Metabolomics profile in depression: a pooled analysis of 230 metabolic markers in 5,283 cases with depression and 10,145 controls

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Abstract

Background: Depression has been associated with metabolic alterations, which adversely impact cardiometabolic health. Here, a comprehensive set of metabolic markers, predominantly lipids, was compared between depressed and non-depressed persons.

Methods: Nine Dutch cohorts were included, comprising 10,145 controls and 5,283 persons with depression, established with diagnostic interviews or questionnaires. A proton nuclear magnetic resonance metabolomics platform provided 230 metabolite measures: 51 lipids, fatty acids and low-molecular-weight metabolites, 98 lipid composition and particle concentration measures of lipoprotein subclasses and 81 lipid and fatty acids ratios. For each metabolite measure logistic regression analyses adjusted for sex, age, smoking, fasting status and lipid-modifying medication were performed within cohort, followed by random-effects meta-analyses.

Results: Twenty-one of the 51 lipids, fatty acids and low-molecular-weight metabolites were significantly related to depression (false discovery rate $q<0.05$). Higher levels of apolipoprotein B, Very Low Density Lipoprotein (VLDL)-cholesterol, triglycerides, diglycerides, total and mono-unsaturated fatty acids, fatty acid chain length, glycoprotein acetyls, tyrosine, and isoleucine, and lower levels of High Density Lipoprotein (HDL)-cholesterol, acetate, and apolipoprotein A1 were associated with increased odds of depression. Analyses of lipid composition indicators confirmed a shift towards less HDL and more VLDL and triglycerides particles in depression. Associations appeared generally consistent across sex, age and body mass index strata, and across cohorts with depressive diagnoses *vs.* symptoms.

Conclusions: This large-scale meta-analysis indicates a clear distinctive profile of circulating lipid metabolites associated with depression, potentially opening new prevention or treatment avenues for depression and its associated cardiometabolic comorbidity.

Introduction

Depression imposes a huge burden on individuals and society.(1) With a high annual (6%) and lifetime (19%) prevalence, depression is among the leading contributors to global disease burden.(1, 2) It has been associated with an increased risk of somatic disease, including cardiometabolic conditions such as metabolic syndrome (3), obesity (4), diabetes mellitus (5), stroke (6), and cardiovascular disease (7), as well as an increased risk of all-cause mortality (8).

Depression is correlated with metabolic alterations in peripheral bodily systems.(1) A systematic review(9) summarizing metabolomics analyses of urine, CSF, and blood samples of depressed patients highlighted a set of altered metabolites implicated in energy metabolism, neuronal integrity and transmission. Meta-analyses showed that depression was associated with increased blood levels of total cholesterol(10) and triglycerides (TG),(3) and decreased low density lipoprotein (LDL) cholesterol,(11) high density lipoprotein (HDL) cholesterol,(3) and omega-3 polyunsaturated fatty acids.(12) However, large heterogeneity was noted between studies, which was partly explained by differential lipid classifications.(11)

Alterations in circulating lipid concentrations may be linked to pathophysiological pathways related to depression, such as chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis or chronic low-grade inflammation (1). Glucocorticoid-induced hypercortisolemia is known to result in lipolysis, the release of fatty acids and synthesis of Very Low Density Lipoprotein (VLDL)(13). Similarly, activation of the pro-inflammatory response leads to a reduction in HDL cholesterol and phospholipids, and an increase in TG, caused by the compensatory production and accumulation of phospholipid-rich VLDL (14). In addition, omega-3 fatty acids have anti-inflammatory properties, impact HPA-axis functioning, promote cell membrane fluidity, and are involved in the regulation of dopaminergic and serotonergic neurotransmission, which can be altered in depression.(15) Alterations of circulating concentrations of lipids may also represent a consequence of depression. Patients with depression are more likely to engage in unhealthy behaviors, such as sedentariness, excessive alcohol use and poor nutrition (with preference

for high palatable food rich in saturated fats), which may lead to dyslipidemia (16), that can result in metabolic syndrome and cardiovascular disease.

