Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5,283 Cases With Depression and 10,145 Control Subjects

Supplementary Information

Contents

Abbreviations 3
Descriptions of studies and measurements 9
Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) 9
The Maastricht Study (TMS) 10
Erasmus Rucphen Family (ERF) study 11
Leiden University Migraine Neuro-Analysis (LUMINA) 12
Netherlands Epidemiology of Obesity Study (NEO) 13
Netherlands Study of Depression and Anxiety (NESDA) 14
Netherlands Twin Registry (NTR) 15
Rotterdam Study (RS) 16
LifeLines-DEEP Study (LLD) 17
Classification of depressed cases and controls 18
Metabolomics assessment 18
Covariates 20
Assessment of potential bias due to metabolites data transformation 21
Table S1. Overview of depression instruments and definitions per study 23
Table S2. Results of pooled analyses for depression for each metabolite 25
Figure S1. Forest plots of the 21 lipids, fatty acids and various low-molecular-weight metabolites significantly associated with depression (FDR<0.05) 26
Figure S2. Pooled odds ratio and 95% confidence intervals for the association of the 81 metabolite ratios with depression 47
Figure S3. Pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with current depression[a] 48
Figure S4. Pooled odds ratio and 95% confidence intervals for the association of the 98 lipid measures of lipoprotein subclasses with current depression 49

Figure S5. Pooled odds ratio and 95% confidence intervals for the association of the 81 metabolite ratios with current depression 50

Figure S6. Pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with depression after excluding antidepressant users 51

Figure S7. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids and various low-molecular-weight metabolites and depression stratified by diagnosis vs. self-reported depression subgroups 52

Figure S8. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by men vs. women subgroups 53

Figure S9. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by age<50 vs. age>=50y subgroups 54

Figure S10. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI<25 vs. BMI 25-29.9 55

Figure S11. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI<25 vs. BMI>=30 56

Figure S12. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI 25-29.9 vs. BMI>=30 57

Table S3. 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 after exclusion of datasets including patients with diabetes 58

Figure S13. Standardized distribution of the (log)values of the metabolites after different data transformations. 59

Figure S14. Comparisons between estimates of the metabolites-depression association obtained after different data transformations. 61

BBMRI-NL Metabolomics Consortium 63

Affiliations 63

Supplemental References 67

Abbreviations

CODAM DM = Cohort on Diabetes and Atherosclerosis Maastricht, subgroup with type 2 diabetes mellitus, CODAM DM = Cohort on Diabetes and Atherosclerosis Maastricht, subgroup without diabetes mellitus, TMS DM = The Maastricht Study, subgroup with type 2 diabetes mellitus, TMS noDM = The Maastricht Study, subgroup 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 Registry, RS = Rotterdam Study.

ApoA1	Apolipoprotein A-I
ApoB	Apolipoprotein B
Serum.C	Serum total cholesterol
EstC	Esterified cholesterol
FreeC	Free cholesterol
Remnant.C	Remnant cholesterol (non-HDL, non-LDL -cholesterol)
VLDL.C	VLDL cholesterol
LDL.C	LDL cholesterol
HDL.C	Total cholesterol in HDL
HDL2.C	Total cholesterol in HDL2 (within the density range of 1.063-1.125 g/mL)
HDL3.C	Total cholesterol in HDL3 (within the density range of 1.125-1.210 g/mL)
VLDL.D	Mean diameter for VLDL particles
LDL.D	Mean diameter for LDL particles
HDL.D	Mean diameter for HDL particles
DAG	Diglycerides
Serum.TG	Serum total triglycerides
VLDL.TG	VLDL triglycerides
LDL.TG	LDL triglycerides
HDL.TG	Triglycerides in HDL
TotPG	Total phosphoglycerides
TotCho	Total cholines
PC	Phosphatidylcholine and other cholines
SM	Sphingomyelins
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids; 16:1, 18:1
PUFA	Polyunsaturated fatty acids
FAw6	Omega-6 fatty acids
FAw3	Omega-3 fatty acids
LA	18:2, linoleic acid

