Outcome of osteoarthritis and arthroplasty from patient perspective to molecular profiling.

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The BBMRI metabolomic consortium: histidine, glutamine and fatty acid make-up associate with the prevalence and progression of hip and knee osteoarthritis.

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Abstract

Objective: Higher body mass index (BMI) is associated with osteoarthritis (OA) in both weight-bearing and non-weight-bearing joints, suggesting a link between OA and poor metabolic health beyond mechanical loading. This risk may be influenced by systemic factors accompanying BMI. We hypothesize that differences in metabolic state contribute to development of OA. This study explores the association of metabolites with radiographic knee/hip OA (HOA/KOA) prevalence and progression.

Methods: A ^{1}H-NMR-metabolomics assay was performed on plasma samples of 1564 cases for prevalent OA and 2125 controls collected from the Rotterdam Study, CHECK, GARP/NORREF and LUMC-arthroplasty cohorts. HOA/KOA was assessed by means of Kellgren-Lawrence (KL) score and the OARSI-atlas. End-stage knee/hip OA was defined as indication for arthroplasty surgery (TKA/THA). OA-progression was defined as an increase in KL-score, to at least ≥2. Controls did not have KOA/HOA at baseline or follow-up. Principal component analysis of 227 metabolites demonstrated 23 factors, of which 19 remained interpretable after quality-control. Associations of factor scores with OA definitions were investigated with logistic regression resulting in odds ratio's (OR) per SD.

Results: Two factors showed consistent associations with prevalence and progression of KOA/HOA and TKA/THA. The "Glutamine and Histidine" factor showed negative associations (HOA: OR=0.7,P<0.001; THA: OR=0.7,P<0.001; KOA: OR=0.8,P=0.004; KOA progression: OR=0.8,P=0.020). The "Fatty-acids make-up" factor, representing chain length, ratio of saturated fatty acids and degree of unsaturation, showed positive associations (THA: OR=1.4,P<0.001; TKA: OR=1.6,P<0.001; HOA-progression: OR=1.2,P=0.047).

Conclusion: Fatty acid-make-up, histidine and glutamine serum levels associate with both prevalence and progression of OA, independent of BMI.

Introduction

Osteoarthritis (OA) is a common, age-related, progressive degenerative disease of articular joints and one of the leading causes of disability and pain worldwide. Due to ageing, increased longevity, and increasing obesity of the population, the OA incidence is expected to rise in the near future.[1-3] Epidemiological studies have shown systemic effects including associations of OA with unfavourable metabolic parameters, such as high body mass index (BMI), waist hip ratio and proportion of fat mass with metabolic diseases, such as hypertension, obesity and diabetes mellitus[4-10] Conversely, weight loss reduces the risk, as well as, relieve the pain and increase the physical function of people with OA.[11-14]

Associations with BMI have been found for OA in both weight-bearing and non-weight bearing joints, suggesting a connection between OA and obesity beyond axial loading.[15-17] In line with this view, OA associates with classical markers of poor metabolic health such as increased circulating levels of (LDL) cholesterol.[18,19] Together these data indicate that the metabolic health of individuals likely affects susceptibility to OA.[16,20-25]

In addition to classical metabolic parameters, such as cholesterol and glucose levels, metabolic health can be assessed by a range of serum metabolites. In the current study we investigated whether prevalence and progression of radiographic knee and hip OA is associated with ^{1}H-NMR based plasma metabolite levels. A well standardized and affordable is that of Nightingale^{Ltd} Finland. The Nightingale platform provides data on 231 metabolites, representing a comprehensive and highly correlated spectrum of amino acids, keton-bodies, lipids, lipoproteins and composite scores such as fatty acid chain length and previously reported to be associated with metabolic syndrome, diabetes and cardiovascular disease.[26,27]

In the current study, we have analysed associations of the Nightingale ^{1}H-NMR based metabolites with prevalent radiographic hip and knee OA, and progression of radiographic knee and hip OA in multiple cohorts participating in the Biobanking and BioMolecular resources Research Infrastructure consortium (BBMRI metabolomics consortium).[28] Identifying an OA-specific metabolite profile independent of BMI would provide further insight into the characteristics of the link between poor metabolism and OA and may eventually help clinicians to better identify those knee and hip OA patients who may benefit most from a lifestyle intervention.

Methods

Study populations

CHECK study: CHECK *(Cohort Hip & Cohort Knee) is a prospective, 10-year follow-up, observational cohort study* of 1002 people aged between 45 and 65 years at the time of inclusion, with pain in their knee(s) and/or hip(s), who had never or not longer than 6 months ago visited a general physician for these complaints.[29] Blood samples were taken non-fasted. Hip and knee radiographs were scored pairwise according to the Kellgren & Lawrence (KL) scoring system. *When scored pairwise, t*hese people did not have obvious radiographic knee or hip OA at baseline (i.e. KL=0 or 1)*. As such, these persons were considered controls for the cross-sectional analyses on OA prevalence at baseline. Those who did not develop OA during follow-up were included as controls in the progression analyses.*

GARP study: The GARP cohort (N=217) consists of patients with advanced radiographic OA at two or more joint sites of hand, spine, knee or hip. Follow-up was performed at 5 years, at which radiographs for hip, knee and hand were scored pairwise using the OARSI Atlas and the KL scoring system. Matched to the GARP study, a normal reference control group (NORREF) was collected using the same protocol and included in this study as controls.[30-32] *Blood was collected non-fasted.*

LUMC Arthroplasty studies: The LUMC arthroplasty studies (N=462) consist of participants of the RAAK, TacTics (NTR309) and TOMaat (NTR303) studies.[33,34] These cross-sectional studies included OA patients who received THA or TKA. Since all participants underwent THA/TKA, all patients are considered as end-stage OA and included in the cross-sectional OA prevalence analysis. Blood samples were collected during surgery while patients were fasted.

Rotterdam Study: The Rotterdam Study (RS) is a large prospective population-based cohort study of men and women aged 55 years and older in the municipality of Rotterdam, the Netherlands. The study design and rationale are described elsewhere in detail.35 In summary, the objective of the study is to investigate the determinants, incidence and progression of chronic disabling diseases in the elderly. Baseline measurements were obtained through a home interview and visits to the

research centre for physical examinations and imaging and laboratory assessments, blood samples were taken while patient was fasted. The present study includes 2802 participants from RS-I (Ergo 4) who were followed for 7 years. *The study included both individuals* with and without OA at baseline with mean follow-up time of 6.51 (0.41) year.

Informed consent was obtained from all included participants according to the Declaration of Helsinki (WHO) and good clinical practise.[36] In addition, approval by the local Medical Ethics Committee was obtained.

Definitions of OA

Prevalent radiographic OA was defined as either having a KL score of ≥2 in hip and/or knee at baseline or having THA or TKA for primary OA.[37] THA/TKA patients were also assessed separately. Controls (N=2125) had no radiographic hip and knee OA (KL<2) and were selected from the Rotterdam Study, CHECK and NORREF as described above.