Emerging technologies allow high-throughput profiling of lipids and other metabolites, which has led to efforts of determining metabolic signatures of various diseases.(17),(18) A few studies have applied this to depression,(19, 20) but their results remain inconsistent,(21, 22) partly due to different methodologies used and different metabolites (lipids, amino acids and other small molecules) analyzed.(23)

This study aims to identify plasma lipids, fatty acids and low-molecular-weight metabolites associated with depression by analyzing data from nine Dutch clinical- and population-based studies, and to assess consistency of findings across studies. A strength of the study is that all metabolites were measured around the same time with the same targeted ^{1}H-NMR platform that quantifies lipids, fatty acids and low-molecular-weight metabolites, including those that have been related to consequences of depression (e.g. insulin resistance (24), onset of cardiovascular events (25), and mortality (26)).

Methods and Materials

Sample description

Eleven datasets from nine cohorts participating in the Biobanking and BioMolecular resources Research Infrastructure-The Netherlands (BBMRI-NL) were included: Cohort on Diabetes and Atherosclerosis Maastricht (CODAM),(27) The Maastricht Study,(28) Erasmus Rotterdam Family study (ERF),(29) Leiden University Migraine Neuro-Analysis (LUMINA),(30) Netherlands Epidemiology of Obesity study (NEO), Netherlands Study of Depression and Anxiety (NESDA), Netherlands Twin Register (NTR),(31) the Rotterdam Study (RS), and Lifelines-DEEP (LLD).(32–34) Both CODAM and The Maastricht Study contributed two datasets stratified by diabetes mellitus status. In total, we included 5,283 persons with depression and 10,145 controls (see Supplement 1 for detailed cohort descriptions). All participants provided written informed consent. Studies were approved by local ethics committees.

Measurements

Depression

The presence of depression was measured either before blood sampling or to a maximum of one month after blood sampling. Subjects were defined as cases when meeting all the criteria required for a diagnosis of major depressive disorder (MDD) in clinical structured interviews in four cohorts, or when scoring above validated clinical cut-off score in depression questionnaires in five cohorts (Supplement 2 for all instruments and definitions). In the main analyses, cases included subjects with any history of depression in lifetime.

Metabolites

Supplement 1 shows details on blood collection (for each cohort), measurement and processing of metabolite measurements. A total of 230 metabolites or metabolite ratios were reliably quantified from EDTA plasma samples using targeted high-throughput proton Nuclear Magnetic Resonance (^{1}H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland).(35). This metabolomics platform has been

used in large-scaled epidemiological studies in the field of diabetes(24), cardiovascular disease(25), mortality(26) and alcohol intake.(36). In order to enhance interpretation, metabolites were classified into three clusters curated by Nightingale Health(37): 1) lipids, fatty acids and low-molecular-weight metabolites (N=51); 2) lipid composition and particle concentration measures of lipoprotein subclasses (N=98); 3) metabolite ratios (N=81). Data were processed according to a shared protocol applied also in other studies of BBMRI-NL.(38) In each cohort, values of metabolites that could not be quantified (≤5 metabolites per cohort) were set as missing for all subjects. Furthermore, metabolites values in subjects with outlying concentrations (±5SDs) were additionally set as missing. A value of 1 was added to all metabolite values (Supplement 1 includes extensive analyses indicating that the degree of bias potentially introduced by this transformation is likely negligible) that were subsequently (natural)log-transformed to approximate normality. The obtained values were scaled to standard deviations units in each cohort to enable comparison.

Statistical analyses

Per-metabolite logistic regression analyses were initially performed in each dataset. The dependent variable was depression, independent variables were the 230 metabolite measurements. For the NTR cohort, logistic regression using generalized estimating equations were conducted, accounting for family-relatedness. All models were adjusted for age, sex, fasting status, use of lipid-modifying drugs listed under ATC (Anatomical Therapeutic Chemical Classification System) code C10 and smoking (Supplement 1 for measurements). All analyses were based on available data per metabolite (pair-wise deletion). Dataset-specific estimates were combined using random-effects meta-analyses (restricted maximum-likelihood estimator) to obtain pooled odds ratios (ORs). Heterogeneity of results between datasets was quantified by I^2(39) along with 95% confidence intervals (CI) as recommended.(40),(41)

