained a small portion of OA disease severity. Furthermore, we observed that the lipid profile is predictive of anti-inflammatory treatment response, and that the concentration of particular lipid species changes upon anti-inflammatory treatment. Although the design of the described studies was exploratory, the results imply that the lipid profile is involved in OA-related inflammatory processes, and suggest a role for lipidomics in future biomarker research. All these small pieces of the puzzle may bring us a step closer to understanding the processes underlying OA pathogenesis, and eventually to the development of disease modifying treatments. We will discuss this in more detail in the later section on future perspectives.

Part 2 – Osteoarthritis disease burden

PROMs provide a standardised method to assess how the patient experiences disease burden. For the assessment of treatment efficacy, it is crucial to be able to reliably measure and adequate interpret these outcomes. Therefore, we assessed in the second part of this thesis the disease burden associated with hand and knee OA in the general population as well as in secondary care patient populations, with a specific focus on improvement of the interpretation of PROMs.

Health-related quality of life in patients with hand OA

The health-related quality of life (HRQoL) has been often studied in patients recruited from secondary or tertiary care. However, these patients may represent a distinct patient group that might differ from the general population, while OA, hand OA in particular, is mostly treated in primary care. Differences that may be encountered between these populations are symptom severity, disabilities, or the co-occurrence of OA in other joints. In patients recruited

from the rheumatology clinic, a reduced physical HRQoL has been reported[23–25], while studies that have investigated the impact of hand OA in the general population have shown no or limited effects on physical HRQoL[26,27]. Similarly, the mental HRQoL may be affected differently in these populations[23–29]. However, previous studies have used a variety of OA definitions and phenotypes, which hinders a valid comparison of findings from different populations. In chapter 9, we combined data from the NEO study and the Hand OSTeoArthritis in Secondary care (HOSTAS) study, which offered the unique opportunity to investigate individuals with hand OA from the general population and from a rheumatology outpatient clinic in the same region. We observed a modest, but clinically relevant lower physical HRQoL in participants with hand OA in the general population, when compared with participants without OA. Moreover, we concluded from data from both cohorts that individuals with hand OA who had consulted a medical specialist for OA complaints, had a lower physical HRQoL than individuals who had not consulted a specialist. Mental HRQoL was not associated with hand OA alone, neither in the general population nor in patients in secondary care. In both patient groups we observed a lower physical HRQoL in patients with concurrent hand and knee OA, compared with patients with hand OA alone. Furthermore, concurrent hand and knee OA was weakly associated with mental HRQoL; however, the impact on mental HRQoL was below the minimal clinically important difference threshold in both populations. These findings emphasize that generalization of findings from different patient populations should be made with caution. Furthermore, these results may raise awareness of concurrent OA in other joints, as this may warrant different patient management.

Physical activity in patients with knee OA

In the absence of disease modifying treatments, other interventions may improve disease burden. Physical activity is a modifiable lifestyle factor that is associated with disease outcomes, and may therefore be a target for intervention. However, current knowledge on the physical activity of individuals with knee OA in the Netherlands is restricted to the elderly population or end-stage disease[30,31]. Ideally, lifestyle interventions are implemented in the middle-aged population, before patients have progressed to severe or end-stage disease. Therefore, we investigated the association of knee OA with physical activity in the general middle-aged Dutch population. Furthermore, in individuals with knee OA, we investigated the association of physical activity with PROMs such as knee pain and function, and HRQoL. In contrast to previous findings, we observed that knee OA was positively associated with self-reported physical activity, which was more evident for clinical than for structural knee OA. Furthermore, in individuals with clinical knee OA no clear association of self-reported physical activity with physical functioning, knee pain, and HRQoL was found. The observed positive association between self-reported physical activity and knee OA, and how these findings diverge from previous studies, may have several explanations. Possibly, the relation between physical activity is different in the middle-aged population from individuals of older age, or with more severe disease. Perhaps, middle-aged individuals with mild knee OA complaints are not able to restrict their activities, are not considerably hindered to be physically active or even well-motivated to address their complaints by a targeted increase in physical activity or by physical therapy. Furthermore, self-reported physical activity is prone to social desirability bias, as well as recall bias, which may cause overestimation of the physical activity reported. This is especially likely in our clinical knee OA group, as painful and arduous activities are likely to be over-reported in questionnaires[32]. We also see this reflected by the weaker association between structural knee OA, which is not primary pain-driven,

and physical activity, than the association of clinical knee OA and physical activity. Therefore, we also investigated an objective physical activity measure, the ActiHeart accelometer, in a subset of the study population. Using an objectively measured outcome, we observed a weak positive association between clinical knee OA and physical activity, which was similar to the association between structural knee OA and self-reported physical activity. This may imply that indeed pain aggravates overreporting of physical activity. However, it also refutes that our findings are fully explained by bias associated with self-reported measures. Finally, our results support that in contrast to other populations there is no association between knee OA and physical activity in the Dutch middle-aged general population.

Improving the interpretation of outcome measures

Knee complaints, often caused by knee OA, are among the most reported complaints of the musculoskeletal system. The KOOS questionnaire has been developed to assess the patients' burden due to knee complaints[33]. The interpretation of PROMs such as the KOOS relies on relevant benchmarks. A suboptimal score may be unrelated to the musculoskeletal condition under investigation, which has been shown by previous studies on different knee-specific questionnaires[34–36]. Therefore, we explored in chapter 11 factors that may influence KOOS scores, and developed percentile curves in the general Dutch population (NEO study). We showed that sex and BMI were strongly associated with KOOS scores, while age was not consistently associated with the KOOS. Therefore, the percentile curves were developed in a sex- and BMI-specific manner. Women scored worse on all KOOS subscales, which is in line with previous research[37–39]. Interestingly, our results indicate that increasing BMI may play an important role in the interpretation of KOOS scores, as a higher BMI was associated with worse scores. Remarkably, the association of BMI with KOOS scores has only been briefly touched upon by a limited number of other studies[38,39], warranting further investigation of the role of BMI on PROMs to verify our results. Possible applications of the percentile curves may be to determine how the KOOS scores of individual patients relate to the reference population, but could also be used to track changes in scores following for example physical therapy or knee surgery. In addition, the curves may be used to get more insight in how the scores of specific patient groups relate to the scores in the general population.

In chapter 12, we elaborate on the applicability of the KOOS percentile curves, by using them to compare real-world clinical data of patients undergoing total knee arthroplasty (TKA) with the general population. We used data from the Longitudinal Leiden Orthopeadics Outcomes of Osteo-Arthritis study (LOAS), including patients between 45 and 65 years and undergoing primary TKA for end-stage knee OA. We plotted the median preoperative and 12 months postoperative KOOS scores on the percentile curves obtained from the general population. Notably, great inter-patient variability was observed for all subscale scores. In comparison to the general population, preoperative median KOOS scores of all subscales were at or below the 2.5th percentile. This finding is according to expectation, as these scores were obtained from patients undergoing TKA for their knee complaints. However, the comparison with the general population provided more insight in the interpretation of the scores in comparison to, for example, a score of 34 out of 100 (the mean preoperative KOOS pain score of the LOAS population). Subsequently, we plotted the 12 months postoperative scores, and observed that median pain scores were around the 25th percentile in men, and between the 25th and 50th percentile in women. Median symptom and ADL function scores increased to around the 25th percentile postoperatively in both men and women. Similarly, postoperative QOL scores were around the 25th percentile in men. In women somewhat higher postoperative QOL

scores were observed, approaching the 50th percentile in women with a high BMI. Sport and recreation scores increased slightly in all patients postoperatively; however, they remained around the 10th percentile of the general population. These findings imply that although KOOS scores improved impressively after surgery, in the majority of patients KOOS scores did not normalize to the median score of the general population. Additionally, we observed that absence of moderate to severe preoperative radiographic OA, as well as the presence of comorbidities, was associated with less improvement after surgery. Visualising the expected improvements after treatment, and differences in treatment benefit based on preoperative patient characteristics, may help making a well-informed patient-centred treatment decision, and may help manage patient expectations to reduce dissatisfaction after surgery[40,41].

In conclusion

There are many PROMs available, each providing insights in OA disease burden. The research described in the second part of this thesis showed that such outcomes may vary between different study populations. We observed that hand OA was most evidently associated with a lower physical HRQoL in individuals seeking specialised care for their complaints, and in co-occurrence of OA in other joints. We described that in the general Dutch population of middle-age knee OA was not associated with lower physical activity levels. An explanation for this finding could be the awareness of the benefits of physical activity for OA outcomes. In addition, this research underscored the lack of generalizability of findings from other countries, such as the United States, to the Dutch population. Since PROMs can be difficult to interpret in the absence of relevant benchmarks, we developed percentile curves for the frequently used KOOS questionnaire based on data from the Dutch general population. Additionally, we investigated the application of the KOOS percentile curves using data from patients with knee OA undergoing TKA. We observed that the KOOS percentile curves provided additional insights and aided the interpretation of the KOOS scores. An alternative format to present PROMs may aid patient-clinician communication and shared decision making.

Methodological considerations

In order to adequately interpret and value the described research findings, it is essential to take some methodological considerations into account. The majority of the results described in this thesis have been obtained from observational cohort studies. The reliability of results from observation studies depends on how challenges to the *internal validity* and *external validity* are handled.