Data on radiographic OA progression were available for GARP, CHECK, and the Rotterdam Study. For GARP, progression of radiographic OA was defined as progression of joint space narrowing (JSN) and/or osteophyte size above the smallest detectable change.[32] For CHECK and the Rotterdam Study, this was defined as an increase in KL-score, resulting in a KL score of ≥2 at follow-up. Thus, both incidence (KL score of 0 or 1 at baseline and ≥2 at follow-up) and progression (increase at KL score with a baseline of ≥2) were defined as progression in our analyses.

Controls were selected from CHECK and the Rotterdam Study and had neither radiographic OA at baseline, nor developed radiographic OA during follow-up.

Metabolite measurement

EDTA plasma samples were taken either at the time of TKA/THA in the LUMC-arthroplasty-studies or at baseline for the cohort studies. Samples were shipped to Nightingale to perform standardized metabolomics analyses on a high throughput platform (Nightingale Ltd, Helsinki, Finland). The ^{1}H-NMR technique used by Nightingale provides simultaneous quantification of routine lipids, lipoprotein subclass profiling with lipid concentration units, resulting in 231 measurements. Details of the techniques have been described before.[38-40]

Statistical Analyses

Analyses were performed per joint (hip or knee) and are also depicted in a flowchart (Figure 1). Since most distributions of metabolites were skewed, metabolite levels were LN-transformed to obtain normal distribution. Metabolite levels below the detection limit were considered missing. Metabolites with more than 5% missing were removed from analysis. Principal component analysis (PCA) was applied to reduce the data dimension of correlated metabolites. Factors were examined by means of scree plots and factors with an eigenvalue above 1 after Varimax rotation with Kaiser Normalization were included in analyses. A metabolite was said to load on a given factor if its factor loading was >0.4 or <-0.4. For each subject, a score was computed for the measures loaded on the factor, representing the linear relationship (Pearson correlation under Varimax rotation) between a factor and variable.

Since some of the used cohorts consist of only controls (NORREF and CHECK at baseline) the presence of cohort effects among controls was assessed by relating each factor to cohort while adjusting for age, sex, BMI and fasting status. Factors with significant cohort effects were removed from the analyses.

The remaining factors were included in logistic regression analyses to assess their association with OA, adjusting for age, sex, BMI and fasting status. Results are expressed as odds ratios per standard deviation and were corrected for multiple testing according to Bonferroni. To assess the modifying role of BMI and fasting, analyses were also performed without an adjustment for BMI or fasting.

Since follow-up was performed at different time points, the progression analyses were done by means of meta-analysis. To increase power by reaching substantial

cases and controls in the analysis, CHECK and GARP were combined. The summary statistics (regression coefficients and standard errors) of GARP+CHECK were then combined with the summary statistics of the Rotterdam Study in a random effects meta-analysis using the “meta package” for R. The individual metabolitesof factors who associate to both cross-sectional OA and progression of OA were LN-transformed and Z-standardized before being included in regression analyses for their association with any OA or any arthroplasty.

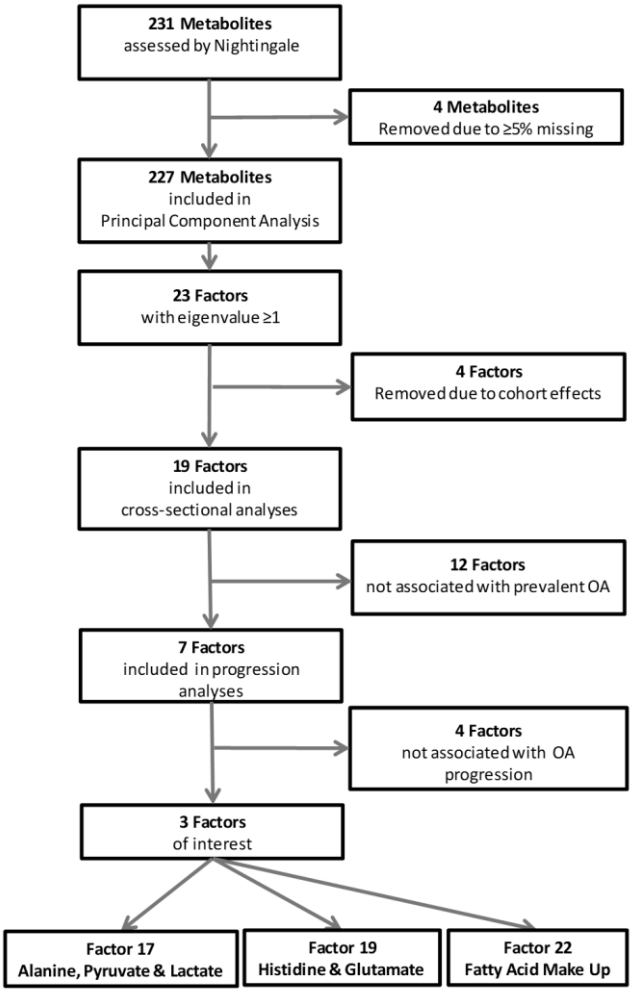

Figure 1 – Flowchart of analyses

Results

Of the 231 metabolites measured on the Nightingale platform, 4 metabolites had more than 5% of the measurements below the detection limit and were removed from analysis. These were conjugated linoleic acid (CLA), the ratio of CLA to total fatty acids (CLAFA), diglycerids (DAG) and ratio DAG to triglycerids (DAG/TG). See Supplementary Table 1 for complete list of assessed metabolites.

Principal component analysis

As shown in Supplementary Table 2, PCA revealed 23 factors with an eigenvalue of ≥1, accounting for 91,4% of the total variance. Notably, the first 2 components explained 56.4% of the variance. Based on the characteristics of the metabolites loading on each of the factors, we distinguished groups of metabolites representing low density lipids (LDL), very low density lipids (VLDL), high density lipids (HDL), fatty acids, and amino acids, se Supplementary Table 1.

Prior to analyses, quality control was performed within the control group with respect to cohort effects and led to removal of factor 2 (representing mostly VLDL-related metabolites), factor 10 and 13 (both representing HDL-related metabolites), and factor 16 (representing triglycerides in large HDL particles), accounting for 28.06% of the variance in the original dataset (see Supplementary Table 3).

Table 1 - Baseline characteristics of subjects in the cross-sectional analyses.

		HOA	THA	KOA	TKA	Controls
Age,	mean (SD)	69,6 (11,9)	68,0 (13,13)	72,5 (8,63)	71,0 (9,26)	66,1 (10,13)
Female,	N (%)	482 (65,6%)	313 (70,2%)	700 (70,8%)	142 (71,7%)	1301 (61,4%)
BMI,	mean (SD)	27,5 (4,4)	27,6 (4,6)	28,7 (4,53)	29,8 (4,1)	26,5 (3,9)
CHECK,	N	0	0	0	0	864
GARP/NORREF,	N	108	34	150	7	34
LUMC-cohorts,	N	302	302	153	153	0
Rotterdam Study,	N	326	111	687	39	1227
Total,	N	736	447	990	199	2125

HOA - Prevalent Radiographic Hip Osteoarthritis
THA – Total Hip Arthroplasty
KOA – Prevalent Radiographic Knee Osteoarthritis
TKA – Total Knee Arthroplasty

Cross-sectional analyses of factors 1, 3-9, 11, 12, 14, 15 and 17-23.