Since body mass index (BMI) has been shown to be associated with depression(4) and metabolites ,(42) we re-run the main analyses adjusting for BMI. Furthermore, to investigate whether metabolic profiles were dependent on recent presence of depression, additional analyses were conducted comparing

current depressed cases (depression present ±1 month around blood sampling) and controls. We conducted sensitivity analyses in which we excluded antidepressant medication (ATC code N06A) users, in order to study the impact of depression apart from its treatment. Here, we *a priori* expected to find a less distinctive metabolomics profile, given that antidepressant medication prescriptions are more likely in depressed individuals with higher depression severity. Correlations between estimates obtained from these sensitivity analyses and those obtained in the main analyses were computed in order to measure the impact of the factors considered.

Four additional sets of stratified analysis were performed to explore whether associations between metabolites and depression were different as a function of (1) depression assessment (diagnosis *vs.* self-report instrument), (2) sex (men *vs.* women), (3) age (<50 years *vs.* ≥50 years) and (4) BMI (normal (18.50-24.9) *vs.* overweight (25.0-29.9) and *vs.* obesity (≥30)). A Wald-test was performed to test differences in effect sizes across these strata,(43) and correlations between estimates obtained across strata were estimated.

The False Discovery Rate (FDR) method(44) was applied to address multiple testing at the meta-analysis level for 230 metabolites. Meta-analyses were conducted with the 'metafor' package (version 2.0.0) in R v3.4.2-3.4.3 (R Project for Statistical Computing).

Results

Overview of cohorts

The study population comprised 15,428 adults from 11 datasets of 9 cohorts. There were 10,145 controls, and 5,283 participants with depression. Table 1 shows the characteristics of the 11 datasets. Across the cohorts, the average age ranged from 40.4-64.8 years, the proportion of women from 32-70%, and the median prevalence of depression was 29.5%.

Associations of the 51 lipids, fatty acids and low-molecular-weight metabolites with depression

Figure 1 shows a polar plot with ORs of meta-analyses investigating associations between depression and the 51 metabolites, after adjustment for sex, age, smoking, lipid modifying drugs, and fasting status. Of these, 21 were associated with depression at FDR-q<0.05 (Table 2; Supplement 4). Metabolites associated with a higher odds for depression were apolipoprotein B, remnant (non-HDL and non-LDL) cholesterol, VLDL cholesterol, mean diameter of VLDL, the glycerides and phospholipid markers diglycerides, TG in LDL, serum TG, TG in HDL, TG in VLDL, the fatty acid measures total fatty acids, monounsaturated fatty acid, and estimated fatty acid chain length, the inflammation marker glycoprotein acetyls, and the amino acids tyrosine and isoleucine. Higher levels of metabolites that were associated with a lower odds for depression were apolipoprotein A1, cholesterol content for HDL (in particular HDL_2- and HDL_3- cholesterol), mean diameter of HDL, and ketone body acetate.

Heterogeneity was small (I^2<25% for 15 out of 21 metabolites) and statistically non-significant in almost all (19 out of 21) analyses. As shown in the related forest plots (Supplement 4) association estimates were quite consistent across the different datasets, including those enriched for cardiometabolic risk. To confirm this,

we re-run the analyses after removing two datasets (CODAM-DM and TMS-DM) including ~900 participants with established diabetes and elevated cardiovascular risk factors. Association estimates were highly concordant with those of the original analyses (r=0.99); all the 21 metabolites detected in the original analyses were associated at nominal level with depression (17 at FDR-q<0.05; Supplement 16).

Additional adjustment for BMI partially reduce the strength of the association of these 21 metabolites with depression (regression slope of the 21 beta's before versus after BMI-adjustment=0.65, whereas a value of 1 would indicate similar average association sizes; correlation r=0.98): of the 21 metabolites associated with depression, 16 remained significantly related to depression at $p<0.05$ and 13 at FDR $q<0.05$ (Table 2). Supplement 3 shows the pooled ORs and heterogeneity findings for all metabolites.

