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Published in:
Biological Psychiatry

DOI:
10.1016/j.biopsych.2019.08.016

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
BBMRI-NL Metabolomics Consortium, Bot, M., Milaneschi, Y., Al-Shehri, T., Amin, N., Garmaeva, S., Onderwater, G. L. J., Pool, R., Thesing, C. S., Vijfhuizen, L. S., Vogelzangs, N., Arts, I. C. W., Demirkan, A., van Duijn, C., van Greevenbroek, M., van der Kallen, C. J. H., Köhler, S., Ligthart, L., van den Maagdenberg, A. M. J. M., ... Penninx, B. W. J. H. (2020). Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biological Psychiatry*, *87*(5), 409-418. https://doi.org/10.1016/j.biopsych.2019.08.016

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Archival Report

Biological Psychiatry

Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls

Mariska Bot, Yuri Milaneschi, Tahani Al-Shehri, Najaf Amin, Sanzhima Garmaeva, Gerrit L.J. Onderwater, Rene Pool, Carisha S. Thesing, Lisanne S. Vijfhuizen, Nicole Vogelzangs, Ilja C.W. Arts, Ayse Demirkan, Cornelia van Duijn, Marleen van Greevenbroek, Carla J.H. van der Kallen, Sebastian Köhler, Lannie Ligthart, Arn M.J.M. van den Maagdenberg, Dennis O. Mook-Kanamori, Renée de Mutsert, Henning Tiemeier, Miranda T. Schram, Coen D.A. Stehouwer, Gisela M. Terwindt, Ko Willems van Dijk, Jingyuan Fu, Alexandra Zhernakova, Marian Beekman, P. Eline Slagboom, Dorret I. Boomsma, and Brenda W.J.H. Penninx, for the BBMRI-NL Metabolomics Consortium

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 nondepressed persons.

METHODS: Nine Dutch cohorts were included, comprising 10,145 control subjects and 5283 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 gender, age, smoking, fasting status, and lipid-modifying medication were performed within cohort, followed by random-effects meta-analyses.

RESULTS: Of the 51 lipids, fatty acids, and low-molecular-weight metabolites, 21 were significantly related to depression (false discovery rate $q < .05$). Higher levels of apolipoprotein B, very-low-density lipoprotein cholesterol, triglycerides, diglycerides, total and monounsaturated fatty acids, fatty acid chain length, glycoprotein acetyls, tyrosine, and isoleucine and lower levels of high-density lipoprotein cholesterol, acetate, and apolipoprotein A1 were associated with increased odds of depression. Analyses of lipid composition indicators confirmed a shift toward less high-density lipoprotein and more very-low-density lipoprotein and triglyceride particles in depression. Associations appeared generally consistent across gender, age, and body mass index strata and across cohorts with depressive diagnoses versus 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.

Keywords: Biomarkers, Cardiovascular, Depression, Metabolites, Metabolomics, Pooled analysis

https://doi.org/10.1016/j.biopsych.2019.08.016

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, cerebrospinal fluid, and blood samples of patients with depression 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 ω-3 polyunsaturated fatty acids (12). However, considerable heterogeneity was noted between studies, which was partly explained by differential lipid classifications (11).

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ISSN: 0006-3223

Alterations in circulating lipid concentrations may be linked to pathophysiological pathways related to depression, such as chronic activation of the hypothalamic-pituitary-adrenal 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 proinflammatory 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, ω-3 fatty acids have anti-inflammatory properties, impact hypothalamic-pituitary-adrenal 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 highly 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 the results remain inconsistent (21,22); this is partly due to different methodologies used and different metabolites (lipids, amino acids, and other small molecules) analyzed (23).