As depicted in Table 1, 736 cases with radiographic hip OA, 990 cases with radiographic knee OA, and 2125 controls without radiographic knee or hip OA at baseline were included in the cross-sectional analysis. These subpopulations differed with regard to age, gender and BMI. Therefore, all analyses were adjusted for age, sex and BMI.

As shown in Table 2, cross-sectional analyses with the remaining 19 factors (explaining 64.1% of variance) showed 3 factors that were positively associated with total joint arthroplasty of both the hip (THA) and knee (TKA) as compared to controls; factor 17 (THA: OR=1.38, 95%CI=1.20-1.59, P=0.002; TKA: OR=1.49, 95%CI=1.21-1.83, P=0.003), factor 22 (THA: OR=1.41, 95%CI=1.23-1.63, P=1.90E-5; TKA: OR=1.61, 95%CI=1.33-1.95, P=1.73E-5) and factor 23 (THA: OR=1.31, 95%CI=1.13-1.51, P=0.005; TKA: OR=1.71, 95%CI=1.40-2.09, P=2.66E-6).

Factor 1 showed a statistically significant association with TKA (OR=0.70, 95%CI=0.58-0.85, P=0.005), but, despite the similar effect size, did not reach statistical significance after correcting for multiple testing for THA (OR=0.85, 95%CI=0.74-0.99, P=0.646).

Three additional factors showed associations with THA, as well as, radiographic hip OA being; factor 4 (HOA: OR=1.37, 95%CI=1.23-1.53, P=4.17E-7; THA: OR=1.33, 95%CI=1.14-1.55, P=0.005), factor 11 (HOA: OR=0.82, 95%CI=0.74-0.91, P=0.002; THA: OR=0.77, 95%CI=0.67-0.88, P=2.00E-4) and factor 19 (HOA: OR=0.68, 95%CI=0.60-0.76, P=3.69E-10; THA: OR=0.65, 95%CI=0.55-0.75, P=5.93E-7).

Concurrent to factor 1, effect sizes of these factors in the association with OA in the knee were similar in direction but did not reach significance.

Table 2 - Outcomes of adjusted cross-sectional analyses

		Factor 1			Factor 17			Factor 22			Factor 23		
		Odds ratio	95% CI	P-value*	Odds ratio	95% CI	P-value*	Odds ratio	95% CI	P-value*	Odds ratio	95% CI	P-value*
Hip	HOA[A]	0,943	0,849-1,048	>1	1,147	1,036-1,270	0,171	1,127	1,015-1,252	0,494	1,088	0,982-1,206	>1
	THA[B]	0,854	0,739-0,988	0,646	1,381	1,198-1,591	0,002	1,414	1,228-1,627	1,90E-05	1,305	1,132-1,505	0,005
Knee	KOA[A]	0,982	0,897-1,075	>1	0,975	0,891-1,067	>1	1,055	0,964-1,155	>1	0,967	0,883-1,059	>1
	TKA[B]	0,703	0,583-0,849	0,005	1,491	1,213-1,834	0,003	1,613	1,333-1,952	1,73E-05	1,708	1,399-2,085	2,66E-06

		Factor 4			Factor 11			Factor 19		
		Odds ratio	95% CI	P-value*	Odds ratio	95% CI	P-value*	Odds ratio	95% CI	P-value*
Hip	HOA[A]	1,368	1,226-1,527	4,17E-07	0,82	0,741-0,906	0,002	0,675	0,601-0,757	3,69E-10
	THA[B]	1,328	1,141-1,546	0,005	0,765	0,665-0,881	0,004	0,645	0,553-0,754	5,93E-07
Knee	KOA[A]	1,18	1,071-1,300	0,019	0,891	0,816-0,973	0,19	0,825	0,745-0,914	0,004
	TKA[B]	1,329	1,059-1,668	0,266	0,809	0,655-0,998	0,912	0,791	0,630-0,993	0,817

Logistic regression analysis relating factor score to OA-phenotype corrected for age, sex, BMI and fasting status.

* P value Bonferroni corrected

A - Radiographic hip or knee OA, defined as Kellgrenn Lawrence score ≥2

B - Total hip or knee arthroplasty for primary OA

Factors 1, 4, 11, 17, 19, 22 & 23 and progression of radiographic hip and knee OA.
Subsequently, we investigated whether the observed associations of factors 1, 4, 11, 17, 19, 22, 23 with prevalent hip and/or knee OA also contributed to OA progression. Progression data were available for the participants of the CHECK, GARP and Rotterdam Study. In total, 282 individuals experienced progression of OA in hip and 463 persons experienced progression of OA in knee and 1244 persons did not have any incidence of OA after 5 to 7 years of follow-up, see Table 3.

Table 3 – Baseline characteristics of subjects in progression analyses.

		Hip Progression	**Knee Progression**	**Progression controls**
CHECK/GARP	N	125	292	523
Age	mean (SD)	58.9 (5.5)	57.4 (5.7)	55.4 (5.2)
Female	N (%)	89 (71.2%)	241 (82.5%)	410 (78.4%)
Body mass index	mean (SD)	26.0 (3.7)	28.0 (5.0)	25.7 (3.8)
Rotterdam Study	N	157	171	721
Age	mean (SD)	72.8 (5.0)	73.13 (5.1)	71.99 (4.6)
Female	N (%)	60.5%	66.1%	53.8%
Body mass index	mean (SD)	27.8 (4.4)	29.09 (4.3)	26.74 (3.5)
Total	N	282	463	1244

Baseline characteristics of persons with radiographic hip and knee progression as well as the controls for the progression analyses. Data are presented per cohort as included in meta-analysis.

A meta-analysis was performed to combine the results of the Rotterdam Study and CHECK and GARP cohorts (see also Table 4, corresponding forrestplots are depicted in Figure 2). Factor 19 associates to lower odds for knee OA (OR=0.84, 95%CI=0.73-0.97, P=0.020). Factor 22, as in the cross-sectional analyses, associates with increased odds for progression of hip OA (OR=1.16, 95%CI=1.00-1.34, P=0.047).

Notably, factor 17 had an inverse effect on progression of hip OA as compared to the cross-sectional analyses (hip progression: OR=0.87, 95%CI=0.75-1.00, P=0.047; cross-sectional hip OA: OR=1.38, 95%CI=1.20-1.59, P=0.002).

Table 4 - Results of meta-analysis for the progression of radiographic hip/knee OA.