Associations of 98 detailed lipid composition and particle concentration measures of lipoprotein subclasses with depression

Figure 2 shows the ORs of the meta-analyses for the 98 lipid measures of the 14 lipoprotein subclasses, ordered from large to small particle size. Generally, there appeared to be a shift in association with depression by lipoprotein classes: VLDL lipoprotein levels were positively related to depression, IDL and LDL lipid levels were not consistently associated, whereas HDL lipoprotein measures were inversely related to depression. Furthermore, depression was related to higher TG levels.

Associations of 81 metabolite ratios with depression

Supplement 5 shows the ORs of the meta-analyses for the 81 metabolite ratios, of which 27 were significant at FDR-$q<0.05$. In general, TG to total lipid ratios were significantly related to an increased odds of depression. Some of the VLDL, IDL, LDL, and HDL lipid measures as percentage of total lipids were positively related to depression, whereas others were inversely related. In general, associations of the metabolite ratios with depression were less pronounced compared to those with absolute metabolite values.

Sensitivity analyses

Current depression

The original 5,283 depressed cases included subjects with any history of depression in lifetime. In 62% of the cases (3,265 subjects) depression was present between one month before to one month after blood draw. We repeated analyses with only these 3,265 current cases with depression (vs. 10,145 controls). Of the 51 lipids, fatty acids and low-molecular weight metabolites, 22 were associated with current depression at FDR-q<0.05 (Supplement 6). Notably, the strength of the associations with the 51 metabolites tended to be stronger for current depression than for the original definition (regression slope of beta's for current versus broadly-defined depression=1.22, r=0.95) (Supplement 3). Supplements 3,7,8 show associations of the 98 lipid measures of lipoprotein subclasses, and the 81 metabolite ratios with current depression, which were largely in line with those of original analyses.

Antidepressant medication

To study whether associations were independent of concurrent antidepressant medication use, we removed 1,597 subjects across cohorts who reported use of antidepressants. The majority were depressed cases (N=1,305), which was expected given that depression is the main indication for receiving antidepressant treatment. Additionally, one study (LLD) was removed because of model convergence issues. In the remaining 3,966 cases and 8,887 controls - representing a 21% drop in effective sample size as compared to the original analyses - associations with the 51 lipids, fatty acids and low-molecular-weight metabolites were generally in the same direction, but the strength of the associations was attenuated (regression slope of betas before and after antidepressant users exclusion=0.60, r=0.88) (Supplement 9). Among the 21 significantly associated metabolites in the overall sample, 8 were still associated at p<0.05, of which 2 (HDL_3- cholesterol, and acetate) at FDR-q<0.05 in the antidepressant-free subsample.

Subgroups

Exploration of consistency of associations across subgroups showed that there were no significant differences (Wald-test, FDR-q>0.05) in the strength of the association between metabolites and

depression across subgroups with depression diagnoses *vs.* self-reported depression (r=0.75, Supplement 10), across men *vs.* women (r=0.64, Supplement 11), across age <50y *vs.* >=50y (r=0.84, Supplement 12), and across BMI groups (normal *vs.* overweight r=0.68, normal *vs.* obese r=0.55, overweight *vs.* obese r=0.71, Supplements 13-15).

Discussion

This meta-analysis of metabolomics and depression, is to our knowledge the largest of its kind. We analyzed data of over 15,000 subjects from nine Dutch clinical and population-based studies in the Netherlands to identify metabolites associated with depression. Our findings showed that depression is associated with a metabolic signature towards less HDL and more VLDL and triglycerides particles. More specifically, 21 plasma lipids, fatty acids and low-molecular-weight metabolites were significantly related to depression: *higher* levels of apolipoprotein B, VLDL cholesterol, triglycerides, diglycerides, total and mono-unsaturated fatty acids, fatty acid chain length, glycoprotein acetyls, tyrosine, and isoleucine, and *lower* levels of HDL cholesterol, acetate, and apolipoprotein A1. Associations were generally consistent across sex, age and body mass index strata, and across cohorts using depression diagnoses *vs.* depressive symptoms. These metabolic alterations in depression could potentially explain part of the increased risk of cardiometabolic disease in depressed individuals.