Causal inference

In order to answer research questions of etiological nature, it is important to consider to what extent causal identification conditions are met. A frequently used phrase is “correlation is not causation”. What this often refers to is a lack of *exchangeability*, most notably the presence of confounding bias. A commonly applied method to identify possible confounding factors is the use of a causal diagram, such as a directed acyclic graph (DAG). In a DAG we include variables which are known to be causally associated with the exposure and the outcome (see chapters 3 and 7 for examples). Unconditional exchangeability is rare in observational studies. With regard to the results presented in this thesis, we assumed conditional exchangeability by identified potential confounders using DAGs, and subsequently adjusting for them in our regression models, or by stratification of the analyses. However, we should always be

aware of the possibility of unmeasured or unknown confounding that might have resulted in misinterpretation of the true association.

Furthermore, especially upon adjusting and stratification, the probability of levels greater than zero for every level of every included variable (a condition known as *positivity*) should be considered. Using study populations of sufficient size such as we have described in this thesis, often solves positivity concerns.

Another important causality condition is *consistency*, which requires no or neglectable variation in the exposure. For instance, patients with hypertension may have a systolic blood pressure of 210 mmHg or 145 mmHg, while most will agree that these blood pressures do not represent similar exposures. Where possible, we have accounted for possible breaches of consistency by using quantitively measured exposures in our regression analyses. For example, in contrast to using the dichotomous variable hypertension, we opted in chapter 7 to use blood pressure as a continuous measure. Furthermore, we quantitively measured lipid levels in blood samples in chapters 3 to 6, and measured the thickness of the vessel walls in chapter 7.

Closely related to a lack of consistency is the problem of variability introduced by *measurement error*. Many epidemiological studies rely in part on self-reported data, often in the form of general or disease specific questionnaires. Self-reported data may be subject to social desirability bias or recall bias, amongst others. In the studies described in this thesis, we have tried to minimize the extent to which information bias introduced by self-report may have affected the reliability of our estimates by using validated questionnaires, which often involve a specific timeframe covering a relatively short period. In addition, measurement error may also arise during analytical measurements. Therefore, to gain more insight in the variability of the main lipidomic platform we have used in this thesis, we have evaluated the variability of the Lipidyzer™ measurements, put extensive effort in eliminating batch effects and including quality controls, as well as addressing the pre-processing of lipidomics data in chapter 4.

The cross-sectional research design that has been used in many studies described in this thesis, inherently hampers causal inference. Key for a causal relation, is that the exposure must have occurred prior to the outcome. In most cases, it is impossible to ensure *temporality* in cross-sectionally designed studies. For this we rely on assumptions and knowledge from previous findings. Additionally, we can perform sensitivity analyses. For example, in chapter 7 we investigated whether cardiovascular markers mediated the association between obesity and OA. Since exposures and outcomes were measured at the same time, we cannot exclude those participants with a history of cardiovascular disease or hypertension have altered their lifestyle, leading to weight reduction. This would have led to an underestimation of the association. Therefore, we repeated the analyses after exclusion of participants with a history of cardiovascular disease, which did not change our results. However, the possibility of *reverse causation* should always be considered when interpreting results from cross-sectional studies.

In addition to *internal validity* considerations, it is crucial to address *external validity*. Findings are externally valid, when the true effect in the study population equals the true effect in the target population. One particular example of how external validity may be compromised, is by the healthy-attendant bias that is often present in population-based studies. The healthy attendant bias, or also sometimes referred to as healthy user bias or healthy worker bias, is a

form of selection that arises from a high response rate of healthy individuals relative to those with disease. We encountered this type of bias in chapter 10 of this thesis. We observed that participants from the NEO study had on average, in comparison to the norm (i.e., the true general population), a higher quality of life. This is crucial to take into account, especially in comparative studies with other (patient) populations, where the healthy-attended bias may be absent or present to a smaller extent. Norm-based scores provide an elegant solution to this problem. To account for the healthy attendant bias, we compared the deviation in scores of the study populations from the norm, in contrast to directly comparing scores from different study populations.

Predictive epidemiology

Predictive epidemiology is another important aspect of clinical research, which may be used to determine disease status (diagnostic testing), predict the development of disease in the future (prognostic testing), or as described in this thesis, discrimination of patient in classes (chapter 5) and the prediction of treatment response (chapter 6). Prediction is a form of noncausal analysis, and therefore warrants a different interpretation of the results. A famous example that clearly explains the difference between causal and predictive epidemiology is the association of gray hair and mortality. While gray hair is a great predictor of mortality, it is obvious that gray hairs do not *cause* death. However, in most research the underlying relationship of exposure and outcome is less evident. Therefore, it is crucial to realize that in predictive research, conditions for causal inference are often not met, and results should not be interpreted as such. An elaborate discussion of prediction models is beyond the scope of this thesis. However, some elements of predictive epidemiology are essential to emphasize to put the results of chapters 5 and 6 into the right perspective.

Similar to causal epidemiology, *internal and external validity* are vital aspects of prediction models. Prediction models are constructed from a single patient population, which leads to the statistical phenomenon of “optimism”, or “overfitting”. To provide more accurate estimations, many methods have been developed to improve the internal validity, such as cross-validation, bootstrapping and permutation testing, some of which have been used in chapters 5 and 6. However, the efficacy of these methods also rely on the data at hand. For example, in chapter 6 the patient population was small, and our models are, despite our efforts to improve the internal validity, likely overfitted to a certain extent.

In addition, external validation entails the evaluation of the prediction models in datasets that were not used to develop the model. Due to the novel use of lipidomics in OA research, we lacked comparative data. Therefore, we were not able to perform external validation of the models described in this thesis, which requires further research.

Future perspectives

The results described in this thesis add to our understanding of how obesity and inflammation-related systemic factors such as lipids might be involved in the pathogenesis of hand and knee OA. Furthermore, our findings contribute to our knowledge on OA disease burden. Both lines of research are essential to get further along the path to new treatments for OA. The first may aid in the discovery of new treatment targets, while the latter improves our interpretation of outcomes investigating treatment benefit. However, there are some limitations to the described studies. Most notably, the majority of the analyses have been performed with

cross-sectionally obtained data, which is limited in several ways as discussed in detail above. In addition, we have used explorative methods in relatively small study populations. These limitations represent challenges that future research should seek to overcome.

Ten-year follow-up of the NEO study
The NEO study has been designed as a prospective cohort study to investigate the pathways that lead to obesity-related diseases and conditions. The NEO study included men and women between 45 and 65 years of age, with an oversampling of participants with overweight or obesity, from 2008 to 2012. The research described in this thesis has been performed using cross-sectional analyses of the baseline data. At the moment, participants are being recalled for 10-year follow-up visits. During this second visit, various OA-related measures used in the current research will be repeated. Similar to the baseline visit, extensive questionnaires regarding hand and knee pain and function will be administered, standardised physical examinations of the joints will be performed to assess palpable warmth, pain, bony deformation and movement restrictions, and in a random subset of the participants an MRI of the knee will be performed. Follow-up measurements of the NEO study will provide a vast amount of data that allow the assessment of OA incidence and progression. The longitudinally obtained data could reveal causal relationships and may be used to confirm findings presented in this thesis.

Prospective results of the APPROACH study
The APPROACH study is a 2-year, prospective multi-centre cohort study. In this thesis, we have described cross-sectional analyses of the baseline data, investigating the association of the lipid profile with knee and hand OA severity. At the moment, the study is in a final phase, performing 2-year follow-up visits in all centres. It will be very interesting to investigate the ability of lipidomics to distinguish patients with a higher probability of structural and pain progression. Furthermore, the longitudinal data will enable etiological research, which may further our knowledge on the role of lipidomics in OA pathogenesis and progression.

Biomarker research
This thesis describes several exploratory studies investigating the use of lipidomics for both causal as predictive epidemiology. Due to several limitations described previously, we should regard the current findings as first steps in lipidomics research in hand and knee OA. Although there still is a long way to go before lipid biomarkers can be implemented in clinical care, our results suggested that lipidomics may be promising for future biomarker development. There are several items that warrant careful consideration for biomarker development, of which the following a just a few examples. One of the important questions that remains unanswered, is in what samples biomarkers for OA should preferable be measured. While blood samples are easy to obtain, synovial fluid may be a better representation of local effects on the joint. On the other hand, OA is frequently concomitant in multiple joint locations, and therefore biomarker development might require a more holistic approach. Another aspect is het use of fasting versus non-fasting lipid measurements. At the moment, most metabolomic and lipidomic research uses fasting (blood) samples to limit short-term dietary fluctuations. However, our current lifestyle results in a postprandial state during most of the day. Therefore, non-fasting measurements may be a better reflection of our lipid profile. In addition, non-fasting samples increases practicality in large epidemiological studies, in which fasted sampling may not be feasible.

Lipid treatment in OA

The 'omics' approaches provide a great amount of information. Lipidomics research in OA is an upcoming field and developing fast, which started from the measurement of a small number of lipids and progressing to the measurement of large unstandardised and standardised lipid platforms. One of the greatest challenges will be the translation of these findings, and to place them into a broad, patient-centred context. The current hypothesis-generating, exploratory research phase will need to be followed by a targeted phase, in which available findings are externally validated in large new cohorts. Subsequently, these hypotheses will need to be tested, preferably in clinical randomised trials. The design, and especially the selection of a suitable patient population for clinical trials presents another major challenge. OA is a complex, heterogeneous, multifactorial disease, in which not only lipids, but also metabolomics, proteomics, (epi)genomics and transcriptomics may be involved. Moreover, there is likely an interaction between all these biological factors and demographic and environmental factors. When all these factors are combined, distinct OA phenotypes may be defined, which may show varying progression trajectories that warrant a personalised treatment approach. The 'one-size fits all' approach that has been adapted historically has not resulted in adequate and effective treatment of OA. Therefore, the identification of well-defined OA phenotypes is essential for future trial designs, as well as clinical observational studies.

