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

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

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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.

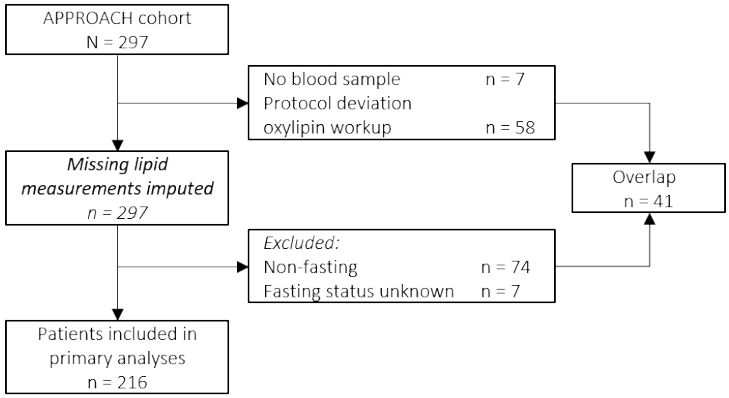

Figure 1. Study populations and exclusions.
In 65 participants there were either missing blood samples, or deviations to the work-up protocol, leading to complete missing lipid data (n=7) or missing oxylipin data (n=58). Since these measurements were regarded as missing completely at random, we mean 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.

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