Our findings that depression is related to higher VLDL, higher TG and lower VLDL are in line with previous research.(3)(11)-(45) In the present study, we predominantly found differences in absolute lipid measures of the VLDL subfractions, whereas findings with lipid measures to lipid ratios in VLDL were less consistently associated with depression. This suggests that the total amount of lipids, rather than the type of lipids, is the main contributor to associations of depression with VLDL. For other metabolites, previous studies indicated more mixed findings. We did not find associations for LDL cholesterol measures, which contrasts with a previous meta-analysis that showed associations between and increased LDL cholesterol.(11) For fatty acids measures, we observed that higher mono unsaturated fatty acids, total fatty acids and estimated fatty acids chain length were associated with an increased odds of depression. Most evidence for links with fatty acids in depression stems from research on omega-3 fatty acids,(12) for which we did not observe a consistent, significant association with depression in the present study. The pro-inflammatory glycoprotein acetyls being positively associated with depression is in line with the large body of evidence linking inflammation to depression.(46) The short chain fatty acid and ketone body acetate was lower in depression. It was hypothesized that a Western-style diet alters gut

microbiome composition, resulting in lower acetate levels, which could subsequently induce depression.(4) Furthermore, a smaller study found lower isoleucine levels in depression,(47) which contrasts our findings. Finally, a review concluded that there was no association between tyrosine and depression,(48) whereas we observed higher tyrosine in depression. Discrepancies could be explained by differences in study characteristics or variation in analyses approaches, such as selection of potentially confounding factors.

We additionally evaluated the impact of the timeframe of depression assessment on the results. In secondary analyses including cases with current depression only, associations tended to become enhanced, suggesting that metabolomics alterations represent state markers reflecting current depression. Nevertheless, a similar profile of associations was found when analyzing depression cases defined in a broader timeframe. The metabolic signature identified may therefore also represent a persisting biological scar after depression remission, or a pre-existing underlying vulnerability factor for depression development.

The impact of antidepressant medication use on the results was explored in secondary analyses, although this observational study precludes definitive conclusions, since depression severity most likely represents the clinical indication for antidepressant treatment (confounded by indication).(49) We reanalyzed data after excluding antidepressant users, and found that the strength of associations was attenuated. Furthermore, the reduction in effective sample size substantially impacted the power to find significant associations. Nevertheless, directions of associations were highly consistent with those obtained in the full sample. Furthermore, the literature shows that potential detrimental effects of antidepressants on dyslipidemia is evident mainly for tricyclic antidepressants (TCA).(50),(51) Data from the NESDA cohort,(51) including patients from mental health care institutions, with the highest prevalence of antidepressant users (27%, Table 1), showed that TCA were prescribed only in 3% of the participants. Since in other cohorts included in the present meta-analysis the overall prevalence of antidepressant use were lower than ~9%, it could be assumed that the number of TCA users may be

limited. This observation, combined with the results of our sensitivity analyses, suggests that antidepressant use is unlikely to be the major driver of the associations between metabolites and depression.

Secondary analyses also indicated that results were generally attenuated when BMI was taken into account, suggesting that part of the differential metabolite levels in depression could be explained by BMI. However, interrelationships between BMI, metabolite, depression and antidepressants are particularly complex. A significant genetic correlation has been found between depression and BMI,(52) indicating that they may emerge from partially shared etiological mechanisms; at the same time BMI has been shown to influence metabolite concentrations.(42) The ability to disentangle different independent effects of this complex network in observational data is limited. Nevertheless, the majority of metabolites were associated with depression after taking into account BMI, indicating that this factor explains only a limited portion of the depression-metabolites link.