References

1 GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017; 390: 1211–59.

2 Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377: 2115–26.

3 Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012; 64: 1697–707.

4 Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis. The Framingham Study. Ann Intern Med 1988; 109: 18–24.

5 Davis MA, Ettinger WH, Neuhaus JM. Obesity and osteoarthritis of the knee: evidence from the National Health and Nutrition Examination Survey (NHANES I). Semin Arthritis Rheum 1990; 20: 34–41.

6 Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. Lancet 1972; 1: 519–22.

7 Yusuf E, Nelissen RG, Ioan-Facsinay A, et al. Association between weight or body mass index and hand osteoarthritis: a systematic review. Ann Rheum Dis 2010; 69: 761–5.

8 Visser AW, Ioan-Facsinay A, de Mutsert R, et al. Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. Arthritis Res Ther 2014; 16: R19.

9 Visser AW, de Mutsert R, le Cessie S, et al. The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. Ann Rheum Dis 2015; 74: 1842–7.

10 Dickson BM, Roelofs AJ, Rochford JJ, Wilson HM, De Bari C. The burden of metabolic syndrome on osteoarthritic joints. Arthritis Res Ther 2019; 21: 289.

11 Ertunc ME, Hotamisligil GS. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. J Lipid Res 2016; 57: 2099–114.

12 Wymann MP, Schneiter R. Lipid signalling in disease. Nat Rev Mol Cell Biol 2008; 9: 162–76.

13 Breier M, Wahl S, Prehn C, et al. Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples. PLoS ONE 2014; 9: e89728.

14 Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995; 62: 572–8.

15 Floegel A, Drogan D, Wang-Sattler R, et al. Reliability of serum metabolite concentrations over a 4-month period using a targeted metabolomic approach. PLoS ONE 2011; 6: e21103.

16 Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE, Turner RC. Within- and between-subject variation in commonly measured anthropometric and biochemical variables. Clin Chem 1999; 45: 561–6.

17 Yu Z, Kastenmüller G, He Y, et al. Differences between human plasma and serum metabolite profiles. PLoS ONE 2011; 6: e21230.

18 Kortekaas MC, Kwok W-Y, Reijnierse M, Watt I, Huizinga TWJ, Kloppenburg M. Pain in hand osteoarthritis is associated with inflammation: the value of ultrasound. Ann Rheum Dis 2010; 69: 1367–9.

19 Keen HI, Wakefield RJ, Grainger AJ, Hensor EMA, Emery P, Conaghan PG. An ultrasonographic study of osteoarthritis of the hand: synovitis and its relationship to structural pathology and symptoms. Arthritis Rheum 2008; 59: 1756–63.

20 Kroon FPB, Kortekaas MC, Boonen A, et al. Results of a 6-week treatment with 10 mg prednisolone in patients with hand osteoarthritis (HOPE): a double-blind, randomised, placebo-controlled trial. The Lancet 2019; 394: 1993–2001.

21 Findlay DM. Vascular pathology and osteoarthritis. Rheumatology (Oxford) 2007; 46: 1763–8.

22 Hussain SM, Dawson C, Wang Y, et al. Vascular Pathology and Osteoarthritis: A Systematic Review. J Rheumatol 2020; 47: 748–60.

23 Kwok WY, Vliet Vlieland TPM, Rosendaal FR, Huizinga TWJ, Kloppenburg M. Limitations in daily activities are the major determinant of reduced health-related quality of life in patients with hand osteoarthritis. Ann Rheum Dis 2011; 70: 334–6.

24 Slatkowsky-Christensen B, Mowinckel P, Loge JH, Kvien TK. Health-related quality of life in women with symptomatic hand osteoarthritis: a comparison with rheumatoid arthritis patients, healthy controls, and normative data. Arthritis Rheum 2007; 57: 1404–9.

25 Moe RH, Grotle M, Kjeken I, Hagen KB, Kvien TK, Uhlig T. Disease impact of hand OA compared with hip, knee and generalized disease in specialist rheumatology health care. Rheumatology (Oxford) 2013; 52: 189–96.

26 Carmona L, Ballina J, Gabriel R, Laffon A, EPISER Study Group. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. Ann Rheum Dis 2001; 60: 1040–5.

27 Lombnæs GØ, Magnusson K, Østerås N, Nordsletten L, Risberg MA, Hagen KB. Distribution of osteoarthritis in a Norwegian population-based cohort: associations to risk factor profiles and health-related quality of life. Rheumatol Int 2017; 37: 1541–50.

28 Cuperus N, Vliet Vlieland TPM, Mahler EAM, Kersten CC, Hoogeboom TJ, van den Ende CHM. The clinical burden of generalized osteoarthritis represented by self-reported health-related quality of life and activity limitations: a cross-sectional study. Rheumatol Int 2015; 35: 871–7.

29 Axford J, Butt A, Heron C, et al. Prevalence

of anxiety and depression in osteoarthritis: use of the Hospital Anxiety and Depression Scale as a screening tool. Clin Rheumatol 2010; 29: 1277–83.
30 Herbolsheimer F, Schaap LA, Edwards MH, et al. Physical Activity Patterns Among Older Adults With and Without Knee Osteoarthritis in Six European Countries. Arthritis Care & Research 2016; 68: 228–36.
31 de Groot IB, Bussmann JB, Stam HJ, Verhaar JAN. Actual everyday physical activity in patients with end-stage hip or knee osteoarthritis compared with healthy controls. Osteoarthritis and Cartilage 2008; 16: 436–42.
32 van Giezen AE, Arensman E, Spinhoven P, Wolters G. Consistency of memory for emotionally arousing events: a review of prospective and experimental studies. Clin Psychol Rev 2005; 25: 935–53.
33 Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998; 28: 88–96.
34 Bremner-Smith AT, Ewings P, Weale AE. Knee scores in a 'normal' elderly population. Knee 2004; 11: 279–82.
35 Demirdjian AM, Petrie SG, Guanche CA, Thomas KA. The outcomes of two knee scoring questionnaires in a normal population. Am J Sports Med 1998; 26: 46–51.
36 Jinks C, Jordan K, Croft P. Measuring the population impact of knee pain and disability with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Pain 2002; 100: 55–64.
37 Paradowski PT, Bergman S, Sundén-Lundius A, Lohmander LS, Roos EM. Knee complaints vary with age and gender in the adult population. Population-based reference data for the Knee injury and Osteoarthritis Outcome Score (KOOS). BMC Musculoskelet Disord 2006; 7: 38.
38 Marot V, Murgier J, Carrozzo A, et al. Determination of normal KOOS and WOMAC values in a healthy population. Knee Surg Sports Traumatol Arthrosc 2018; published online Sept 24. DOI:10.1007/s00167-018-5153-6.
39 Williamson T, Sikka R, Tompkins M, Nelson BJ. Use of the Knee Injury and Osteoarthritis Outcome Score in a Healthy United States Population. Am J Sports Med 2016; 44: 440–6.
40 Yang LY, Manhas DS, Howard AF, Olson RA. Patient-reported outcome use in oncology: a systematic review of the impact on patient-clinician communication. Support Care Cancer 2018; 26: 41–60.
41 Scott CEH, Howie CR, MacDonald D, Biant LC. Predicting dissatisfaction following total knee replacement: a prospective study of 1217 patients. J Bone Joint Surg Br 2010; 92: 1253–8.

13

Nederlandse samenvatting

Introductie

Wereldwijd lijden er ongeveer 300 miljoen mensen aan artrose. Dit maakt artrose een van de meest voorkomende reumatische aandoeningen van het bewegingsapparaat. Artrose is niet te genezen, en is daarmee een chronische ziekte. Belangrijke risicofactoren voor artrose zijn ouder worden en zwaarlijvigheid. Omdat we steeds ouder worden, en helaas een steeds groter deel van onze bevolking te zwaar is, is de verwachting dat in de komende jaren het aantal mensen met artrose nog verder zal toenemen. Artrose is een grote belasting voor de patiënt; het veroorzaakt pijn, stijfheid, verlies van functioneren en een vermindering van de kwaliteit van leven. Artrose treft het hele gewricht; het leidt tot kraakbeenschade, veranderingen in botvorming, vorming van benige uitsteeksels (osteofyten), schade aan ligamenten en ontsteking van het synoviale weefsel. Het kan in elk gewricht voorkomen, maar wordt het vaakst gezien in de handen en knieën. Momenteel zijn de behandelmogelijkheden voor artrose beperkt tot verlichting van symptomen zoals pijn. Er is dan ook een dringende noodzaak voor de ontwikkeling van behandelingen die verergering van de ziekte kunnen stoppen, of idealiter de opgelopen schade kunnen herstellen.

