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

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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.

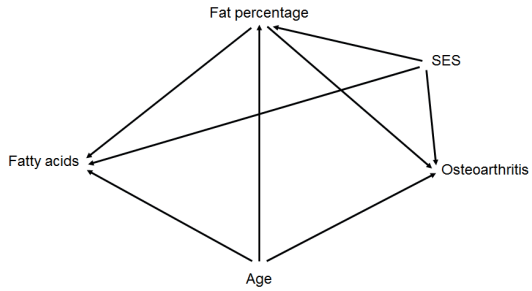

Figure 1. Causal diagram illustrating confounding of the association between fatty acids and osteoarthritis by age, total body fat and social economic status (SES).

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.

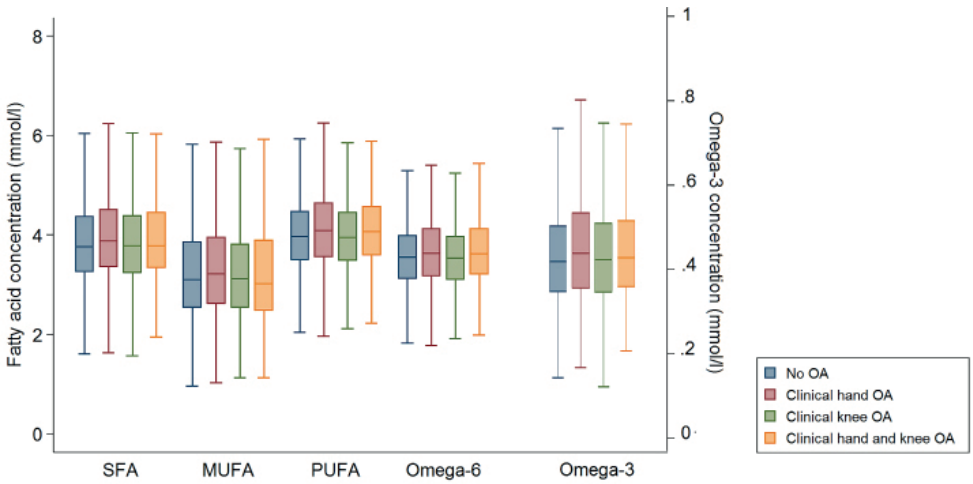

Figure 2. Postprandial fatty acid concentrations stratified by clinical OA phenotype.
Results are based on analyses weighted towards the BMI distribution of the general population. Boxes and bars represent median and interquartile range respectively. Abbreviations: SFA= saturated fatty acid, MUFA= monounsaturated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

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