The present findings may be explained by three, non-mutually exclusive, scenarios. First, alterations of lipids may be a consequence of depression. Depressed persons are more likely to engage in unhealthy behaviors such as sedentariness, excessive alcohol use and poor nutrition (e.g. saturated fats), which may lead to dyslipidemia.(16) Second, lipid dysregulations may be part of the pathophysiological pathways implicated in depression, such as chronic HPA-axis and inflammatory activity, resulting in lipolysis, release of fatty acids, synthesis of VLDL, hypertriglyceridemia and reduction in HDL cholesterol. Finally, metabolomic alterations in depression may represent epiphenomena stemming from the same root, such as a common genetic factor. A recent genome-wide association study (GWAS) of major depression involving >450,000 participants, reported a significant genetic correlation (r_g=0.14, p=7.8x10^{-7}) with high TG levels, but not with LDL or HDL.(53) Furthermore, no genetic correlations emerged with metabolites of the same panel that we found to be associated with depression, although the relatively smaller sample size (~25,000) of the metabolomics GWAS may substantially limit the ability to detect correlation; the only exception was a nominally significant correlation with glycoprotein acetyls (r_g=0.15, p=0.03), with the same direction of the phenotypic association we identified. Further

experimental studies and genetic-informed designs such as Mendelian randomization may disentangle whether depression and lipid dysregulations emerge from shared etiology, and whether depression causally determines lipid alterations or *vice versa*.

The study has some limitations. Due to limited availability or differences in assessment across datasets we cannot rule out confounding by other health-related or lifestyle factors, such as chronic cardiometabolic conditions, alcohol use or specific food intake prior to sample collection. Nevertheless, the associations between depression and metabolites were consistent across datasets, including those enriched for traits such as diabetes, cardiovascular risk factors and migraine. Furthermore, alcohol use may represent a mediating mechanism rather than a confounder in the metabolites-depression association, as recent evidence(54) showed that heavy-drinking is to quite some extent caused by depression. Analyses were adjusted for fasting status (>94% of subjects were fasting, Table 1), but both fasting and non-fasting samples can be reliably analyzed by the metabolomics platform utilized(26, 35). We could not examine whether the associations with metabolites detected vary as a function of specific depression clinical characteristics. Strengths of the study (large samples, metabolites data generated for all studies with the same platform) have enabled the identification of the most reliable metabolic signals associated with depression. These are worth further examination in relation to clinically-relevant phenotypes (e.g. age of onset, recurrence, duration, symptom profiles) in future studies based on psychiatrically well-characterized samples.

This large-scale meta-analysis including over 15,000 participants identified a metabolomics signature associated with depression. This biological signature is partially shared with other conditions such as diabetes, obesity and cardiovascular diseases(3)(5)(6)(7) that commonly co-occur with depression, heavily burdening public health resources. Alterations in the lipid spectrum identified in the present study may represent a substrate linking depression to cardiometabolic diseases and, therefore, a potential target for prevention and treatment of depression and its detrimental somatic sequelae.

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Disclosures

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Overview of tables and figures

Table 1. Characteristics of the study populations

Table 2. Overview of the 21 lipids, fatty acids and various low-molecular-weight metabolites that are significantly related to depression in the pooled analysis at FDR q<0.05

Figure 1. Polar plot illustrating pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with depression

*significant at FDR q<0.05.

Dotted circle indicates an OR of 1.

Figure legend

C=cholesterol, TG=triglycerides, VLDL=very-low-density lipoprotein, LDL=low-density lipoprotein, IDL=intermediate-density lipoprotein, HDL=high-density lipoprotein, FA=fatty acids

ApoA1=apolipoprotein A-I, ApoB=apolipoprotein B, Est=esterified, Remnant=non-HDL, non-LDL –cholesterol, D=mean diameter, DAG=diglycerides, TotPG=total phosphoglycerides, TotCho=total cholines, PC= phosphatidylcholine and other cholines, SM=sphingomyelins, SFA=saturated FA, MUFA=monounsaturated FA (16:1,18:1), PUFA=polyunsaturated FA, FAw6=omega-6 FA, FAw3=omega-3 FA, LA=linoleic acid (18:2), CLA=conjugated linoleic acids, DHA=docosahexaenoic acid, TotFA=total FA, FALen=estimated FA chain length, UnsatDeg=estimated degree of unsaturation, Alb=albumin, Crea=creatinine, Cit=citrate, Glc=glucose, Lac=lactate, Gp=glycoprotein acetyls, mainly a1-acid glycoprotein, bOHBut=3-hydroxybutyrate, Ace=acetate, AcAce=Acetoacetate, Ala=alanine, Gln=glutamine, His=histidine, Phe=phenylalanine, Tyr=tyrosine, Ile=isoleucine, Leu=leucine, Val=valine

Density: HDL2 (1.063-1.125 g/mL) HDL3 (1.125-1.210 g/mL)

Figure 2. Pooled odds ratio's and 95% confidence intervals for the association of the 98 lipid measures of lipoprotein subclasses with depression

*significant at FDR q<0.05.