Hoewel er in de afgelopen jaren grote stappen zijn gemaakt om het ziektebeeld artrose beter te begrijpen, weten we nog steeds niet goed hoe artrose precies ontstaat. Wel weten we dat overgewicht, in de ernstige vorm vaak obesitas genoemd, een belangrijke rol speelt in het ontstaan van artrose. Tot voor kort werd er gedacht dat de relatie tussen obesitas en artrose volledig verklaard werd door een toename van lichaamsgewicht drukkend op de gewrichten. Echter, meer recente onderzoeksresultaten laten zien dat er tevens een relatie is tussen obesitas en gewrichten die geen lichaamsgewicht dragen, zoals de handen. Om die reden is het waarschijnlijk dat obesitas ook andere effecten moet hebben op artrose. Hierbij wordt gedacht aan effecten door het hele lichaam, ook wel systemische effecten genoemd. Mogelijke factoren die een rol spelen zijn stoffen die zorgen voor ontstekingen (cytokines). Ook het vetweefsel scheidt dit soort ontstekingveroorzakende stoffen uit (adipokines), net als vetten, ook wel lipiden genoemd. Ons begrip over deze factoren, en hoe die betrokken zijn bij het ontstaan en verergeren van artrose staat nog in de kinderschoenen. Hoewel de kennis door laboratoriumonderzoek en dierproeven flink is gegroeid, weten we weinig van dit onderwerp uit wetenschappelijke onderzoeken in mensen. Inzichten uit klinische onderzoek kunnen ons helpen om de ontstaanswijze van artrose beter te begrijpen, en nieuwe aangrijpingspunten te ontdekken voor de behandeling. In het eerste deel van dit proefschrift onderzochten we daarom hoe lipiden gerelateerd zijn aan hand- en knieartrose. Een ander belangrijk aspect in de ontwikkeling van nieuwe behandelingen is het kiezen van geschikte meetinstrumenten. In het tweede deel van dit proefschrift besteedden we aandacht aan verscheidene patiënt-gerapporteerde uitkomsten, en hoe die inzicht bieden in hoe de patiënt de ziektelast van hand- en knieartrose ervaart.

Deel 1 - Lipiden en andere systemische factoren in artrose

Kennis over of, en hoe, systemische- en ontstekingsfactoren betrokken zijn bij artrose kan ons helpen om de ontstaanswijze van artrose beter te begrijpen en aangrijpingspunten te vinden voor de behandeling van artrose. In het eerste deel van dit proefschrift onderzochten we daarom hoe lipiden en uitingen van een verstoorde vetstofwisseling gerelateerd zijn aan hand- en knieartrose.

Vetzuren

Het proefschrift begint met een samenvatting van de beschikbare literatuur over de rol van vetzuren in de ontwikkeling en verergering van artrose, om vast te stellen wat de huidige kennis over dit onderwerp is. De onderzoeksresultaten die we hierin samengevat hebben, laten zien dat vetzuren zowel betrokken zijn bij symptomen van artrose, als bij structurele veranderingen in het gewricht. Opmerkelijk is dat de verschillende vetzuren een verschillend effect lijken te hebben. Bijvoorbeeld, het stimuleren van kraakbeencellen (chondrocyten) met omega-3 meervoudig onverzadigde vetzuren verlaagt de aanwezigheid van ontstekingsfactoren en kraakbeenafbraak. Daarentegen zorgt stimulatie van chondrocyten met omega-6 meervoudig onverzadigde vetzuren en verzadigde vetzuren juist voor meer ontsteking en kraakbeenafbraak. Dierproeven laten zien dat het toevoegen van omega-3 meervoudig onverzadigde vetzuren aan het dieet leidt tot minder schade in het gewricht ten gevolge van artrose, terwijl diëten met meer omega-6 meervoudig onverzadigde vetzuren en verzadigde vetzuren zorgen voor meer ontsteking en schade aan het kraakbeen. Het is echter moeilijk om bevindingen uit laboratoriumonderzoek en diermodellen te vertalen naar relevante inzichten voor de mens. Helaas waren humane studies slechts mondjesmaat terug te vinden. Hoewel het geringe aantal humane studies wel gelijkaardige resultaten laat zien als het experimenteel onderzoek, is het duidelijk dat meer klinische studies noodzakelijk zijn om definitieve conclusies te kunnen trekken over de relatie tussen vetzuren en artrose in de mens. Daarnaast zijn de huidige onderzoeken beperkt tot patiënten met knie- en heupartrose. Dit terwijl systemische factoren mogelijk juist een prominente rol zouden kunnen spelen in gewrichten die geen gewicht dragen, zoals de hand. Daarom is het aangewezen te onderzoeken of vetzuren ook een rol spelen in patiënten met handartrose.

In hoofdstuk 3 hebben we dan ook juist die onderzoeksvraag aangekaart. In een groot bevolkingsonderzoek, de Nederlandse Epidemiologie van Obesitas (NEO) studie, hebben we bij alle deelnemers onderzocht of er pijnklachten waren van de handen en knieën. Daarnaast hebben we deze gewrichten onderzocht op typische bevindingen van artrose zoals pijn, bewegingsbeperkingen, zwelling en warmte. Bij alle deelnemers is bloed afgenomen en hierin hebben we de verschillende vetzurengroepen (verzadigd, enkelvoudig onverzadigd, omega-3 en omega-6 meervoudig onverzadigd) bepaald. De statistische analyses lieten zien dat in het bloed van mannen met handartrose de concentraties van zowel verzadigde vetzuren, als totaal en omega-3 meervoudig onverzadigde vetzuren hoger waren dan in mannen zonder artrose. Opmerkelijk genoeg zagen we deze relatie niet bij vrouwen. Ook zagen we geen relatie tussen de concentratie van vetzuren en hand- of kniepijn. De verschillen tussen mannen en vrouwen waren opvallend, en zijn niet eenvoudig te verklaren. Zulke sekseverschillen worden echter vaker gezien. Een mogelijke verklaring is een verschillende relatieve bijdrage van systemische versus mechanische effecten van obesitas tussen mannen en vrouwen kunnen zijn. Een andere verklaring kan gevonden worden in verschillen in de vetstofwisseling tussen mannen en vrouwen.

Reproduceerbaarheid van lipidenmetingen

In het lichaam komen veel verschillende soorten lipiden voor. Deze worden veelal gegroepeerd zoals gepresenteerd in het NEO onderzoek (enkelvoudig/meervoudig, verzadigd/onverzadigd). Echter het is ook mogelijk al deze lipiden apart te onderzoeken. Lipidomics, een samenvoeging van lipide (vetdeeltje) en -omics (naar het van Sanskriet afgeleide “Om” dat vrij vertaald allesomvattend betekend), is een onderzoeksveld dat poogt al deze lipiden tegelijk te onderzoeken. Lipiden zijn stoffen die belangrijk zijn voor veel

lichamelijke functies, maar lipiden zijn ook betrokken bij ziekteprocessen. Lipidomics, ook wel het lipidenprofiel van een patiënt genoemd, kan daarom mogelijk een rol als biomarker vervullen. Biomarkers zijn specifieke metingen die gebruikt worden om de aanwezigheid een ziekte aan te tonen, de ernst van een ziekte te meten of het ontstaan of verergeren van een ziekte te voorspellen. Om een biomarker voor dit soort doeleinden in te zetten, is het belangrijk om een betrouwbare meting te kunnen nemen. Metingen van de concentraties van een dergelijke biomarker kunnen verschillen, bijvoorbeeld door de manier waarop een (bloed)monster wordt afgenomen, verwerkt of opgeslagen. Ook kunnen de concentraties van een biomarker fluctueren door normale lichamelijke processen binnen een individu. Bij de ontwikkeling van een geschikte biomarker zijn dit belangrijke aspecten om mee te nemen. Bij lipidomics is nog weinig gekeken naar de reproduceerbaarheid van herhaalde metingen. Ook zijn niet alle verschillende monstertypes zoals de verschillen in betrouwbaarheid tussen plasma en rode bloedcellen reeds onderzocht. In hoofdstuk 4 hebben we onderzocht wat de reproduceerbaarheid is van metingen met een groot lipidenplatform. Met dit Lipidyzer™ platform kunnen meer dan duizend verschillende lipiden tegelijk gemeten worden. Hierbij hebben we zoveel mogelijk alle andere factoren, zoals de bloedafnameprocedure en verwerking en opslag van de monsters, constant gehouden om de natuurlijk voorkomende variatie binnen een individu te onderzoeken. We hebben dit onderzocht in twee monstertypes: plasma en rode bloedcellen. Hoewel plasma een gemakkelijk verkrijgbare en veelgebruikte component van het bloed is, hadden we op voorhand de hypothese dat in rode bloedcellen de metingen wellicht minder variabel zouden zijn doordat rode bloedcellen een lange levensduur hebben. We zagen dat in plasma meer verschillende lipiden voorkomen dan in rode bloedcellen, en dat over het geheel genomen in plasma de metingen beter reproduceerbaar waren. Echter de reproduceerbaarheid was sterk afhankelijk van het type lipide, en sommige lipidenmetingen waren juist beter reproduceerbaar in rode bloedcellen. De resultaten van dit onderzoek kunnen richting bieden bij biomarkeronderzoek met het Lipidyzer™ platform, waarbij de interesse in specifieke lipiden of lipidenklassen leidend kan zijn voor de keuze van een monstertype.