This study aimed to identify plasma lipids, fatty acids, and low-molecular-weight metabolites associated with depression by analyzing data from 9 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 proton nuclear magnetic resonance 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 9 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 Rucphen Family (ERF) study (29), Leiden University Migraine Neuro-Analysis (30), Netherlands Epidemiology of Obesity (NEO) study, Netherlands Study of Depression and Anxiety (NESDA), Netherlands Twin Register (31), the Rotterdam Study, and Lifelines DEEP (LLD) (32–34). Both CODAM and The Maastricht Study contributed 2 datasets stratified by diabetes mellitus status. In total, we included 5283 persons with depression and 10,145 control subjects (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 up to a maximum of 1 month after blood sampling. Subjects were defined as cases when meeting all the criteria required for a diagnosis of major depressive disorder in clinical structured interviews in 4 cohorts or when scoring above a validated clinical cutoff score in depression questionnaires in 5 cohorts (see Table S1 in Supplement 1 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. Using targeted high-throughput proton nuclear magnetic resonance metabolomics (Nightingale Health Ltd., Helsinki, Finland), 230 metabolites or metabolite ratios were reliably quantified from ethylenediamine tetraacetate plasma samples (35). This metabolomics platform has been used in large-scale epidemiological studies of diabetes (24), cardiovascular disease (25), mortality (26), and alcohol intake (36). To enhance interpretation, metabolites were classified into 3 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); and 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, metabolite values in subjects with outlying concentrations (± 5 SD) 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 deviation 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, and independent variables were the 230 metabolite measurements. For the Netherlands Twin Register cohort, logistic regression using generalized estimating equations were conducted, accounting for family relatedness. All models were adjusted for age, gender, fasting status, use of lipid-modifying drugs listed under Anatomical Therapeutic Chemical Classification System code C10, and smoking (see Supplement 1 for measurements). All analyses were based on available data per metabolite (pairwise 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 as recommended (40,41).

As body mass index (BMI) has been shown to be associated with depression (4) and metabolites (42), we reran 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 subjects using antidepressant medication (Anatomical Therapeutic Chemical code N06A) 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 individuals with higher depression severity. Correlations between estimates obtained from these sensitivity analyses and estimates obtained in the main analyses were computed 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) gender (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 version 3.4.2-3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Overview of Cohorts

The study population comprised 15,428 adults from 11 datasets of 9 cohorts. There were 10,145 control subjects and 5283 participants with depression. Table 1 shows the characteristics of the 11 datasets. Across the cohorts, the average age ranged from 40.4 to 64.8 years, the proportion of women ranged from 32% to 70%, and the median prevalence of depression was 29.5%.

Associations of 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 gender, age, smoking, lipid-modifying drugs, and fasting status. Of these, 21 metabolites were associated with depression at FDR $q < .05$ (Table 2; Figure S1 in Supplement 1). Metabolites associated with a higher odds for depression were apolipoprotein B; remnant (non-HDL and non-LDL) cholesterol, VLDL cholesterol, and mean diameter of VLDL; the glycerides and phospholipid markers diglycerides; TG in LDL, serum TG, TG in HDL, and 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

Table 1. Characteristics of Study Populations (*N* = 15,428)

	CODAM DM	CODAM noDM	TMS DM	TMS noDM	ERF	LUMINA	NEO	NESDA	NTR	RS	LLD
Total Number	139	416	775	723	346	231	6554	2509	1523	1188	1024
Gender, Women, *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 (9.0)	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)
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)	NA	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)