Dotted circle indicates an OR of 1.

Figure legend

XXL=extremely large, XL=very large, L=large, M=medium, S=small, XS=very small

VLDL=very-low-density lipoprotein, LDL=low-density lipoprotein, IDL=intermediate-density lipoprotein, HDL=high-density lipoprotein

CE=cholesterol ester, FC=free cholesterol, P=particle concentration, PL=phospholipids, C=total cholesterol, L=total lipids, TC=triglycerides

Particle sizes: XXL.VLDL (>75nm), XL.VLDL (64nm), L.VLDL (53.6nm), M.VLDL (44.5nm), S.VLDL (36.8nm), XS.VLDL (31.3nm), IDL (28.6nm), L.LDL (25.5nm), M.LDL (23.0nm), S.LDL (18.7nm), XL.HDL (14.3nm), L.HDL (12.1nm), M.HDL (10.9nm), S.HDL (8.7 nm)

Table 1. Characteristics of the study populations (N=15,428)

	CODAM DM	CODAM noDM	TMS DM	TMS noDM	ERF	LUMINA	NEO	NESDA	NTR	RS	LLD
Total N	139	416	775	723	346	231	6554	2509	1523	1188	1024
Female, n (%)	46 (33.1)	168 (40.4)	248 (32.0)	455 (62.9)	198 (57.2)	136 (58.9)	3433 (52.4)	1680 (67.0)	1072 (70.4)	755 (63.6)	596 (58.2)
Age (years), mean (SD)	61.2 (6.2)	59.0 (7.1)	62.7 (7.5)	58.8 (8.0)	48.0(14.0)	41.2 (12.2)	55.8 (6.0)	41.8 (13.0)	40.4 (13.2)	64.8 (5.8)	44.9 (13.2)
Current smoker, n (%)	26 (18.7)	86 (20.7)	122 (15.7)	94 (13.0)	127 (36.7)	25 (10.8)	1071 (16.3)	978 (39.0)	74(4.9)	161 (13.6)	204 (19.9)
Use of lipid-modifying medications n (%)	35 (25.2)	69 (16.6)	578 (74.6)	162 (22.4)	31 (8.95)	2 (0.9)	1024 (15.6)	177 (7.0)	77 (5.1)	257 (21.6)	45 (4.4)
Fasting, n (%)	139 (100)	416 (100)	775 (100)	723 (100)	344 (99.4)	230 (99.5)	6554 (100)	2403 (95.8)	1441 (94.6)	1113 (93.7)	1013 (98.9)
BMI (kg/m^2), mean (SD)	30.3 (4.7)	28.0 (4.1)	29.8 (4.9)	29.3 (3.6)	27.2 (4.5)	23.6 (2.4)	30.1 (4.8)	25.6 (5.0)	24.7 (4.1)	27.4 (4.3)	25.2 (4.1)
No depression, n (%)	105 (75.5)	338 (81.3)	503 (64.9)	480 (66.4)	193 (55.8)	172 (74.5)	4620 (70.5)	634 (44.8)	1353 (88.8)	737 (62.0)	1010 (98.6)
Depression, n (%)	34 (24.5)	78 (18.8)	272 (35.1)	243 (33.6)	153 (44.2)	59 (25.5)	1934 (29.5)	1875 (74.7)	170 (11.2)	451 (38.0)	14 (1.4)
Of which current depression, n (%)	34 (24.5)	78 (18.8)	46 (8.4)	24 (4.8)	25 (7.2)	14 (6.1)	1934 (29.5)	782 (55.2)	N.A.	314 (26.4)	14 (1.4)
Antidepressant use, n (%)	10 (7.2)	20 (4.8)	63 (8.1)	64 (8.9)	24(6.9)	3 (1.3)	534 (8.1)	683 (27.2)	73 (4.8)	77(6.5)	46 (4.5)