Lipidenprofiel en ernst van artrose

In hoofdstuk 5 keken we wederom verder dan alleen de grote vetzuurgroepen, en onderzoeken we de relatie tussen de lipiden gemeten met het Lipidyzer™ platform in plasma en de mate van schade door artrose gezien op röntgenfoto's van handen en knieën, als ook de mate van pijn en functieverlies. We gebruikten hiervoor gegevens uit de Applied Public-Private Research enabling OsteoArthritis Clinical Headway (APPROACH) studie, een samenwerkingsverband tussen vijf ziekenhuizen in Europa. In dit onderzoek zagen we dat het lipidenprofiel slechts een klein deel van de verschillen in de ernst van de artrose tussen patiënten kon verklaren. Voor de verschillende uitkomsten zagen we het sterkste verband tussen het lipidenprofiel met handpijn. Verrassend genoeg zagen we geen verband tussen het lipidenprofiel en knieartrose. Dit sluit aan bij eerdere onderzoeksresultaten dat het verband tussen overgewicht en knieartrose in vooral verklaard wordt door het extra gewicht dat op het kniegewricht drukt en niet zozeer door de met overgewicht geassocieerde verstoorde vetstofwisseling. De APPROACH studie heeft patiënten gedurende 2 jaar opgevolgd. Helaas waren deze gegevens ten tijde van dit schrijven nog niet voorhanden. Deze gegevens bieden de mogelijkheid om in de toekomst te onderzoeken of het lipidenprofiel verband houdt met het verergeren van artrose.

Lipidomics en ontstekingsremmende medicatie
Naast het gebruik van lipiden als biomarkers voor de ernst van een ziekte, zoals we in hoofdstuk 5 hebben onderzocht, kan lipidomics tevens gebruikt worden om te voorspellen of een patiënt baat zal hebben bij een bepaald medicijn. Op dit moment is er geen medicament beschikbaar dat het ziektebeloop van artrose kan beïnvloeden. Eerder onderzoek heeft laten zien dat ontsteking een rol speel in artrose, en dat deze ontsteking gecorreleerd is met pijn. Dit heeft tot de onderzoeksvraag geleid of ontsteking een aangrijpingspunt zou kunnen zijn voor de behandeling van artrose. De Hand Osteoarthritis Prednisolone Efficacy (HOPE) studie is een gerandomiseerd dubbelblind onderzoek waarin is aangetoond dat behandeling met prednison leidt tot een significante vermindering van pijn. Echter, niet alle patiënten toonden verbetering met de behandeling. Verschillen in behandelresponse tussen patiënten ondanks dezelfde behandeling is een obstakel bij vele behandelingen. We hypothetiseerden dat het lipidenprofiel van de patiënt voorspelt of een patiënt baat zal hebben bij de behandeling met prednison. De resultaten van dit onderzoek hebben we beschreven in hoofdstuk 6. We zagen dat het toevoegen van lipidomics aan algemene patiëntkarakteristieken inderdaad de voorspelling verbeterde ten opzichte van een voorspelmodel met alleen algemene patiëntkarakteristieken. Echter, dit was een exploratief onderzoek, met een kleine onderzoeksgroep. Het is daarom belangrijk dat onze resultaten bevestigd worden met onderzoek in een grotere populatie. Tevens bestaat de onderzoeksgroep van de HOPE studie uit een selectieve groep patiënten met handartrose en bewezen ontstekingen, daarom gelden onze resultaten mogelijk niet voor patiënten met andere vormen van artrose. Desalniettemin ondersteunen de resultaten van dit onderzoek een rol voor lipidomics als biomarker voor de behandelresponse op ontstekingsremmende medicatie, wat de ontwikkeling van gepersonaliseerde behandeling weer een stap dichterbij kan brengen.

Vervolgens onderzochten we in hoofdstuk 7 met gegevens uit de HOPE studie hoe prednison het lipidenprofiel beïnvloedt. Hiervoor hebben we gekeken naar verschillen in veranderingen van de lipidenconcentratie na 6 weken behandeling met prednison, ten opzichte van het lipidenprofiel in patiënten behandeld met placebo. Zoals ook aangekaart in hoofdstuk 4, is het belangrijk om in dit soort onderzoek alleen biomarkers te onderzoeken die reproduceerbaar te meten zijn. Daarom hebben we voor dit onderzoek eerst gekeken naar de veranderingen over 6 weken tijd in de onbehandelde (placebo) groep, en alleen de lipiden meegenomen in de statistische analyses die weinig variatie lieten zien tussen de tijdspunten in de onbehandelde patiënten. We zagen dat de concentratie in het bloed van drie fosfolipiden (met verschillende vetzuurketens) significant veranderden door de behandeling met prednison. Mogelijk zijn deze lipiden betrokken bij de ontstekings- of pijnprocessen in patiënten met handartrose. Dat zou wederom impliceren dat lipiden een interessant aangrijpingspunt zijn voor toekomstig onderzoek naar de ontwikkeling van nieuwe medicatie voor artrose.

Metabole consequenties
Obesitas leidt frequent tot verstoringen in de stofwisseling (metabolisme). Verstoringen van de vetstofwisseling worden gezien bij vele metabole aandoeningen, zoals ook bij cardiovasculaire ziekten. Cardiovasculaire ziekten zijn, net als artrose, veelal chronische aandoeningen met een lange preklinische fase waarin de ziekte reeds in ontwikkeling is maar vaak nog onmerkbaar. Belangrijke markers en risicofactoren van preklinische cardiovasculaire ziekte zijn een verhoogde bloeddruk, verstijving van de bloedvaten en aderverkalking (atherosclerose). Eerder onderzoek heeft laten zien dat er een relatie bestaat tussen markers van cardiovasculaire ziekte en artrose. Een verklaring voor deze relatie kan zijn dat

atherosclerose en de bijbehorende vernauwing van de bloedvaten leidt tot verminderde bloedtoevoer naar het gewricht. Hierdoor komen er minder voedingsstoffen bij het kraakbeen en het ondergelegen bot, wat mogelijk leidt tot artrose. Deze hypothese wordt ondersteund door een recent literatuuroverzicht, dat liet zien dat vaatziekte gecorreleerd is met hand- en knieartrose. Echter, in hoeverre cardiovasculaire ziekte de tussenliggende oorzaak is die de relatie tussen obesitas en artrose verklaard, is eerder niet onderzocht. In hoofdstuk 8 onderzochten we of de bloeddruk, stijfheid van de aorta en atherosclerose van de halsvaten en een bloedvat in de knie, de relatie tussen obesitas en artrose kan verklaren. Door de onderzoeksopzet van de NEO studie is er veel informatie verzameld over verschillende obesitas-gerelateerde ziekten. Daarom hebben we voor dit onderzoek wederom gegevens uit de NEO studie gebruikt. We zagen dat de vaatwanddikte (een surrogaatmaat voor atherosclerose) van de halsvaten hooguit een fractie verklaarde van de relatie tussen obesitas en artrose. Dit effect was dusdanig klein, dat het hoogstwaarschijnlijk niet klinisch relevant is. Ook de andere preklinische cardiovasculaire markers boden geen verklaring voor hoe obesitas leidt tot artrose. Mogelijk komt het ontbreken van een relatie in de NEO studiepopulatie doordat er in deze groep van middelbare leeftijd preklinische cardiovasculaire ziekte voornamelijk in milde vorm aanwezig was, zodat een duidelijk verband niet zichtbaar werd. Ook zijn de gebruikte gegevens verzameld op één tijdspunt (dwarsdoorsnede onderzoek), wat het aantonen van een oorzakelijk verband bemoeilijkt.

Concluderend

Als we alle bevindingen van de hoofdstukken uit het eerste deel van dit proefschrift tezamen nemen, kunnen we concluderen dat onze kennis omtrent de relatie tussen lipiden en hand- en knieartrose is gegroeid. Door eerst de beschikbare literatuur samen te vatten zagen we waar de grootste gaten in ons begrip over de relatie tussen lipiden en hand- en knieartrose zaten. We zagen dat er met name een gebrek was aan bevindingen van klinische studies. De onderzoeksresultaten van de opvolgende hoofdstukken hebben deze gaten wellicht niet gedicht, maar wel kleiner gemaakt. We hebben laten zien welke lipiden, gemeten met een groot gestandaardiseerd en commercieel beschikbaar lipidenplatform, betrouwbaar te meten zijn over de tijd in twee verschillende typen bloedmonsters. Deze bevindingen kunnen sturing geven bij het opzetten van toekomstig onderzoek naar lipidomics. We zagen het sterkste verband tussen het lipidenprofiel en handpijn, en niet tussen het lipidenprofiel en knieartrose. Dit sluit aan bij onze hypothese dat in gewrichten die geen gewicht dragen, zoals de handen, de met obesitas geassocieerde verstoorde vetstofwisseling belangrijk is. Tevens suggereren resultaten beschreven in deze thesis dat het lipidenprofiel betrokken is bij artrose-gerelateerde ontstekingsprocessen. Dit blijkt uit de bevinding dat het lipidenprofiel voorspellend was voor een goede response op ontstekingsremmende medicatie, en dat het lipidenprofiel veranderde bij het gebruik van deze medicatie. Hoewel de opzet van de beschreven onderzoeken exploratief van aard was, zijn de resultaten veelbelovend voor een rol voor lipidomics in toekomstig biomarkeronderzoek. Al deze puzzelstukjes bij elkaar brengen ons weer een stapje dichterbij het begrijpen van de onderliggende processen betrokken bij artrose, en uiteindelijk bij de ontwikkeling van behandelingen die artrose een halt toe kunnen roepen.