		OR	95% CI	P-Value
Factor 1 (LDL)	hip	0,878	0,757-1,017	0,083
	knee	0,910	0,805-1,029	0,134
Factor 4 (LDL)	hip	1,064	0,872-1,298	0,541
	knee	0,963	0,849-1,093	0,560
Factor 11 (Fatty Acids)	hip	0,948	0,818-1,098	0,476
	knee	1,042	0,917-1,184	0,527
Factor 17 (Amino Acids)	**hip**	**0,855**	**0,749-0,998**	**0,047**
	knee	0,870	0,694-1,091	0,228
Factor 19 (Amino Acids)	hip	0,916	0,653-1,286	0,614
	knee	**0,844**	**0,732-0,973**	**0,020**
Factor 22 (Fatty Acids)	**hip**	**1,156**	**1,002-1,334**	**0,047**
	knee	1,045	0,925-1,180	0,483
Factor 23 (Amino Acids)	hip	0,962	0,828-1,119	0,617
	knee	0,918	0,806-1,046	0,201

Meta-analysis combining the results for the relation of factor to progression of hip/ knee OA from the Rotterdam Study and CHECK+GARP cohorts. Factors were studied when they had significant associations with prevalent radiographic hip or knee osteoarthritis. OR= odds ratio, 95%CI=95% confidence interval.

Assessment of individual metabolites of factors 17, 19 and 22.

Successively, we explored whether individual metabolites of the factors which go both with cross-sectional OA and progression of OA, drive any of the found associations. As shown in Supplementary Table 3, for Factor 17 the strongest effect was found in Pyruvate in any OA, whereas this effect got even stronger in arthroplasty (OR=1.21, 95%CI= 1.12-1.30, P=<0.001 and OR=1.93, 95%CI=1.72-2.16, P<0.001, respectively). Factor 19 appears to be mainly driven by Glutamine, which was negatively associated with both OA and TJA (OR=0.70, 95%CI=0.64-0.76, P<0.001 and OR=0.65, 95%CI=0.58-0.74, P<0.001, respectively). Of factor 22 was FALen consequently associated with both OA and arthroplasty (OR=1.26, 95%CI=1.16-1.36, P<0.001 and OR=1.83, 95%CI=1.64-2.05, P<0.001, respectively).

Assessment of individual metabolites of factors 17, 19 and 22 with OA-progression did not result in an obvious independent effect of any of the metabolites, nonetheless again the effect size of FALen was relatively large, albeit not statistically significant (Supplementary Table 4).

The effects of BMI and statins for factor 17, 19 & 22

To explore possible confounding effects of BMI in the associations observed, we performed analyses with and without adjustment for BMI. As shown in Supplementary Table 5, the effect sizes got slightly larger when omitting an adjustment for BMI.

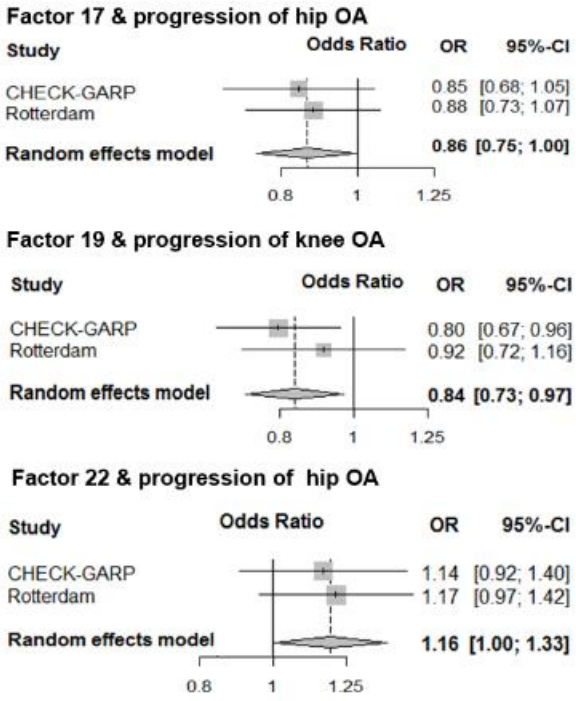

Figure 2 – Forrest plots from meta-analysis combining the results for the factors of interest with the progression of hip or knee OA from the Rotterdam Study and CHECK+GARP cohorts.

Moreover, as statins might affect the concentrations of metabolites, we performed sensitivity analyses to assess their influence on our outcomes.[41] The use of statins was known for all included studies except CHECK. Sensitivity analysis with subjects that did not use statins revealed only minor changes in the effect sizes (results not shown).

The modifying effect of fasting on associations of factor 17, 19 and 22 with OA

As some cohorts were fasted and others were unfasted, we also assessed in similar fashion the effects of fasting on the outcomes. Supplementary Table 6 shows the outcomes for the cross-sectional analyses for factors 17, 19 and 22 with and without the addition of fasting to the analysis. Although fasting had a strong significant effect in the analyses, the odds ratio's for factor and OA-phenotype were only marginally altered between the analyses.

Discussion

Serum metabolomics assay of hip and knee OA cases and controls were assessed by means of the Nightingale ^{1}H-NMR platform, resulting in 227 different metabolite measurements. These metabolites were included in PCA-analyses which identified 19 factors explaining 71.9% of variance eligible for further regression analyses. Of these, seven factors (1, 4, 11, 17, 19, 22 and 23) were associated with cross-sectional OA and three factors (17, 19 and 22) were found to be associated with cross-sectional hip or knee OA, as well as its progression. All associations were assessed independent of age, sex, fasting and BMI. As such, this study places composite scores of fatty acid-make-up, energy balance, histidine and glutamine at the heart of the link of osteoarthritis and metabolites.

Factor 19, composed of 2 amino acids: glutamine and histidine showed a consistent association with a decreased risk for hip and knee OA in the cross-sectional analysis (radiographic hip OA: OR=0.68, 95%CI=0.60-0.76, radiographic knee OA: OR=0.83, 95%CI=0.75-0.91 and THA OR=0.65, 95%CI=0.55-0.75) as well as for the knee progression analyses (OR=0.84, 95%CI=0.73-0.97). These findings are consistent with a previously performed NMR-based urine metabolomics study which found that low levels of histidine are associated to OA.[42] Another study by *Loeser et al*[43] *identified that histidine (as well as alanine) measured in urine is an important metabolite to discriminate between persons with knee OA progression as compared to BMI matched controls.* However, the exact nature of the underlying pathophysiological mechanism of the association between OA and histidine and glutamine remains to be elucidated.

Factor 22 represents measures of the make-up of fatty acids: fatty acid chain length, saturated fatty acids to total fatty acids ratio and the level of unsaturation. The latter 2 measures contributed in opposite fashion to the factor score. Factor 22 was associated with a higher risk for end stage hip (OR=1.41, 95%CI=1.23-1.63) and knee OA (OR=1.61, 95%CI=1.33-1.95), as well as a higher risk for hip OA progression (OR=1.16, 95%CI=1.00-1.34). Notably, fatty acid chain length is, in the analysis of individual metabolites, independent and strongly associated to the prevalent cross-sectional OA-phenotypes and seems to drive factor 22.