Abbreviations: BMI = Body mass index, CODAM DM =Cohort on Diabetes and Atherosclerosis Maastricht, participants with type 2 diabetes mellitus, CODAM noDM =Cohort on Diabetes and Atherosclerosis Maastricht, participants without diabetes mellitus, TMS DM = The Maastricht Study, participants with type 2 diabetes mellitus, TMS noDM = The Maastricht Study, participants without diabetes mellitus, ERF = Erasmus Rucphen Family study, LLD = Lifelines Deep, LUMINA = Leiden University Migraine Neuro-Analysis, NEO = The Netherlands Epidemiology of Obesity Study, NESDA = Netherlands Study of Depression and Anxiety, NTR = Netherlands Twin Register, RS = Rotterdam Study. N.A.=not available.

Table 2. Overview of the 21 lipids, fatty acids and various low-molecular-weight metabolites that are significantly related to depression in the pooled analysis at FDR q<0.05

	Model 1			Model 2*		
Metabolite	Pooled OR	p-value	FDR q-value	Pooled OR	p-value	FDR q-value
Apolipoproteins						
Apolipoprotein A1	0.90	2.71×10^{-7}	2.50×10^{-6}	0.94	0.007	0.021
Apolipoprotein B	1.08	2.40×10^{-4}	6.90×10^{-4}	1.05	0.014	0.040
Cholesterol						
Remnant cholesterol	1.07	0.003	0.006	1.05	0.014	0.038
VLDL cholesterol	1.10	1.68×10^{-4}	5.03×10^{-4}	1.07	0.001	0.002
HDL cholesterol	0.86	1.24×10^{-12}	9.47×10^{-11}	0.91	2.03×10^{-5}	2.59×10^{-4}
HDL_2 cholesterol	0.89	5.78×10^{-6}	2.79×10^{-5}	0.93	0.001	0.003
HDL_3 cholesterol	0.90	2.18×10^{-5}	8.37×10^{-5}	0.93	4.91×10^{-4}	0.002
Mean diameter of VLDL	1.13	1.30×10^{-6}	8.82×10^{-6}	1.08	2.39×10^{-4}	0.001
Mean diameter of HDL	0.91	2.10×10^{-4}	6.10×10^{-4}	0.96	0.104	0.222
Di- and triglycerides						
Diglycerides	1.09	2.56×10^{-5}	9.65×10^{-5}	1.07	0.003	0.008
Serum total TG	1.11	3.29×10^{-5}	1.15×10^{-4}	1.08	1.92×10^{-4}	0.001
VLDL TG	1.11	8.68×10^{-5}	2.77×10^{-4}	1.08	1.76×10^{-4}	0.001
LDL TG	1.05	0.015	0.032	1.04	0.101	0.218
HDL TG	1.09	0.007	0.015	1.07	0.029	0.072
Fatty acids						
Mono Unsaturated FA	1.09	7.13×10^{-6}	3.35×10^{-5}	1.06	0.004	0.012
Total FA	1.05	0.013	0.027	1.03	0.102	0.219
Estimated FA chain length	1.10	0.020	0.043	1.08	0.060	0.140
Inflammation						
Glycoprotein acetyls	1.13	0.003	0.007	1.09	0.028	0.071
Ketone bodies						
Acetate	0.91	0.003	0.006	0.93	0.038	0.092
Amino acids						
Tyrosine	1.07	0.013	0.028	1.02	0.552	0.760
Isoleucine	1.14	8.26×10^{-6}	3.71×10^{-5}	1.08	0.001	0.004

Model 1: adjusted for sex, age, smoking, lipid modifying drugs, fasting status; Model 2: adjusted for model 1 and body mass index; Abbreviations: FDR=false discovery rate, FA=fatty acids, HDL=high-density lipoprotein, LDL=low-density lipoprotein, OR=odds ratio, TG=triglycerides, VLDL=very-low-density lipoprotein.