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

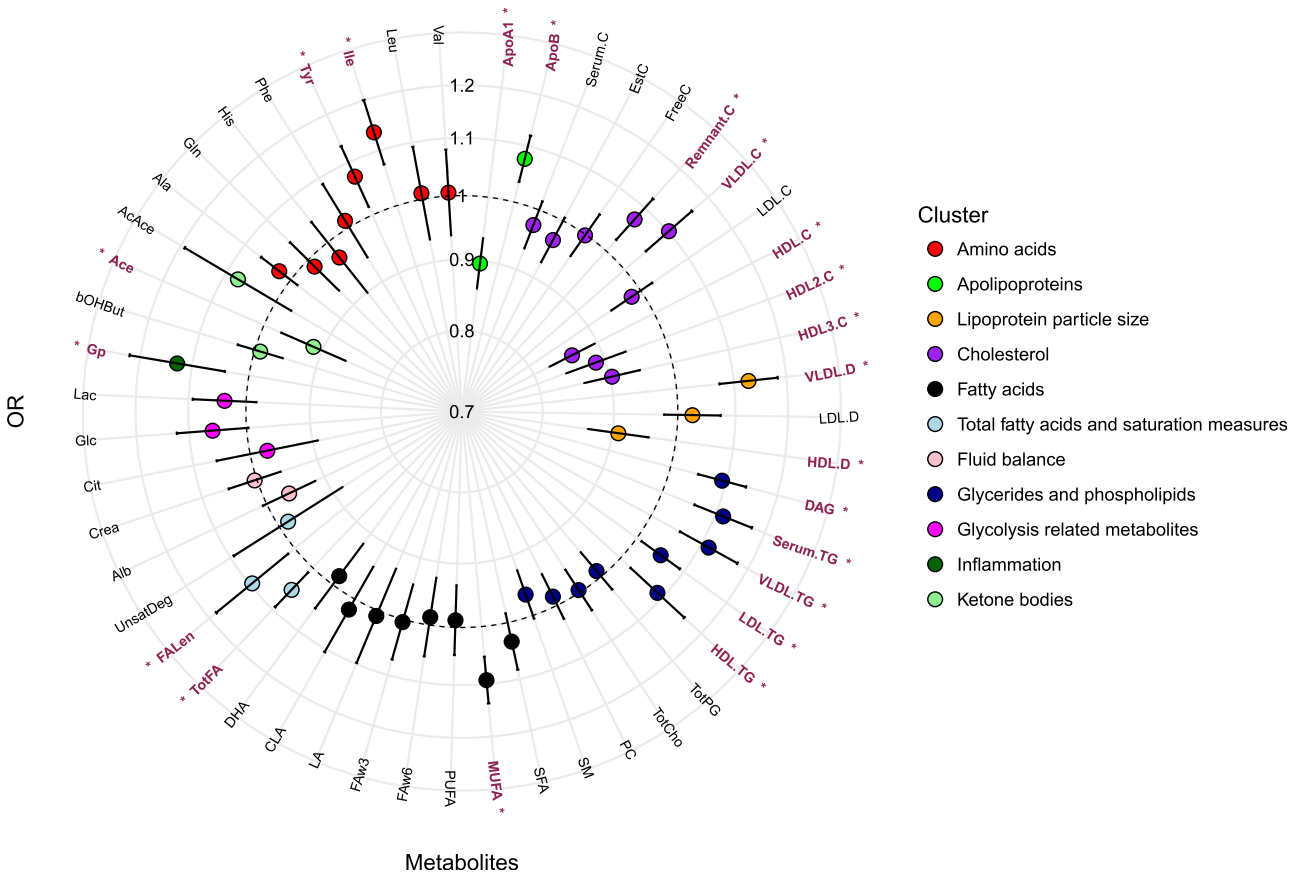

Figure 1. Polar plot illustrating pooled odds ratios (OR) and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with depression. *Significant at false discovery rate $q < .05$. Dotted circle indicates an OR of 1. Density: high-density lipoprotein (HDL) subfraction 2 (HDL_2), 1.063–1.125 g/mL; HDL_3, 1.125–1.210 g/mL. AcAce, acetoacetate; Ace, acetate; Ala, alanine; Alb, albumin; ApoA1, apolipoprotein A-I; ApoB, apolipoprotein B; bOHBut, 3-hydroxybutyrate; C, cholesterol; Cit, citrate; CLA, conjugated linoleic acids; Crea, creatinine; D, mean diameter; DAG, diglycerides; DHA, docosahexaenoic acid; Est, esterified; FA, fatty acids; FALen, estimated fatty acids chain length; FAw3, ω-3 fatty acids; FAw6, ω-6 fatty acids; Glc, glucose; Gln, glutamine; Gp, glycoprotein acetyls, mainly α1-acid glycoprotein; His, histidine; IDL, intermediate-density lipoprotein; Ile, isoleucine; LA, linoleic acid (18:2); Lac, lactate; Leu, leucine; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids (16:1, 18:1); PC, phosphatidylcholine and other cholines; Phe, phenylalanine; PUFA, polyunsaturated fatty acids; Remnant, non-HDL, non-LDL cholesterol; SFA, saturated fatty acids; SM, sphingomyelins; TG, triglycerides; TotCho, total cholines; TotFA, total fatty acids; TotPG, total phosphoglycerides; Tyr, tyrosine; UnsatDeg, estimated degree of unsaturation; Val, valine; VLDL, very-low-density lipoprotein.

HDL_2 and HDL_3 cholesterol) and mean diameter of HDL, and ketone body acetate.

Heterogeneity was small (I^2 <25% for 15 of 21 metabolites) and statistically nonsignificant in almost all (19 of 21) analyses. As shown in the related forest plots (Figure S1 in Supplement 1), association estimates were quite consistent across the different datasets, including the datasets enriched for cardiometabolic risk. To confirm this, we reran the analyses after removing 2 datasets (CODAM subgroup with type 2 diabetes mellitus and The Maastricht Study subgroup with type 2 diabetes mellitus) containing approximately 900 participants with established diabetes and elevated cardiovascular risk factors. Association estimates were highly concordant with estimates of the original analyses ($r = .99$); all 21 metabolites detected in the original analyses were associated at nominal level with depression (17 at FDR $q < .05$) (Table S3 in Supplement 1).