Nevertheless, in the OA-progression analyses this was less clear. A recent study has shown that longer-chain dietary fatty acids in rats induce both metabolic syndrome and OA like knee changes.[44] Fatty acids are known to play a role in a broad range of cardiovascular diseases as well as to the immune system, which might hint that there is a more generic pathway underlying the association of OA and fatty acid make up.[45,46]

Open for discussion is factor 17, representing alanine, lactate and pyruvate, which are produced during glycolysis in cells in aerobic and anaerobic conditions.[47-49] Factor 17 is associated with a higher risk for cross sectional hip OA (OR=1.38, 95%CI=1.20-1.59), knee OA (OR=1.49, 95%CI=1.21-1.83) and arthroplasty but with a decreased risk for hip OA progression (OR=0.86, 95%CI=0.75-0.99). This association of factor 17 with OA may be a reflection of different types of energy consumption in play as chondrocytes in OA switch from oxidative phosphorylation to glycolysis as their main source of energy metabolism.[50] Nevertheless, the opposite relation in the cross sectional and progression analyses is a result that we currently cannot explain. The association of factor 17 could therefore also be spurious and needs confirmation in additional cohorts.

In this study we choose to differentiate between patients who underwent total hip or knee arthroplasty from patients with radiographic signs of OA. This because THA and TKA patients are essentially in a different stage of the disease, their OA-symptoms were clinically assessed and severe enough to undergo arthroplasty surgery. In contrast, patients with radiological OA represent a range of patients who may not (yet) be severe enough for an indication for arthroplasty. The fact that we observed more consistent associations with arthroplasty patients justifies this approach. Although we found significant associations between some factors and knee or hip OA progression, none of the individual metabolites reached a statistically significant level for progression. This indicates that the baseline level of individual metabolites might be less informative then a complete metabolite profile.
A strength of our study was that this study comprises a large sample size of which a subset was followed overtime, enabling us to follow progression over time. The combination of different studies to reach more power also meant that we

incorporated some studies with only cases or controls, raising the chance of cohort effects. However, we did assess the presence of cohort effects within the control phenotype, where no differences should be present between the cohorts as all samples are the exact same phenotype. The factors which were free of cohort effects were included in our analyses.

We adjusted our analyses for BMI measured at baseline, however we cannot exclude the bias in our findings due to the effect of weight loss or gain right before blood collection. To obtain more insight in the modifying / confounding role of BMI in our analyses, we compared analyses with and without adjustment for BMI (Supplementary Table 5). The odds ratios between these analyses were only marginally altered, with the biggest change found for TKA in factor 19, where the odds ratio went from 0.791 (adjusted for age, sex, fasting and BMI) to 0.565 (adjusted for age, sex and fasting). As such we concluded that the observed metabolite associations with OA were independent or only slightly modified by BMI.

Moreover, the fact that our study included both fasted and nonfasted samples and metabolites are very sensitive to fasting status, the adjustment for fasting status may not have been sufficient to properly correct for dietary influences. To obtain more insight in the modifying role of fasting status, our metabolite factors and OA, we performed analyses with and without fasting adjustment. As shown in Supplementary Table 6, the odds ratios for OA in the two analyses showed only marginally changes i.e. effect sizes were very similar. Analyses were stratified by fasting status for HOA and factor 19, we found that the effect size was larger in the non-fasted samples as compared to the fasted samples (fasted samples: HOA OR=0.77, 95%CI=0.68-0.88, P=0.003; non-fasted samples: HOA OR=0.49, 95%CI=0.37-0.63, P<0.001).

The current paper is to our knowledge the first large scale hypothesis free approach in search for metabolites that associate to OA in a cross-sectional as well as a follow-up design. Future research should particularly focus on replication of the found results and, if this succeeds, further elucidate the mechanisms behind the association of the identified metabolites and OA should be performed.

Eventually, these studies could lead up to the identification of lifestyle changes which might alter the predisposition for OA. Identifying lifestyle changes such as different levels of fatty acid intake or physical training to improve the switch between aerobe/anaerobe metabolism may lessen the burden of OA. In conclusion, the current study identified a number of metabolic factors associated with OA, independent of BMI. This indicates that there is an altered metabolic state in patients with OA as compared to controls without OA. This is another token that OA should be seen as a component of poor metabolic health.

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Supplementary Table 1

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
AcetoAcetate	AcAce																							0,63
Acetate	Ace																							0,77
Alanine	Ala																	0,62						
Albumine	Alb																		0,69					
3-hydroxybutyrate	bOHBut																				0,59			0,41
Citrate	Cit																				0,60			
Creatinine	Crea																							
Glucose	Glc																							
Glutamine	Gln																			0,77				
Glycoprotein	Gp		0,58																					
Histidine	His																			0,67				
Isoleucine	Ile		0,50						0,73															
Lactate	Lac																	0,82						
Leucine	Leu								0,86															
Phenylalanine	Phe								0,70															
Pyruvate	Pyr																	0,81						
Tyrosine	Tyr								0,71															
Valine	Val								0,83															

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Esterified Cholesterol	EstC	0,91																						
Free Cholesterol	FreeC	0,89																						
HDL2-C hdl2 cholesterol particle density	HDL2C		-0,43	0,80																				
HDL3-C hdl3 cholesterol particle density	HDL3C	0,73																						
HDL-C hdl cholesterol	HDLC			0,79																				
Rem t-C non-hdl / ldl cholesterol	RemtC	0,79	0,58																					
Serum-C cholesterol	SerumC	0,91																						
Triglycerides	SerumTG		0,90																					

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Apolipoprotein A-I	ApoA1			0,80		0,41																		
Apolipoprotein B	ApoB	0,77	0,60																					
ApoB / ApoA1	ApoB/ApoA1	0,56	0,62	-0,48																				
Docosahexaenoic acid 22:6	DHA							0,86																
DHA / total fatty acids	DHA FA							0,94																
Estimated fatty chain length	Falen	-0,52																					0,46	
omega 3 fatty acid	FAw3							0,81																
FAw3 / total fatty acids	FAw3 fa							0,94																
omega 6 fatty acid	FAw6	0,74										0,46												
FAw6 / total fatty acids	FAw6 FA		-0,59									0,61												
Linoleic acid 18:2	LA	0,69										0,55												
LA / total fatty acids	LA FA		-0,42									0,67												
Monounsaturated fatty acids 16:1 18:1	MUFA		0,80																					
MUFA / total fatty acids	MUFAFA		0,60									-0,58												
Phosphatidycholine and other cholines	PC	0,52		0,59																				
polyunsaturated fatty acids	PUFA	0,74										0,42												
PUFA / total fatty acids	PUFA FA		-0,62									0,58												
Saturated fatty acids	SFA	0,48	0,70																					
SFA / total fatty acids	SFA FA																						-0,83	
sphingomyelines	SM	0,71																						
triglycerides / phosphoglycerides	TG PG		0,81	-0,40																				
cholines	TotCho	0,63		0,58																				
total fatty acids	TotFA	0,56	0,69																					
phosphoglycerides	TotPG	0,55		0,56																				
estimated degree of unsaturaization	UnsatDeg		-0,54					0,45															0,44	