Deel 2 – Ziektelast van artrose

Patiënt-gerapporteerde uitkomsten bieden op een gestandaardiseerde manier inzicht in hoe de patiënt de ziektelast van hand- en knieartrose ervaart. Bij onderzoek naar de effectiviteit van (nieuwe) medicijnen is het dan ook van groot belang deze uitkomsten goed te kunnen meten en interpreteren.

Kwaliteit van leven in patiënten met hand artrose
Een belangrijke uitkomstmaat van de invloed van ziekte op een patiënt is de ziekte-gerelateerde kwaliteit van leven. Deze uitkomstmaat is frequent bestudeerd in handartrose patiënten gerekruteerd vanuit de tweedelijns- of derdelijnszorg. Uit deze onderzoeken bleek dat patiënten met handartrose een verminderde kwaliteit van leven hebben. Echter, handartrose is een veelvoorkomende aandoening en wordt veelal reeds herkend door patiënten zelf of de huisarts (eerstelijnszorg). Patiënten uit gespecialiseerde zorg kunnen daarom wezenlijk verschillen van individuen met handartrose in de algemene bevolking, bijvoorbeeld door verschillen in ziekteduur en ziekte-ernst, fysieke beperkingen of het tegelijkertijd voorkomen van artrose in verscheidene gewrichten. Het is vaak niet goed mogelijk om de resultaten van onderzoeken verkregen uit verschillende studiepopulaties onderling met elkaar te vergelijken, omdat verschillende definities voor artrose worden gebruikt en verschillende soorten artrose worden onderzocht. In hoofdstuk 9 hebben we gegevens gecombineerd van handartrosepatiënten van de polikliniek in het Leids Universitair Medisch Centrum (tweede- en derdelijnszorg), en individuen uit de algemene bevolking, uit dezelfde regio in Nederland (Leiden en omgeving). In dit onderzoek zagen we dat er een bescheiden, klinisch irrelevant, lager dan gemiddelde lichamelijke kwaliteit van leven was in individuen met handartrose in de algemene bevolking. Echter, indien er sprake was van een verwijzing naar een specialist in het ziekenhuis voor de artroseklachten, was de lichamelijke kwaliteit van leven wel duidelijk gereduceerd. Ook zagen we dat indien er tevens sprake was van knieartrose, de lichamelijke kwaliteit van leven nog minder was. De aanwezigheid van handartrose was in geen van beide onderzoekspopulaties geassocieerd met de geestelijke kwaliteit van leven. Deze onderzoeksresultaten benadrukken dat onderzoeksresultaten van verschillende studiepopulaties niet altijd direct met elkaar vergelijkbaar zijn. Daarnaast impliceren de resultaten dat het belangrijk is om na te gaan of er sprake is van artrose in meerdere gewrichten, aangezien dit invloed lijkt te hebben op de kwaliteit van leven van de patiënt.

Fysieke activiteit in patiënten met knieartrose
Naast medicijnen zijn er mogelijk ook andere manieren om de ziektelast te verminderen. Fysieke activiteit is een levensstijlfactor die niet alleen is geassocieerd met de ziektelast door artrose, maar ook te beïnvloeden is met gerichte interventies. Hoewel er eerder onderzoek is gedaan naar het verband tussen knieartrose en fysieke activiteit, komen veel van de beschikbare resultaten van studies uit het buitenland. Wegens culturele verschillen en verschillen in levensstijl, zijn deze bevindingen mogelijk niet van toepassing op Nederlandse patiënten. De enkele beschikbare Nederlandse resultaten betreffen zeer oude patiënten, of werden verkregen voorafgaande aan een knievervangende operatie. Idealiter worden levensstijlinterventies toegepast in een populatie van middelbare leeftijd en in een vroeg ziektestadium. In hoofdstuk 10 beschrijven we hoe knieartrose samenhangt met fysieke activiteit in de algemene Nederlandse bevolking van middelbare leeftijd. We zagen, in tegenstelling tot onze verwachtingen op basis van eerdere onderzoeken, dat individuen met

knieartrose juist een iets hoger niveau van fysieke activiteit vertoonden dan gemiddeld, zowel op basis van vragenlijstonderzoek, als door middel van een meting van het energieverbruik met accelerometrie. Mogelijk is de relatie die wij hebben gevonden anders dan eerder beschreven resultaten, doordat we een studiepopulatie van middelbare leeftijd hebben onderzocht, waarin individuen minder mogelijkheden hadden om hun fysieke activiteit af te schalen (werkende populatie), of ze nog weinig werden gehinderd in hun fysieke activiteit doordat ze een milde vorm van artrose hadden. Ook is het mogelijk dat juist in deze groep de fysieke activiteit hoger ligt dankzij adviezen die patiënten met artrose meekrijgen om fysiek actief te blijven. Een andere mogelijkheid is dat door de pijn in de knie de individuen met knieartrose hun fysieke activiteit overschatten, iets wat 'herinneringsbias' wordt genoemd, of dat ze geneigd zijn meer fysieke activiteit te rapporteren vanwege het advies tot bewegen van hun arts ('sociale wenselijkheidsbias'). Echter, we zagen ook met de metingen van het energieverbruik geen lagere fysieke activiteit in individuen met knieartrose dan individuen zonder knieartrose, en we zagen geen relatie van fysieke activiteit met pijn of functie van de knie. Dit weerlegt dat bias aan de resultaten ten grondslag ligt, en suggereert dat deze relatie werkelijk anders is in de algemene Nederlandse bevolking van middelbare leeftijd dan bij eerdere onderzoekspopulaties.

Verbeteren van de interpretatie van uitkomstmaten
Knieklachten behoren tot de meest gerapporteerde klachten van het bewegingsapparaat, en ontstaan vaak ten gevolge van knieartrose. De Knee injury and Osteoarthritis Outcome Score (KOOS) is een vragenlijst die is ontwikkeld om de ziektelast van de patiënt ten gevolge van knieklachten te onderzoeken. De interpretatie van patiënt-gerapporteerde uitkomsten zoals de KOOS is afhankelijk van relevante referentiematen. Een suboptimale vragenlijstscore kan verscheidene oorzaken hebben, en is niet altijd het gevolg van de aandoening van het bewegingsapparaat waar de vragenlijst op gericht is, een fenomeen dat eerder werd gezien bij andere knie-specifieke vragenlijsten. Daarom hebben we in hoofdstuk 11 onderzocht wat voor patiëntkarakteristieken de KOOS score beïnvloeden. We zagen hierbij dat geslacht en body mass index (BMI) sterk geassocieerd waren met KOOS scores, terwijl leeftijd geen consistente correlatie met de KOOS scores liet zien. Vervolgens hebben we percentielcurves (vergelijkbaar met de groeicurves bij kinderen) ontwikkeld met gegevens uit de algemene Nederlandse bevolking, waarin we de verschillen tussen mannen en vrouwen, als ook met oplopende BMI inzichtelijk hebben gemaakt. We zagen dat vrouwen gemiddeld slechtere KOOS scores hadden dan mannen, een bevinding die ook in eerdere literatuur is beschreven. Tevens zagen we dat individuen met een hogere BMI slechtere KOOS scores hadden. Hoewel dit vanuit artrose perspectief wellicht voor de hand ligt, is er opmerkelijk weinig beschreven over de associatie tussen BMI en KOOS scores in eerdere onderzoeken in referentiepopulaties. Meer onderzoek is dan ook nodig om onze bevindingen te ondersteunen. De KOOS percentielcurves kunnen toegepast worden om te bepalen hoe patiëntscores afwijken van de algemene bevolking en om veranderingen over tijd gedurende een behandeling of na een operatie tegen deze referentie af te zetten.

In hoofdstuk 12 gaan we op het voorgaande onderzoek verder, door een toepassing van de KOOS percentielcurves met patiëntgegevens te onderzoeken. Hiervoor maakten we gebruik van gegevens van patiënten uit de Longitudinal Leiden Orthopeadics Outcomes of Osteo-Arthritis study (LOAS), waarbij op gestandaardiseerde tijdspunten de KOOS vragenlijst is afgenomen. Zoals verwacht zagen we dat de KOOS scores van patiënten voor een gewrichtsvervangende operatie lager waren dan de KOOS scores in de algemene bevolking.

Het plotten van de scores op de grafieken, met die van de algemene bevolking, zorgde voor een eenvoudigere (visuele) interpretatie dan een individuele score van een patiënt zonder de directe visuele vergelijking met scores uit de algemene bevolking. Vervolgens hebben we ook de postoperatieve KOOS scores in de grafieken opgenomen. Dit liet zien dat de postoperatieve scores achterbleven ten opzichte van de scores van de algemene bevolking. Namelijk, 7 tot 9 op de 10 Nederlanders ervaart minder klachten dan knieartrose patiënten ná een knievervangende operatie. De aanwezigheid van co-morbiditeiten was met name oorzaak van minder dan verwachte postoperatieve verbetering. Deze inzichten, de stand van de patiënt ten opzichte van de algemene bevolking, en de vereenvoudigde interpretatie door de visualisatie van de scores, kunnen bijdragen in de communicatie van behandelperspectieven naar de patiënt, om zo gezamenlijk tot een goed geïnformeerd behandelbesluit te komen. Ook kan het gebruik van de KOOS percentielcurves helpen in het verwachtingsmanagement met betrekking tot de uitkomsten van een behandeling of operatie.