CLA	Conjugated linoleic acids
DHA	Docosahexaenoic acid
TotFA	Total fatty acids
FALen	Estimated fatty acid 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 (acetic acid)
AcAce	Acetoacetate or acetoacetic acid
Ala	Alanine
Gln	Glutamine
His	Histidine
Phe	Phenylalanine
Tyr	Tyrosine
Ile	Isoleucine
Leu	Leucine
Val	Valine

XXL.VLDL.CE	Cholesterol ester in extremely large VLDL particles (>75nm)
XXL.VLDL.FC	Free cholesterol in extremely large VLDL particles (>75nm)
XXL.VLDL.P	Particle concentration of extremely large VLDL particles (>75nm)
XXL.VLDL.PL	Phospholipids in extremely large VLDL particles (>75nm)
XXL.VLDL.C	Total cholesterol in extremely large VLDL particles (>75nm)
XXL.VLDL.L	Total lipids in extremely large VLDL particles (>75nm)
XXL.VLDL.TG	Triglycerides in extremely large VLDL particles (>75nm)
XL.VLDL.CE	Cholesterol ester in very large VLDL particles (64 nm)
XL.VLDL.FC	Free cholesterol in very large VLDL particles (64 nm)
XL.VLDL.P	Particle concentration of very large VLDL particles (64 nm)
XL.VLDL.PL	Phospholipids in very large VLDL particles (64 nm)
XL.VLDL.C	Total cholesterol in very large VLDL particles (64 nm)
XL.VLDL.L	Total lipids in very large VLDL particles (64 nm)
XL.VLDL.TG	Triglycerides in very large VLDL particles (64 nm)
L.VLDL.CE	Cholesterol ester in large VLDL particles (53.6 nm)
L.VLDL.FC	Free cholesterol in large VLDL particles (53.6 nm)
L.VLDL.P	Particle concentration of large VLDL particles (53.6 nm)
L.VLDL.PL	Phospholipids in large VLDL particles (53.6 nm)

L.VLDL.C	Total cholesterol in large VLDL particles (53.6 nm)
L.VLDL.L	Total lipids in large VLDL particles (53.6 nm)
L.VLDL.TG	Triglycerides in large VLDL particles (53.6 nm)
M.VLDL.CE	Cholesterol ester in medium VLDL particles (44.5 nm)
M.VLDL.FC	Free cholesterol in medium VLDL particles (44.5 nm)
M.VLDL.P	Particle concentration of medium VLDL particles (44.5 nm)
M.VLDL.PL	Phospholipids in medium VLDL particles (44.5 nm)
M.VLDL.C	Total cholesterol in medium VLDL particles (44.5 nm)
M.VLDL.L	Total lipids in medium VLDL particles (44.5 nm)
M.VLDL.TG	Triglycerides in medium VLDL particles (44.5 nm)
S.VLDL.CE	Cholesterol ester in small VLDL particles (36.8 nm)
S.VLDL.FC	Free cholesterol in small VLDL particles (36.8 nm)
S.VLDL.P	Particle concentration of small VLDL particles (36.8 nm)
S.VLDL.PL	Phospholipids in small VLDL particles (36.8 nm)
S.VLDL.C	Total cholesterol in small VLDL particles (36.8 nm)
S.VLDL.L	Total lipids in small VLDL particles (36.8 nm)
S.VLDL.TG	Triglycerides in small VLDL particles (36.8 nm)
XS.VLDL.CE	Cholesterol ester in very small VLDL particles (31.3 nm)
XS.VLDL.FC	Free cholesterol in very small VLDL particles (31.3 nm)
XS.VLDL.P	Particle concentration of very small VLDL particles (31.3 nm)
XS.VLDL.PL	Phospholipids in very small VLDL particles (31.3 nm)
XS.VLDL.C	Total cholesterol in very small VLDL particles (31.3 nm)
XS.VLDL.L	Total lipids in very small VLDL particles (31.3 nm)
XS.VLDL.TG	Triglycerides in very small VLDL particles (31.3 nm)
IDL.CE	Cholesterol ester in intermediate-density lipoprotein particles (28.6 nm)
IDL.FC	Free cholesterol in intermediate-density lipoprotein particles (28.6 nm)
IDL.P	Particle concentration of intermediate-density lipoprotein (28.6 nm)
IDL.PL	Phospholipids in intermediate-density lipoprotein particles (28.6 nm)
IDL.C	Total cholesterol in intermediate-density lipoprotein particles (28.6 nm)
IDL.L	Total lipids in intermediate-density lipoprotein particles (28.6 nm)
IDL.TG	Triglycerides in intermediate-density lipoprotein particles (28.6 nm)
L.LDL.CE	Cholesterol ester in large LDL particles (25.5 nm)
L.LDL.FC	Free cholesterol in large LDL particles (25.5 nm)
L.LDL.P	Particle concentration of large LDL particles (25.5 nm)
L.LDL.PL	Phospholipids in large LDL particles (25.5 nm)
L.LDL.C	Total cholesterol in large LDL particles (25.5 nm)
L.LDL.L	Total lipids in large LDL particles (25.5 nm)
L.LDL.TG	Triglycerides in large LDL particles (25.5 nm)
M.LDL.CE	Cholesterol ester in medium LDL particles (23.0 nm)
M.LDL.FC	Free cholesterol in medium LDL particles (23.0 nm)
M.LDL.P	Particle concentration of medium LDL particles (23.0 nm)
M.LDL.PL	Phospholipids in medium LDL particles (23.0 nm)
M.LDL.C	Total cholesterol in medium LDL particles (23.0 nm)