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
HDL mean diameter hdl particle	HDL-D		-0,43	0,87																				
HDL Triglycerides	HDL-TG		0,74		0,42																			
XL-HDL Total Cholesterol	XL HDL C			0,81																				
XL-HDL Total Choleseterol to total Lipids	XL HDL C %			-0,67												0,43								
XL-HDL Cholesterol Esters	XL HDL CE			0,78																				
XL-HDL CholesterolEsters to total Lipids	XL HDL CE %			-0,72																				
XL-HDL Free Cholesterol	XL HDL FC			0,85																				
XL-HDL Free Cholesterol to total lipids	XL HDL FC %										-0,58													
XL-HDL Total lipids	XL HDLL			0,89																				
XL-HDL Particle concent n	XL HDL P			0,89																				
XL-HDL Phospholipids	XL HDL PL		-0,44	0,85																				
XL-HDL Phospholipids to total Lipids	XL HDL PL %		-0,47	0,64																				

XL-HDL Triglycerids	XL HDL TG		0,57	0,50																				
XL-HDL TriGlycerides to total Lipids	XL HDL TG %		0,78																					
L-HDL Total Cholesterol	L HDL C		-0,49	0,80																				
L-HDL Total Choleseterol to total Lipids	L HDL C %		-0,42							0,78														
L-HDL Cholesterol Esters	L HDL CE		-0,48	0,79																				
L-HDL CholesterolEsters to total Lipids	L HDL CE %									0,81														
L-HDL Free Cholesterol	L HDL FC		-0,49	0,78																				
L-HDL Free Cholesterol to total lipids	L HDL FC %		-0,45	0,50						0,48														
L-HDL Total lipids	L HDLL		-0,46	0,83																				
L-HDL Particle concent n	L HDL P		-0,45	0,83																				
L-HDL Phospholipids	L HDL PL		-0,44	0,81																				
L-HDL Phospholipids to total Lipids	L HDL PL %									-0,70														
L-HDL Triglycerids	L HDL TG			0,58														0,42						
L-HDL TriGlycerides to total Lipids	L HDL TG %		0,59																					
M-HDL Total Cholesterol	M HDL C			0,60		0,75																		
M-HDL Total Choleseterol to total Lipids	M HDL C %		-0,52											-0,72										
M-HDL Cholesterol Esters	M HDL CE			0,57		0,75																		
M-HDL CholesterolEsters to total Lipids	M HDL CE %		-0,45		-0,42									-0,65										
M-HDL Free Cholesterol	M HDL FC			0,59		0,64																		
M-HDL Free Cholesterol to total lipids	M HDL FC %	0,41																						
M-HDL Total lipids	M HDLL			0,58		0,79																		
M-HDL Particle concent n mol / L	M HDL P			0,57		0,79																		
M-HDL Phospholipids	M HDL PL			0,58		0,77																		
M-HDL Phospholipids to total Lipids	M HDL PL %													0,91										
M-HDLTriglycerids	M HDL TG		0,76																					
M-HDLTriGlycerides to total Lipids	M HDL TG %		0,77																					
S-HDL Total Cholesterol	S HDL C	0,62				0,44																		
S-HDL Total Choleseterol to total Lipids	S HDL C %	0,68																						
S-HDL Cholesterol Esters	S HDL CE	0,68																						
S-HDL CholesterolEsters to total Lipids	S HDL CE %	0,69																						
S-HDL Free Cholesterol	S HDL FC					0,80																		
S-HDL Free Cholesterol to total lipids	S HDL FC %																0,50							
S-HDL Total lipids	S HDLL					0,91																		
S-HDL Particle concent n mol / L	S HDL P					0,89																		
S-HDL Phospholipids	S HDL PL	-0,43				0,78																		
S-HDL Phospholipids to total Lipids	S HDL PL %	-0,69																						
S-HDL Triglycerids	S HDL TG		0,73		0,49																			
S-HDL TriGlycerides to total Lipids	S HDL TG %		0,70		0,46																			

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
LDL cholestrol	LDL C	0,99																						
LDL mean diameter ldl particle	LDL D										0,63													
LDL triglycerides	LDL TG	0,48			0,77																			
L-LDL Total Cholesterol	LLDL C	0,98																						
L-LDL Total Choleseterol to total Lipids	LLDL C %	0,79			-0,55																			
L-LDL Cholesterol Esters	LLDL CE	0,98																						
L-LDL CholesterolEsters to total Lipids	LLDL CE %	0,87																						

L-LDLFree Cholesterol	LLDL FC	0,97																						
L-LDL Free Cholesterol to total lipids	LLDL FC %		-0,73		-0,47																			
L-LDL Total lipids	LLDLL	0,98																						
L-LDL Particle concent n mol / L	LLDL P	0,97																						
L-LDL Phospholipids	LLDL PL	0,97																						
L-LDL Phospholipids to total Lipids	LLDL PL %	-0,95																						
L-LDL Triglycerids	LLDL TG	0,52			0,77																			
L-LDL TriGlycerides to total Lipids	LLDL TG %	-0,41			0,86																			
M-LDL Total Cholesterol	MLDL C	0,99																						
M-LDL Total Choleseterol to total Lipids	MLDL C %	0,83																						
M-LDL Cholesterol Esters	MLDL CE	0,99																						
M-LDL CholesterolEsters to total Lipids	MLDL CE %	0,90																						
M-LDL Free Cholesterol	MLDL FC	0,95																						
M-LDL Free Cholesterol to total lipids	MLDL FC %	-0,85																						
M-LDL Total lipids	MLDLL	0,97																						
M-LDL Particle concent n mol / L	MLDL P	0,97																						
M-LDL Phospholipids	MLDL PL	0,89																						
M-LDL Phospholipids to total Lipids	MLDL PL %	-0,92																						
M-LDL Triglycerids	MLDL TG	0,47			0,79																			
M-LDL TriGlycerides to total Lipids	MLDL TG %				0,89																			
S-LDL Total Cholesterol	SLDL C	0,98																						
S-LDL Total Choleseterol to total Lipids	SLDL C %	0,84																						
S-LDL Cholesterol Esters	SLDL CE	0,99																						
S-LDL CholesterolEsters to total Lipids	SLDL CE %	0,90																						
S-LDL Free Cholesterol	SLDL FC	0,90																						
S-LDL Free Cholesterol to total lipids	SLDL FC %	-0,78																						
S-LDL Total lipids	SLDLL	0,95																						
S-LDL Particle concent n mol / L	SLDL P	0,94																						
S-LDL Phospholipids	SLDL PL	0,82																						
S-LDL Phospholipids to total Lipids	SLDL PL %	-0,94																						
S-LDL Triglycerids	SLDL TG		0,57		0,69																			
S-LDL TriGlycerides to total Lipids	SLDL TG %		0,49		0,76																			

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
IDL Total Cholesterol	IDL C	0,96																						
IDL Total Choleseterol to total Lipids	IDL C %	0,45			-0,83																			
IDL Cholesterol Esters	IDL CE	0,94																						
IDL CholesterolEsters to total Lipids	IDL CE %				-0,81																			
IDL Free Cholesterol	IDL FC	0,96																						
IDL Free Cholesterol to total lipids	IDL FC %	0,56	-0,54																					
IDL Total lipids	IDLL	0,96																						
IDL Particle concent n mol / L	IDL P	0,96																						
IDL Phospholipids	IDL PL	0,98																						
IDL Phospholipids to total Lipids	IDL PL %		-0,78																					
IDL Triglycerids	IDL TG	0,44	0,43		0,76																			
IDL TriGlycerides to total Lipids	IDL TG %	-0,41			0,81																			