Concluderend

Er bestaan veel verschillende patiënt-gerapporteerde uitkomsten, elk met hun eigen toepassingsgebied. Het onderzoek beschreven in het tweede deel van dit proefschrift laat zien dat zulke uitkomsten sterk verschillen tussen verschillende onderzoekspopulaties, onder andere door welke gewrichten worden onderzocht, en verschillen in cultuur en levensstijl van de onderzoekspopulatie. We zagen dat handartrose met name leidde tot verminderde lichamelijke kwaliteit van leven in individuen die hiervoor gespecialiseerde zorg zochten, en indien artrose voorkwam in meer dan één gewricht. We beschreven dat in de algemene Nederlandse bevolking van middelbare leeftijd knieartrose geen negatieve relatie heeft met fysieke activiteit. Een verklaring is wellicht dat men zich er in Nederland van bewust is dat fysieke activiteit gunstig is voor patiënten met artrose, en dat adviezen hierover worden opgevolgd. We concludeerden uit deze resultaten tevens dat onderzoek naar fysieke activiteit uit andere landen, zoals de Verenigde Staten, slecht te extrapoleren is naar de Nederlandse situatie. Omdat in afwezigheid van referentiematen scores uit vragenlijsten moeilijk te interpreteren zijn, hebben we voor een veelgebruikte knieklachten-specifieke vragenlijst, de KOOS, percentielcurves ontwikkeld die de spreiding van scores in de algemene Nederlandse bevolking laten zien. Vervolgens hebben we een toepassing van deze grafieken onderzocht met gegevens van knieartrose patiënten voor en na hun knievervangende operatie. Het gebruik van de KOOS percentielcurves leverde extra inzichten op en vereenvoudigde de interpretatie door de scores te visualiseren. Een dergelijke alternatieve weergave van patiënt-gerapporteerde uitkomsten kan eraan bijdragen om samen met de patiënt een weloverwogen behandelbesluit te nemen.

Toekomstperspectieven

De resultaten beschreven in dit proefschrift vergroten ons begrip over hoe obesitas- en ontstekingsgerelateerde factoren zoals lipiden betrokken zijn bij hand- en knieartrose. Daarnaast hebben we gekeken naar de door de patiënt ervaren ziektelast door artrose. Hiervoor hebben we verschillende patiënt-gerapporteerde uitkomsten onderzocht, en gepoogd de interpretatie van deze uitkomsten te verbeteren. Het onderzoek in beide onderdelen van dit proefschrift is, elk op zijn eigen wijze, essentieel om verder te komen in de ontwikkeling van nieuwe behandelingen voor artrose. Enerzijds door ons te richten op mogelijke nieuwe aangrijpingspunten voor de behandeling, anderzijds door behandeluitkomsten voor klinische studies te onderzoeken. Echter, de beschreven bevindingen staan niet op zichzelf, en er zijn ook beperkingen aan te wijzen die een eenduidige conclusie van de resultaten verhinderen. De belangrijkste beperkingen van de beschreven onderzoeken zijn inherent aan de gebruikte onderzoeksmethoden. Hierbij valt te denken aan het gebruik van gegevens verkregen op één tijdspunt, in tegenstelling tot het over de tijd volgen van patiënten, en het gebruik van nieuwe, exploratieve methodes in kleine onderzoekspopulaties. Deze beperkingen vormen uitdagingen die toekomstige onderzoekers moeten zien te overbruggen.

Tien jaar vervolgonderzoek van de NEO studie
De NEO studie is ontworpen als een prospectieve cohort studie, met als doel te onderzoeken hoe obesitas-gerelateerde aandoeningen ontstaan. De NEO studie heeft tussen 2008 en 2012 mannen en vrouwen tussen de 45 en 65 jaar geïncludeerd voor het eerste onderzoeksvisite (baseline). De resultaten beschreven in dit proefschrift zijn gebaseerd op analyses met gegevens verkregen van dit eerste tijdspunt. Momenteel worden deelnemers teruggevraagd voor een onderzoeksvisite 10 jaar na het baseline bezoek. Tijdens dit tweede bezoek zullen verscheidene artrose-gerelateerde maten die in de huidige onderzoeken staan beschreven worden herhaald. Net als tijdens het baseline bezoek zullen uitgebreide vragenlijsten worden afgenomen betreffende hand- en kniepijn en functie, zullen er gestandaardiseerde lichamelijke onderzoeken van de gewrichten plaatsvinden om onder andere pijn, benige veranderingen en bewegingsrestricties te registreren, en zal in een subgroep van de deelnemers wederom een knie MRI worden gemaakt. Deze vervolgmetingen van de NEO studie leiden tot een grote hoeveelheid nieuwe gegeven, die gebruikt kunnen worden om het ontstaan en de verergering van artrose te onderzoeken. De gegevens over een tienjaars periode zullen bijdragen aan het leggen van oorzakelijke verbanden, en kunnen gebruikt worden om de bevindingen zoals beschreven in dit proefschrift te bevestigen.

Prospectieve resultaten van de APPROACH studie
De APPROACH studie is een tweejarig, prospectief cohortonderzoek dat in vijf Europese ziekenhuizen plaatsvindt. Omdat de 2-jaars gegevens nog niet beschikbaar waren ten tijde van deze analyse, is er in dit proefschrift een dwarsdoorsnedeonderzoek van de baselinegegevens verricht. Het combineren van de gegevens over de gehele studieduur levert ongetwijfeld nieuwe inzichten op met betrekking tot oorzakelijke verbanden, en kan bijdragen aan onze kennis over de rol van lipidomics in de ontstaanswijze van artrose en progressie van de ziekte. Met de vervolggegevens kan tevens onderzocht worden of lipidomics voorspellend zijn voor de verergering van artrose op röntgenfoto's en MRI, en voor de verergering van patiënt-gerapporteerde uitkomsten zoals pijn en functie.

Biomarker onderzoek

Dit proefschrift bevat de beschrijving van verscheidene exploratieve onderzoeken naar het gebruik van lipidomics. De getoonde resultaten zijn slechts de eerste voorzichtige stappen, gezien de beperkingen die beschreven staan in de betreffende hoofdstukken. Hoewel er nog een lange weg te bewandelen is voordat er een klinisch bruikbare (lipiden) biomarker beschikbaar zal zijn, suggereren onze resultaten dat lipidomics een veelbelovend veld is voor verder biomarkeronderzoek. Er zijn echter nog belangrijke punten waar toekomstig onderzoek zich aan dient te wijden. Onduidelijkheid bestaat nog over wat voor soort monsters het beste gebruikt kunnen worden om biomarkers in te meten. Hoewel bloedmonsters eenvoudig te verkrijgen zijn, is synoviaal vocht (vocht uit het gewricht), mogelijk een betere representatie van lokale processen in het gewricht. Aan de andere kant kan ook beargumenteerd worden dat biomarkeronderzoek beter af is met een meer holistische benadering, omdat artrose vaak in verscheidene gewrichten tegelijkertijd voorkomt, en dus bloedmonsters de voorkeur hebben. Daarnaast is bekend dat lipidenconcentraties veranderen bij zowel normale lichamelijke processen als ziekteprocessen. Het is dan ook van cruciaal belang om over langere tijd gegevens te verzamelen over de veranderingen in lipidenconcentraties tijdens de ontwikkeling en verergering van artrose. Dit zou het mogelijk kunnen maken om met lipidomics een onderscheid te maken tussen normale processen, processen die van belang zijn voor het ontstaan van artrose, en processen die het ziekteproces gaande houden.

Appendices

Curriculum Vitae
List of publications
Dankwoord

Curriculum vitae

Marieke Loef is geboren op 6 januari 1991 te Jakarta. Na het behalen van haar gymnasium diploma in 2009 aan het Ostrea Lyceum te Goes, is zij gestart met de studie Biomedische Wetenschappen aan de Universiteit Leiden. In het derde jaar van haar studie deed zij haar Bachelor onderzoek bij de afdelingen Reumatologie en Epidemiologie in het Leids Universitair Medisch Centrum (LUMC), gericht op de rol van obesitas en vetverdeling in hand en knie artrose. Na het behalen van de Bachelor Biomedische Wetenschappen in 2012, stroomde Marieke in als dubbeltraject student bij de Bachelor Geneeskunde, die ze afrondde in 2013. Aansluitend startte ze simultaan met de Masters Biomedische Wetenschappen en Geneeskunde, waarvoor ze twee wetenschappelijke stages doorliep. Op de afdeling Endocrinologie in het LUMC deed zij onderzoek naar methoden om de invloed van bruin vet op het energieverbruik en lipiden metabolisme te analyseren. Vervolgens deed Marieke haar afsluitende Masterstage op de afdeling Reumatologie in het LUMC, waarbij ze onderzocht wat het effect van anti-tumour necrosis factor alpha behandeling is op het ontstaan en verergeren van hand artrose in vroege reumatoïde artritis patiënten. Na het afronden van beide Master studies in 2017, startte zij als arts-onderzoeker met haar promotietraject op de afdeling Reumatologie van het LUMC, onder supervisie van prof. dr. M. Kloppenburg en prof. dr. F.R. Rosendaal. Tevens heeft zij tijdens deze periode de opleiding tot epidemioloog B gevolgd via de afdeling Klinische Epidemiologie van het LUMC onder supervisie van prof.dr. F.R. Rosendaal.