Additional adjustment for BMI partially reduced the strength of the association of these 21 metabolites with depression (regression slope of the 21 β values before vs. after BMI adjustment = .65, whereas a β value of 1 would indicate similar average association sizes; correlation $r = .98$): of the 21 metabolites associated with depression, 16 remained significantly related to depression at $p < .05$ and 13 at FDR $q < .05$ (Table 2). Table S2 in Supplement 2 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 levels were positively related to depression, intermediate-density lipoprotein and LDL levels were not consistently associated,

Table 2. Overview of 21 Lipids, Fatty Acids, and Various Low-Molecular-Weight Metabolites That Are Significantly Related to Depression in Pooled Analysis at FDR $q < .05$

	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	.007	.021
Apolipoprotein B	1.08	2.40×10^{-4}	6.90×10^{-4}	1.05	.014	.040
Cholesterol						
Remnant cholesterol	1.07	.003	.006	1.05	.014	.038
VLDL cholesterol	1.10	1.68×10^{-4}	5.03×10^{-4}	1.07	.001	.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	.001	.003
HDL_3 cholesterol	0.90	2.18×10^{-5}	8.37×10^{-5}	0.93	4.91×10^{-4}	.002
Mean diameter of VLDL	1.13	1.30×10^{-6}	8.82×10^{-6}	1.08	2.39×10^{-4}	.001
Mean diameter of HDL	0.91	2.10×10^{-4}	6.10×10^{-4}	0.96	.104	.222
Diglycerides and Triglycerides						
Diglycerides	1.09	2.56×10^{-5}	9.65×10^{-5}	1.07	.003	.008
Serum total TG	1.11	3.29×10^{-5}	1.15×10^{-4}	1.08	1.92×10^{-4}	.001
VLDL TG	1.11	8.68×10^{-5}	2.77×10^{-4}	1.08	1.76×10^{-4}	.001
LDL TG	1.05	.015	.032	1.04	.101	.218
HDL TG	1.09	.007	.015	1.07	.029	.072
Fatty Acids						
Monounsaturated FA	1.09	7.13×10^{-6}	3.35×10^{-5}	1.06	.004	.012
Total FA	1.05	.013	.027	1.03	.102	.219
Estimated FA chain length	1.10	.020	.043	1.08	.060	.140
Inflammation						
Glycoprotein acetyls	1.13	.003	.007	1.09	.028	.071
Ketone Bodies						
Acetate	0.91	.003	.006	0.93	.038	.092
Amino Acids						
Tyrosine	1.07	.013	.028	1.02	.552	.760
Isoleucine	1.14	8.26×10^{-6}	3.71×10^{-5}	1.08	.001	.004