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
VLDLCholesterol	VLDL C	0,55	0,80																					
VLDL mean diameter vldl particles	VLDL D		0,90																					
VLDLTriglycerides	VLDL TG		0,91																					
XXL-VLDL Total Cholesterol	XXLVLDL C		0,93																					
XXL-VLDL Total Choleseterol to total Lipids	XXLVLDL C %						0,54						0,68											
XXL-VLDL Cholesterol Esters	XXLVLDL CE		0,87																					
XXL-VLDL CholesterolEsters to total Lipids	XXLVLDL CE %						0,62						0,44											
XXL-VLDL Free Cholesterol	XXLVLDL FC		0,95																					
XXL-VLDL Free Cholesterol to total lipids	XXLVLDL FC %												0,68											
XXL-VLDL Total lipids	XXLVLDLL		0,93																					
XXL-VLDL Particle concent n mol / L	XXLVLDL P		0,93																					
XXL-VLDL Phospholipids	XXLVLDL PL		0,92																					
XXL-VLDL Phospholipids to total Lipids	XXLVLDL PL %												0,44											
XXL-VLDL Triglycerids	XXLVLDL TG		0,92																					
XXL-VLDL TriGlycerides to total Lipids	XXLVLDL TG %												-0,81											
XL-VLDL Total Cholesterol	XLVLDL C		0,96																					
XL-VLDL Total Choleseterol to total Lipids	XLVLDL C %		-0,54				0,73																	
XL-VLDL Cholesterol Esters	XLVLDL CE		0,95																					
XL-VLDL CholesterolEsters to total Lipids	XLVLDL CE %		-0,46				0,71																	
XL-VLDL Free Cholesterol	XLVLDL FC		0,94																					
XL-VLDL Free Cholesterol to total lipids	XLVLDL FC %		-0,46				0,61																	
XL-VLDL Total lipids	XLVLDLL		0,96																					
XL-VLDL Particle concent n mol / L	XLVLDL P		0,96																					
XL-VLDL Phospholipids	XLVLDL PL		0,93																					
XL-VLDL Phospholipids to total Lipids	XLVLDL PL %														0,46									
XL-VLDL Triglycerids	XLVLDL TG		0,93																					
XL VLDL TG %	XLVLDL TG %						-0,68																	
L-VLDL Total Cholesterol	LVLDL C		0,96																					
L-VLDL Total Choleseterol to total Lipids	LVLDL C %						0,69																	
L-VLDL Cholesterol Esters	LVLDL CE		0,95																					
L-VLDL CholesterolEsters to total Lipids	LVLDL CE %						0,72																	
L-VLDL Free Cholesterol	LVLDL FC		0,94																					
L-VLDL Free Cholesterol to total lipids	LVLDL FC %														0,69									
L-VLDL Total lipids	LVLDLL		0,94																					
L-VLDL Particle concent n mol / L	LVLDL P		0,93																					
L-VLDL Phospholipids	LVLDL PL		0,92																					
L-VLDL Phospholipids to total Lipids	LVLDL PL %		0,41																			0,63		
L-VLDL Triglycerids	LVLDL TG		0,91																					
L-VLDL TriGlycerides to total Lipids	LVLDL TG %						-0,52								-0,47									

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
M-VLDL Total Cholesterol	MVLDL C		0,91																					
M-VLDL Total Choleseterol to total Lipids	MVLDL C %	0,49				0,55																		
M-VLDL Cholesterol Esters	MVLDL CE	0,43	0,86																					
M-VLDL CholesterolEsters to total Lipids	MVLDL CE %	0,48	-0,41			0,55																		
M-VLDL Free Cholesterol	MVLDL FC		0,92																					
M-VLDL Free Cholesterol to total lipids	MVLDL FC %														0,45									
M-VLDL Total lipids	MVLDLL		0,93																					
M-VLDL Particle concent n mol / L	MVLDL P		0,92																					
M-VLDL Phospholipids	MVLDL PL		0,92																					
M-VLDL Phospholipids to total Lipids	MVLDL PL %																					0,75		
M-VLDL Triglycerids	MVLDL TG		0,89																					
M-VLDL TriGlycerides to total Lipids	MVLDL TG %	-0,44				-0,54																		

Metabolite		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
S-VLDL Total Cholesterol	SVLDL C	0,69	0,65																					
S-VLDL Total Choleseterol to total Lipids	SVLDL C %	0,51	-0,58																					
S-VLDL Cholesterol Esters	SVLDL CE	0,78	0,52																					
S-VLDL Cholesterol Esters to total Lipids	SVLDL CE %	0,50	-0,56																					
S-VLDL Free Cholesterol	SVLDL FC	0,48	0,77																					
S-VLDL Free Cholesterol to total lipids	SVLDL FC %		-0,50																					
S-VLDL Total lipids	SVLDLL		0,83																					
S-VLDL Particle concent n mol / L	SVLDL P		0,84																					
S-VLDL Phospholipids	SVLDL PL		0,80																					
S-VLDL Phospholipids to total Lipids	SVLDL PL %					0,45																0,41		
S-VLDL Triglycerids	SVLDL TG		0,85																					
S-VLDL TriGlycerides to total Lipids	SVLDL TG %	-0,41	0,63																					
XS-VLDL Total Cholesterol	XSVLDL C	0,89																						
XS-VLDL Total Choleseterol to total Lipids	XSVLDL C %		-0,60		-0,62																			
XS-VLDL Cholesterol Esters	XSVLDL CE	0,85																						
XS-VLDL CholesterolEsters to total Lipids	XSVLDL CE %		-0,50		-0,61																			
XS-VLDL Free Cholesterol	XSVLDL FC	0,89																						
XS-VLDL Free Cholesterol to total lipids	XSVLDL FC %		-0,48																					
XS-VLDL Total lipids	XSVLDLL	0,84	0,42																					
XS-VLDL Particle concent n mol / L	XSVLDL P	0,81	0,47																					
XS-VLDL Phospholipids	XSVLDL PL	0,93																						
XS-VLDL Phospholipids to total Lipids	XSVLDL PL %	0,79																						
XS-VLDL Triglycerids	XSVLDL TG		0,74		0,50																			
XS-VLDL TriGlycerides to total Lipids	XSVLDL TG %		0,62		0,53																			

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a Rotation converged in 18 iterations.

Supplementary Table 2 – Variance explained by factors.