M.LDL.L	Total lipids in medium LDL particles (23.0 nm)
M.LDL.TG	Triglycerides in medium LDL particles (23.0 nm)
S.LDL.CE	Cholesterol ester in small LDL particles (18.7 nm)
S.LDL.FC	Free cholesterol in small LDL particles (18.7 nm)
S.LDL.P	Particle concentration of small LDL particles (18.7 nm)
S.LDL.PL	Phospholipids in small LDL particles (18.7 nm)
S.LDL.C	Total cholesterol in small LDL particles (18.7 nm)
S.LDL.L	Total lipids in small LDL particles (18.7 nm)
S.LDL.TG	Triglycerides in small LDL particles (18.7 nm)
XL.HDL.CE	Cholesterol ester in very large HDL particles (14.3 nm)
XL.HDL.FC	Free cholesterol in very large HDL particles (14.3 nm)
XL.HDL.P	Particle concentration of very large HDL particles (14.3 nm)
XL.HDL.PL	Phospholipids in very large HDL particles (14.3 nm)
XL.HDL.C	Total cholesterol in very large HDL particles (14.3 nm)
XL.HDL.L	Total lipids in very large HDL particles (14.3 nm)
XL.HDL.TG	Triglycerides in very large HDL particles (14.3 nm)
L.HDL.CE	Cholesterol ester in large HDL particles (12.1 nm)
L.HDL.FC	Free cholesterol in large HDL particles (12.1 nm)
L.HDL.P	Particle concentration of large HDL particles (12.1 nm)
L.HDL.PL	Phospholipids in large HDL particles (12.1 nm)
L.HDL.C	Total cholesterol in large HDL particles (12.1 nm)
L.HDL.L	Total lipids in large HDL particles (12.1 nm)
L.HDL.TG	Triglycerides in large HDL particles (12.1 nm)
M.HDL.CE	Cholesterol ester in medium HDL particles (10.9 nm)
M.HDL.FC	Free cholesterol in medium HDL particles (10.9 nm)
M.HDL.P	Particle concentration of medium HDL particles (10.9 nm)
M.HDL.PL	Phospholipids in medium HDL particles (10.9 nm)
M.HDL.C	Total cholesterol in medium HDL particles (10.9 nm)
M.HDL.L	Total lipids in medium HDL particles (10.9 nm)
M.HDL.TG	Triglycerides in medium HDL particles (10.9 nm)
S.HDL.CE	Cholesterol ester in small HDL particles (8.7 nm)
S.HDL.FC	Free cholesterol in small HDL particles (8.7 nm)
S.HDL.P	Particle concentration of small HDL particles (8.7 nm)
S.HDL.PL	Phospholipids in small HDL particles (8.7 nm)
S.HDL.C	Total cholesterol in small HDL particles (8.7 nm)
S.HDL.L	Total lipids in small HDL particles (8.7 nm)
S.HDL.TG	Triglycerides in small HDL particles (8.7 nm)

