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

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## 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 2.5$^{th}$ 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 25$^{th}$ percentile in men, and between the 25$^{th}$ and 50$^{th}$ percentile in women. Median symptom and ADL function scores increased to around the 25$^{th}$ percentile postoperatively in both men and women. Similarly, postoperative QOL scores were around the 25$^{th}$ 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.

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