Model 1 was adjusted for gender, age, smoking, lipid-modifying drugs, and fasting status; model 2 was adjusted for model 1 and body mass index.

FA, fatty acids; FDR, false discovery rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; TG, triglycerides; VLDL, very-low-density lipoprotein.

whereas HDL measures were inversely related to depression. Furthermore, depression was related to higher TG levels.

Associations of 81 Metabolite Ratios With Depression

Figure S2 in Supplement 1 shows the ORs of the meta-analyses for the 81 metabolite ratios, of which 27 were significant at FDR $q < .05$. In general, TG-to-total lipid ratios were significantly related to an increased odds of depression. Some of the VLDL, intermediate-density lipoprotein, LDL, and HDL 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 with those with absolute metabolite values.

Sensitivity Analyses

Current Depression. The original 5283 depression cases included subjects with any lifetime history of depression. In 62% of the cases (3265 subjects), depression was present between 1 month before and 1 month after blood draw. We repeated analyses with only these 3265 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 $q < .05$ (Figure S3 in Supplement 1). Notably, the strength of the associations with the 51 metabolites tended to be greater for current depression than for the original definition (regression slope of β values for current vs. broadly defined depression = 1.22, $r = .95$) (Table S2 in Supplement 2). Table S2 in Supplement 2 and Figures S4 and S5 in Supplement 1 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 1597 subjects across cohorts who reported use of antidepressants. The majority were depression