In mei 2021 is Marieke gestart met de opleiding tot reumatoloog in het LUMC. Momenteel volgt zij de vooropleiding Interne Geneeskunde in het Alrijne ziekenhuis te Leiderdorp (opleider: dr. L. Hardi).

List of publications

Loef M, van de Stadt L, Böhringer S, Bay-Jensen AC, Mobasheri A, Larkin J, Lafeber FPJG, Blanco FJ, Berenbaum F, Giera M, Ioan-Facsinay A, Kloppenburg M. The association of the lipid profile with knee and hand osteoarthritis severity: the IMI-APPROACH cohort. Osteoarthritis and Cartilage. 2022;30:1062-1069

van Helvoort EM, Welsing PMJ, Jansen MP, Gielis WP, **Loef M**, Kloppenburg M, Blanco F, Haugen IK, Berenbaum F, Bay-Jensen AC, Ladel C, Lalande A, Larkin J, Loughlin J, Mobasheri A, Weinans H, Lafeber F, Eijkelkamp N, Mastbergen S. Neuropathic pain in the IMI-APPROACH knee osteoarthritis cohort: prevalence and phenotyping. RMD Open. 2021;7:e002025

Loef M, Gademan MGJ, Latijnhouwers DAJM, Kroon HM, Kaptijn HH, Marijnissen WJCM, Nelissen RGHH, Vliet Vlieland TPM, Kloppenburg M; LOAS Study Group. Comparison of KOOS Scores of Middle-Aged Patients Undergoing Total Knee Arthroplasty to the General Dutch Population Using KOOS Percentile Curves: The LOAS Study. J Arthroplasty. 2021;36:2779-2787

Loef M, Faquih TO, von Hegedus JH, Ghorasaini M, Ioan-Facsinay A, Kroon FPB, Giera M, Kloppenburg M. The lipid profile for the prediction of prednisolone treatment response in patients with inflammatory hand osteoarthritis: the HOPE study. Osteoarthritis and Cartilage OPEN. 2021

Terpstra SES, van der Velde JHPM, de Mutsert R, Schiphof D, Reijnierse M, Rosendaal FR, van de Stadt LA, Kloppenburg M, **Loef M.** The association of clinical and structural knee osteoarthritis with physical activity in the middle-aged population: The NEO study. Osteoarthritis Cartilage. 2021;29:1507-1514

Loef M, van der Geest RJ, Lamb HJ, de Mutsert R, le Cessie S, Rosendaal FR, Kloppenburg M. Mediation of the association between obesity and osteoarthritis by blood pressure, vessel wall stiffness and subclinical atherosclerosis. Rheumatology (Oxford). 2021;60:3268-3277

Loef M, von Hegedus JH, Ghorasaini M, Kroon FPB, Giera M, Ioan-Facsinay A, Kloppenburg M. Reproducibility of Targeted Lipidome Analyses (Lipidyzer) in Plasma and Erythrocytes over a 6-Week Period. Metabolites. 2020;11:26.

Helvoort EM, Hodgins D, Mastbergen S, Marijnissen AK, Guehring H, **Loef M**, et al. Relationship between motion, using the GaitSmart™, and radiographic knee osteoarthritis: an explorative analysis in the IMI-APPROACH cohort. Rheumatology. 2021;60:3588-3597

Helvoort EM, van Spil WE, Jansen MP, Welsing PMJ, Kloppenburg M, **Loef M**, et al. Cohort profile: The Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) study: a 2-year, European, cohort study to describe, validate and predict phenotypes of osteoarthritis using clinical, imaging and biochemical markers. BMJ Open. 2020;10: e035101

Loef M, Kroon FPB, Böhringer S, Roos EM, Rosendaal FR, Kloppenburg M. Percentile curves for the Knee injury and Osteoarthritis Outcome Score in the middle-aged Dutch population. Osteoarthritis Cartilage. 2020;28:1046-54

Loef M, Ioan-Facsinay A, Mook-Kanamori DO, Willems van Dijk K, de Mutsert R, Kloppenburg M, et al. The association of plasma fatty acids with hand and knee osteoarthritis: the NEO study. Osteoarthritis Cartilage. 2020;28:223–30.

Loef M, Damman W, de Mutsert R, Rosendaal FR, Kloppenburg M. Health-related quality of life in patients with hand osteoarthritis from the general population and the outpatient clinic. J Rheumatol. 2020;47:1409-1415.

Loef M, Schoones JW, Kloppenburg M, Ioan-Facsinay A. Fatty acids and osteoarthritis: different types, different effects. Jt Bone Spine. 2019;86:451–8.

Loef M, van Beest S, Kroon FPB, Bloem JL, Dekkers OM, Reijnierse M, et al. Comparison of histological and morphometrical changes underlying subchondral bone abnormalities in inflammatory and degenerative musculoskeletal disorders: a systematic review. Osteoarthritis Cartilage. 2018;26:992–1002.

Loef M, Kroon FPB, Bergstra SA, van der Pol JA, Lems WF, Kerstens PJSM, et al. TNF inhibitor treatment is associated with a lower risk of hand osteoarthritis progression in rheumatoid arthritis patients after 10 years. Rheumatol Oxf Engl. 2018;57:1917–24.

Loef M, Geijteman ECT, Beelen KJ, Bornebroek M, Schweitzer DH. [Relapse of chronic inflammatory demyelinating polyneuropathy following treatment with zoledronic acid]. Ned Tijdschr Geneeskd. 2017;161:D1747.

Kooijman S, van den Berg R, Ramkisoensing A, Boon MR, Kuipers EN, **Loef M**, et al. Prolonged daily light exposure increases body fat mass through attenuation of brown adipose tissue activity. Proc Natl Acad Sci U S A. 2015;112:6748–53.

Visser AW, de Mutsert R, **Loef M**, le Cessie S, den Heijer M, Bloem JL, et al. The role of fat mass and skeletal muscle mass in knee osteoarthritis is different for men and women: the NEO study. Osteoarthritis Cartilage. 2014;22:197–202.

Visser AW, Ioan-Facsinay A, de Mutsert R, Widya RL, **Loef M**, de Roos A, et al. Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study. Arthritis Res Ther. 2014;16:R19.

Dankwoord

Bij de totstandkoming van dit proefschrift hebben vele mensen bijgedragen, waarvan de volgende personen hieronder in het bijzonder wil bedanken.

Ik ben veel dank verschuldigd aan alle patiënten voor hun vrijwillige en onbaatzuchtige inzet om de wetenschap vooruit te helpen.

Prof.dr. Kloppenburg, beste Margreet, bedankt voor je vertrouwen in mij als beginnende onderzoeker en voor alle energie en tijd die je hebt geïnvesteerd om mij te kneden tot de klinisch onderzoeker die ik geworden ben. Jouw toewijding voor het artrose onderzoek zijn een grote bron van inspiratie. Prof.dr. Rosendaal, beste Frits, bedankt voor de epidemiologische inzichten die hebben bijgedragen aan het vormgeven van verscheidene projecten. De kennis die ik op heb gedaan tijdens de scholing tot epidemioloog is me zeer waardevol. Prof.dr. Huizinga, beste Tom, bedankt voor jouw altijd aanwezige en aanstekelijke enthousiasme voor de wetenschap.

Onderzoek is teamwerk, daarom wil ik alle coauteurs bedanken die hebben meegewerkt om dit proefschrift tot een hoger niveau te tillen.

Daarnaast bedank ik alle onderzoeksverpleegkundigen die geholpen hebben bij het verzamelen van de patiëntgegevens, en in het bijzonder Bernadette, Iris en Anita, voor hun werk in het APPROACH studie team. Hierbij mag dank aan Jessica als studiecoördinator, Jozé en Cedric als datamanagement, en het Center for Proteomics and Metabolomics voor de laboratoriummetingen, ook niet ontbreken.

Nancy, Sandra en Joyce, bedankt voor jullie luisterend oor en de hulp bij administratieve rompslomp.

Dank aan alle promovendi van de reumatologie en epidemiologie. De kletspraatjes maakten het werk nog leuker, jullie boden altijd ruimte om te ventileren en hielpen mij verder wanneer het tegenzat. In het bijzonder de “artrose groep”: Féline, bedankt voor je motiverende begeleiding tijdens mij masterstage en tips in het begin van mijn promotietraject. Sjoerd, Lotte en Coen, bedankt voor alle nuttige bijdragen tijdens onze overlegmomenten en de fijne samenwerking.

Nienke en Marlon, dank voor jullie hulp in de voorbereidingen en tijdens de verdediging van dit proefschrift in de rol als paranimf.

Veel dank gaat uit naar mijn ouders, die mij met hun liefde en opvoeding altijd het gevoel hebben gegeven dat met de juiste toewijding alles mogelijk is. Bedankt dat jullie mij vrij lieten te ontdekken, mijn eigen keuzes te maken en voor het stukje Zeeuwse nuchterheid bij twijfel.

Wilko, bedankt dat ik bij je kan lachen, klagen en huilen. Bedankt dat je er altijd voor me bent, zowel om de mooie momenten te delen, als om me op de moeilijke momenten te helpen alles weer in het juiste perspectief te zetten.