Factor	Eigenvalue	% of Variance Explained
1	70.95	31.26
2	57.17	25.19
3	19.79	8.71
4	11.08	4.88
5	8.55	3.77
6	4.75	2.09
7	4.08	1.80
8	3.67	1.62
9	3.04	1.34
10	2.85	1.25
11	2.72	1.20
12	2.50	1.10
13	2.05	0.90
14	1.87	0.82
15	1.85	0.81
16	1.63	0.72
17	1.60	0.70
18	1.48	0.65
19	1.38	0.61
20	1.25	0.55
21	1.16	0.51
22	1.11	0.49
23	1.00	0.44

Eigenvalues and percentage of variance explained by the factors identified by principal component analysis on the included metabolites. The total variance explained by the 23 factors with an eigenvalue of >1 was 91,4%.

Supplementary Table 3 – Assessment of cohort effects in controls**.**

Factor	Effect size	95% CI	P-value*
1	-0,453	-0,846 - -0.060	0,552
2	**0,607**	**0,220 – 0.994**	**0,046**
3	0,382	0,025 – 0.740	0,828
4	0,113	-0,262 – 0.489	>1
5	0,328	-0,059 – 0.715	>1
6	-0,094	-0,484 – 0.296	>1
7	-0,418	-0,827 - -0.009	>1
8	0,139	-0,230 – 0.507	>1
9	0,157	-0,208 – 0.523	>1
10	**-0,796**	**-1,177 - -0.415**	**0,001**
11	-0,200	-0,589 – 0.189	0,314
12	0,075	-0,292 – 0.441	>1
13	**-0,649**	**-1,034 - -0.265**	**0,023**
14	0,106	-0,304 – 0.517	>1
15	-0,530	-0,917 - -0.143	0,161
16	**1,225**	**0,858 – 1.592**	**$1{,}64 \times 10^{-9}$**
17	0,249	-0,141 – 0.639	>1
18	-0,474	-0,831 - -0.117	0,207
19	-0,371	-0,708 - -0.033	0,713
20	-0,255	-0,626 – 0.116	>1
21	-0,560	-0,934 - -0.187	0,069
22	-0,236	-0,619 – 0.147	>1
23	-0,111	-0,489 – 0.268	>1

Association of cohort with the factor within controls. After adjusting for age, sex, BMI and fasting, four factors had a significant cohort effect and were removed from further analyses.
* Pvalue Bonferroni corrected

Supplementary Table 4: Individual metabolites and cross-sectional outcomes

Factor	Metabolite	Hip or knee OA			Hip or Knee arthroplasty		
		OR	95%CI	P*	OR	95%CI	P*
17	Alanine	0.91	0.84-0.98	0.088	0.82	0.72-0.93	0.008
	Lactate	1.00	0.92-1.08	>1	1.47	1.31-1.65	$7.0x10^{-10}$
	Pyruvate	1.21	1.12-1.30	$5.1x10^{-6}$	1.93	1.72-2.16	$8.0x10^{-20}$
19	Glutamine	0.70	0.64-0.76	$8.8x10^{-15}$	0.65	0.58-0.74	$2.0x10^{-11}$
	Histidine	0.91	0.84-0.98	0.120	0.92	0.81-1.04	>1
22	Fatty Acid Chain Length	1.26	1.16-1.36	$4.8x10^{-8}$	1.83	1.64-2.05	$8.0x10^{-20}$
	Saturated Fatty Acids Ratio	1.01	0.93-1.08	>1	0.95	0.84-1.07	>1
	Degree of Unsaturation	1.05	0.97-1.14	>1	1.12	0.99-1.28	0.560

* Pvalue Bonferroni corrected

Supplementary Table 5 – Individual metabolites and progression

Factor	Metabolite	Hip Progression			Knee Progression		
		Beta	SE	P*	Beta	SE	P*
17	Alanine	-0,126	0,334	>1	-0,609	0,27	0,192
	Lactate	-0,605	0,247	0,120	-0,569	0,448	>1
	Pyruvate	-0,518	0,393	>1	-0,307	0,168	0,544
19	Glutamine	-0,452	0,991	>1	-0,488	0,798	>1
	Histidine	-0,411	0,339	>1	-0,295	0,29	>1
22	Fatty Acid Chain Length	7,97	3,742	0,264	4,123	4,309	>1
	Saturated Fatty Acids Ratio	-1,907	1,593	>1	-0,102	1,377	>1
	Degree of Unsaturation	-0,028	1,432	>1	1,047	1,314	>1

* Pvalue Bonferroni corrected

Supplementary Table 6 – with and without adjustment for BMI or fasting

	Factor 17								
	OA ~ Factor + age + sex + fasting + BMI			***Excluding BMI***			***Excluding fasting***		
	OR	95%CI	P-value*	OR	95%CI	P-value*	OR	95%CI	P-value*
HOA	1,15	1,04-1,27	0.171	1.25	1.14-1.37	0.008	1,14	1,03 - 1,26	0.228
THA	1,38	1,20-1,59	1.5x10-4	1.52	1.35-1.72	2.28x10-10	1,34	1,17 - 1,55	7.4x10-4
KOA	0,98	0,89-1,07	>1	1.04	0.96-1.14	>1	0,97	0,89 - 1,06	>1
TKA	1,49	1,21-1,83	0.003	1.56	1.31-1.85	9.69x10-6	1,42	1,16 - 1,74	0.019
	Factor 19								
	OA ~ Factor + age + sex + fasting + BMI			***Excluding BMI***			***Excluding fasting***		
	OR	95%CI	P-value*	OR	95%CI	P-value*	OR	95%CI	P-value*
HOA	0,68	0,60-0,76	3.6x10-10	0,59	0,53-0,65	2.30x10-24	0,67	0,60 - 0,75	1.0x10-10
THA	0,65	0,55-0,75	5.9x10-7	0,52	0,46-0,59	6.8x10-24	0,62	0,53 - 0,72	5.6x10-9
KOA	0,83	0,75-0,91	0.004	0,71	0,65-0,78	1.7x10-11	0,82	0,74 - 0,91	0.003
TKA	0,79	0,63-0,99	0.817	0,57	0,48-0,67	3.2x10-10	0,76	0,61 - 0,95	0.266
	Factor 22								
	OA ~ Factor + age + sex + fasting + BMI			***Excluding BMI***			***Excluding fasting***		
	OR	95%CI	P-value*	OR	95%CI	P-value*	OR	95%CI	P-value*
HOA	1,13	1,02-1,25	0.494	1,20	1,09-1,32	0.004	1.13	1.02-1.26	0.380
THA	1,41	1,23-1,63	1.9x10-5	1,45	1,29-1,63	3.1x10-8	1.44	1.25-1.65	7.0x10-6
KOA	1,06	0,96-1,16	>1	1,06	0,97-1,16	>1	1.06	0.97-1.16	>1
TKA	1,61	1,33-1,95	1.7x10-5	1,46	1,24-1,72	7.6x10-5	1.67	1.38-2.02	1.8x10-6

Analyses performed with logistic regression analyse relating factor-score to the OA-phenotype. Standard analyse was adjusted for age, sex, fasting and BMI, whereas the extra analyse was adjusted for age, sex either BMI or fasting.

* Pvalue Bonferroni corrected.

HOA - Radiographic Hip OA; THA - Total Hip Arthroplasty for primary hip OA; KOA - radiographic Knee OA; TKA - Total Knee Arthroplasty for primary knee OA