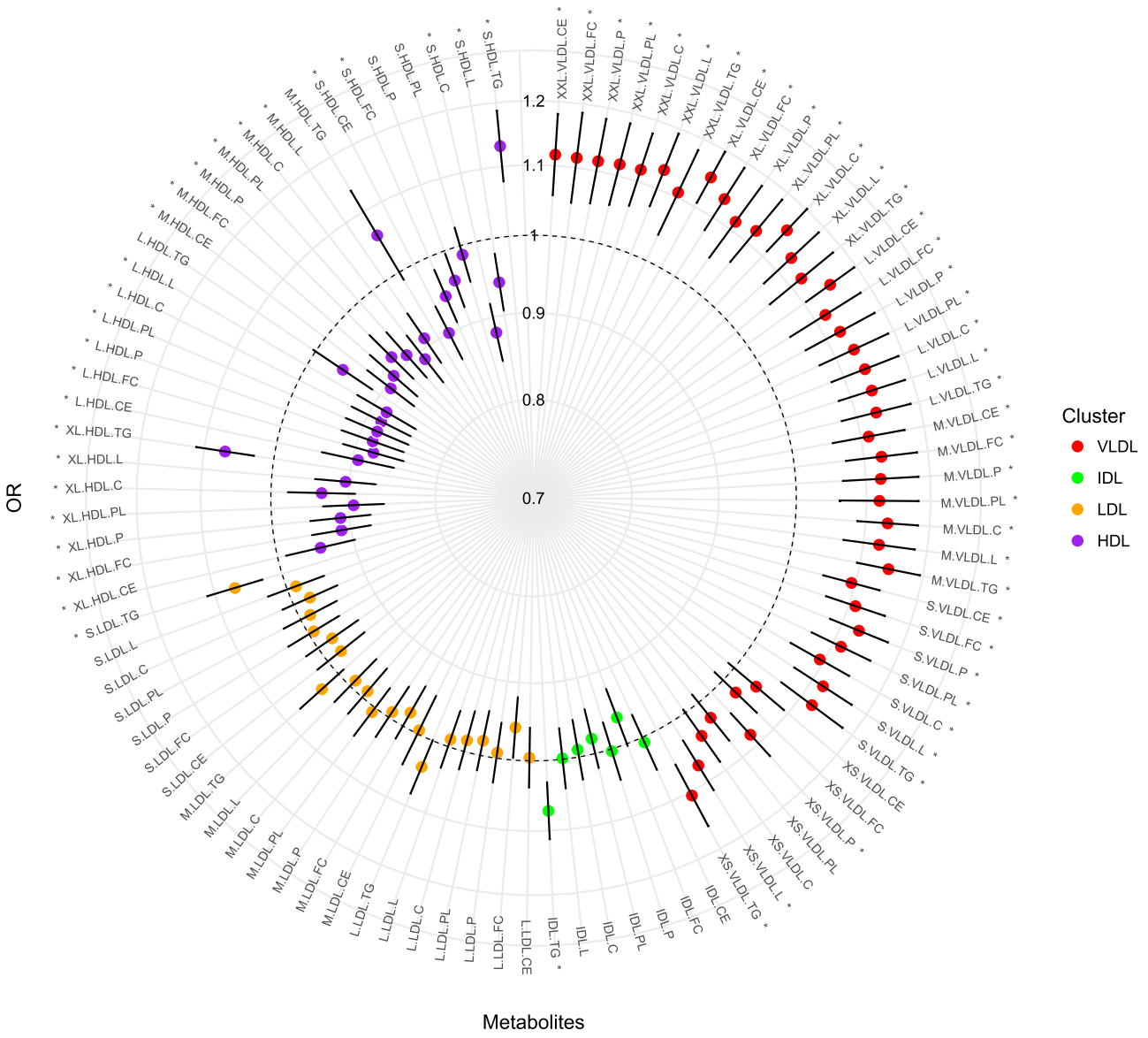

Figure 2. Pooled odds ratios (OR) and 95% confidence intervals for the association of the 98 lipid measures of lipoprotein subclasses with depression. *Significant at false discovery rate $q < .05$. Dotted circle indicates an OR of 1. Particle sizes: extremely large (XXL) very-low-density lipoprotein (VLDL), >75 nm; very large (XL) VLDL, 64 nm; large (L) VLDL, 53.6 nm; medium (M) VLDL, 44.5 nm; small (S) VLDL, 36.8 nm; very small (XS) VLDL, 31.3 nm; intermediate-density lipoprotein (IDL), 28.6 nm; L low-density lipoprotein (LDL), 25.5 nm; M LDL, 23.0 nm; S LDL, 18.7 nm; XL high-density lipoprotein (HDL), 14.3 nm; L HDL, 12.1 nm; M HDL, 10.9 nm; S HDL, 8.7 nm. C, total cholesterol; CE, cholesterol ester; FC, free cholesterol; L, total lipids; P, particle concentration; PL, phospholipids; TC, triglycerides.

cases (n = 1305), 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 3966 cases and 8887 controls, representing a 21% decrease in effective sample size compared with 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 β values before and after exclusion of antidepressant users = .60, r = .88) (Figure S6 in Supplement 1). Among the 21 significantly associated metabolites in the overall sample, 8 were still associated at $p < .05$, of which 2 (HDL_3 cholesterol and acetate) at FDR $q < .05$ in the antidepressant-free subsample.

Subgroups. Exploration of consistency of associations across subgroups showed that there were no significant differences (Wald test, FDR $q > .05$) in the strength of the association between metabolites and depression across subgroups with depression diagnoses versus self-reported depression (r = .75) (Figure S7 in Supplement 1), across men versus women (r = .64) (Figure S8 in Supplement 1), across age

<50 years versus ≥50 years (r = .84) (Figure S9 in Supplement 1), and across BMI groups (normal vs. overweight [r = .68], normal vs. obese [r = .55], overweight vs. obese [r = .71]) (Figures S10–S12 in Supplement 1).

DISCUSSION

This meta-analysis of metabolomics and depression is, to our knowledge, the largest of its kind. We analyzed data of more than 15,000 subjects from 9 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 toward less HDL and more VLDL and TG 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, TG, diglycerides, total and monounsaturated 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 gender, age, and BMI strata and across cohorts using depression diagnoses versus depressive symptoms. These metabolic alterations in depression could potentially explain part of the increased risk of cardiometabolic disease in individuals with depression.

Our findings that depression is related to higher VLDL, higher TG, and lower HDL 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 depression and increased LDL cholesterol (11). For measures of fatty acids, we observed that higher monounsaturated 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 ω-3 fatty acids (12), for which we did not observe a consistent, significant association with depression in the present study. The finding of proinflammatory 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 analytic approaches, such as selection of potentially confounding factors.

We additionally evaluated the impact of the time frame 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 time frame. The metabolic signature identified may therefore also represent a persisting biological scar after remission of depression or a preexisting underlying vulnerability factor for development of depression.

The impact of antidepressant medication use on the results was explored in secondary analyses, although this observational study precludes definitive conclusions, as 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 (50,51). Data from the NESDA cohort (51), including patients from mental health care institutions and with the highest prevalence of antidepressant users (27%) (Table 1), showed that tricyclic antidepressants were prescribed only in 3% of the participants. As the overall prevalence of antidepressant use in other cohorts included in the present meta-analysis was lower than approximately 9%, it could be assumed that the number of users of tricyclic antidepressants 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, metabolites, 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 3 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 hypothalamic-pituitary-adrenal axis and inflammatory activity, resulting in lipolysis, release of fatty acids, synthesis of VLDL, hypertriglyceridemia, and reduction in HDL cholesterol. Third, metabolomic alterations in depression may represent epiphenomena stemming from the same root, such as a common

genetic factor. A recent genome-wide association study of major depression involving >450,000 participants reported a significant genetic correlation (r_g = .14, $p = 7.8 \times 10^{-7}$) 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 (approximately 25,000) of the metabolomics genome-wide association study may substantially limit the ability to detect correlations; the only exception was a nominally significant correlation with glycoprotein acetyls (r_g = .15, p = .03), with the same direction of the phenotypic association we identified. Further experimental studies and genetically 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 present study has some limitations. Owing 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 before 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 alcohol dependence 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 nonfasting samples can be reliably analyzed by the metabolomics platform used (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 more than 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 (3,5–7) 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.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was performed within the framework of the BBMRI Metabolomics Consortium funded by BBMRI-NL, a research infrastructure financed by the Dutch government through Netherlands Organisation for Scientific Research (NWO) (Grant Nos. 184.021.007 and 184033111).

NESDA: The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Grant No. 10-000-1002) and financial contributions by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Leiden University Medical Center, Leiden University, GGZ Rivierduinen, University Medical Center Groningen, University of Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Rob Giel Onderzoekscentrum).

CODAM: The initiation of the CODAM study was supported by NWO (Grant No. 940-35-034) and the Dutch Diabetes Research Foundation (Grant No. 98.901). The work of NV was supported through a grant from the Maastricht University Medical Center+.

Leiden University Migraine Neuro-Analysis: Leiden University Migraine Neuro-Analysis is supported by the Netherlands Organisation for Health Research and Development (Vidi Grant No. 91711319 [to GMT]), Centre for Medical Systems Biology and Netherlands Consortium for Systems Biology, both within the framework of the Netherlands Genomics Initiative/NWO (to AMJMvdM), and Seventh Framework Programme EU project EUROHEADPAIN (Grant No. 602633 [to AMJMvdM and GMT]).

NEO study: The NEO study is supported by the participating Departments, Division, and Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. DOM-K is supported by Dutch Science Organization (ZonMW-VENI Grant No. 916.14.023).

The Maastricht Study: The Maastricht Study was supported by the European Regional Development Fund via OP-Zuid, Province of Limburg, Dutch Ministry of Economic Affairs (Grant No. 31O.041), Stichting De Weijerhorst, Pearl String Initiative Diabetes, Cardiovascular Center, Cardiovascular Research Institute Maastricht, School for Public Health and Primary Care, School for Nutrition, Toxicology and Metabolism, Stichting Annadal, and Health Foundation Limburg and by unrestricted grants from Janssen-Cilag B.V., Novo Nordisk Farma B.V., and Sanofi-Aventis Netherlands B.V.

Netherlands Twin Register: Funding was obtained from the NWO and MagW/ZonMW (Grant Nos. 904-61-090, 985-10-002, 904-61-193,480-04-004, l400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192), BBMRI-NL (Grant No. 184.021.007), European Community's Seventh Framework Programme (2007–2013) ENGAGE (HEALTH-F4-2007-201413), European Science Council (ERC Advanced Grant No. 230374), Rutgers University Cell and DNA Repository (National Institute of Mental Health Grant No. U24 MH068457-06), Avera Institute, and National Institutes of Health (Grant Nos. R01D0042157-01A and MH081802, Grand Opportunity Grant No. 1RC2 MH089951). We gratefully acknowledge NWO Grant No. 480-15-001/674: Netherlands Twin Register Repository: researching the interplay between genome and environment.

ERF: The ERF study as a part of European Special Populations Research Network was supported by European Commission FP6 STRP Grant No. 018947 (LSHG-CT-2006-01947) and received funding from the European Community's Seventh Framework Programme (2007–2013) HEALTH-F4-2007-201413 by the European Commission under the program "Quality of Life and Management of the Living Resources" of Fifth Framework Programme (Grant No. QLG2-CT-2002-01254). High-throughput analysis of the ERF data was supported by a joint grant from NWO and the Russian Foundation for Basic Research (Grant No. 047.017.043).

Rotterdam Study: The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organisation for the Health Research and Development, Research Institute for Diseases in the Elderly, Ministry of Education, Culture and Science, Ministry for Health, Welfare and Sports, European Commission (DG XII), and Municipality of Rotterdam.

LLD study: LLD is a subcohort of the Lifelines cohort study, with additional measurements and sample collection funded by CardioVasculair Onderzoek Nederland (Grant No. 2012-03), European Science Council (Grant No. 715772 [to AZ]), and NWO (Vidi Grant No. 016.178.056 [to AZ] and Grant No. 864.13.013 [to JF]. SG holds scholarships from the Graduate School of Medical Sciences, University of Groningen.

See Supplement 1 for BBMRI-NL Metabolomics Consortium collaborators and their affiliations.

We thank the BBMRI-NL Metabolomics Consortium (see Supplement 1).

The authors of the NEO study thank all participants, all participating general practitioners for inviting eligible participants, all research nurses for data collection, and the NEO study group: Pat van Beelen, Petra

Noordijk, and Ingeborg de Jonge for coordination, laboratory, and data management.

The authors of the ERF study thank all study participants and their relatives; general practitioners and neurologists for their contributions; and P. Veraart for help in genealogy, J. Vergeer for supervision of the laboratory work, and P. Snijders for help in data collection.

The authors of the Rotterdam Study thank the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists.

DOM-K works as a part-time clinical research consultant for Metabolon, Inc. BWJHP received (nonrelated) research funding from Janssen Research and Boehringer. All other authors report no biomedical financial interests or potential conflicts of interest.

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Received Mar 5, 2019; revised and accepted Aug 19, 2019.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2019.08.016.

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