XXL.VLDL.CE%	Cholesterol esters to total lipids ratio in chylomicrons and extremely large VLDL
XXL.VLDL.FC%	Free cholesterol to total lipids ratio in chylomicrons and extremely large VLDL
XXL.VLDL.PL%	Phospholipids to total lipids ratio in chylomicrons and extremely large VLDL
XXL.VLDL.C%	Total cholesterol to total lipids ratio in chylomicrons and extremely large VLDL
XXL.VLDL.TG%	Triglycerides to total lipids ratio in chylomicrons and extremely large VLDL

XL.VLDL.CE%	Cholesterol esters to total lipids ratio in very large VLDL
XL.VLDL.FC%	Free cholesterol to total lipids ratio in very large VLDL
XL.VLDL.PL%	Phospholipids to total lipids ratio in very large VLDL
XL.VLDL.C%	Total cholesterol to total lipids ratio in very large VLDL
XL.VLDL.TG%	Triglycerides to total lipids ratio in very large VLDL
L.VLDL.CE%	Cholesterol esters to total lipids ratio in large VLDL
L.VLDL.FC%	Free cholesterol to total lipids ratio in large VLDL
L.VLDL.PL%	Phospholipids to total lipids ratio in large VLDL
L.VLDL.C%	Total cholesterol to total lipids ratio in large VLDL
L.VLDL.TG%	Triglycerides to total lipids ratio in large VLDL
M.VLDL.CE%	Cholesterol esters to total lipids ratio in medium VLDL
M.VLDL.FC%	Free cholesterol to total lipids ratio in medium VLDL
M.VLDL.PL%	Phospholipids to total lipids ratio in medium VLDL
M.VLDL.C%	Total cholesterol to total lipids ratio in medium VLDL
M.VLDL.TG%	Triglycerides to total lipids ratio in medium VLDL
S.VLDL.CE%	Cholesterol esters to total lipids ratio in small VLDL
S.VLDL.FC%	Free cholesterol to total lipids ratio in small VLDL
S.VLDL.PL%	Phospholipids to total lipids ratio in small VLDL
S.VLDL.C%	Total cholesterol to total lipids ratio in small VLDL
S.VLDL.TG%	Triglycerides to total lipids ratio in small VLDL
XS.VLDL.CE%	Cholesterol esters to total lipids ratio in very small VLDL
XS.VLDL.FC%	Free cholesterol to total lipids ratio in very small VLDL
XS.VLDL.PL%	Phospholipids to total lipids ratio in very small VLDL
XS.VLDL.C%	Total cholesterol to total lipids ratio in very small VLDL
XS.VLDL.TG%	Triglycerides to total lipids ratio in very small VLDL
IDL.CE%	Cholesterol esters to total lipids ratio in IDL
IDL.FC%	Free cholesterol to total lipids ratio in IDL
IDL.PL%	Phospholipids to total lipids ratio in IDL
IDL.C%	Total cholesterol to total lipids ratio in IDL
IDL.TG%	Triglycerides to total lipids ratio in IDL
L.LDL.CE%	Cholesterol esters to total lipids ratio in large LDL
L.LDL.FC%	Free cholesterol to total lipids ratio in large LDL
L.LDL.PL%	Phospholipids to total lipds ratio in large LDL
L.LDL.C%	Total cholesterol to total lipids ratio in large LDL
L.LDL.TG%	Triglycerides to total lipids ratio in large LDL
M.LDL.CE%	Cholesterol esters to total lipids ratio in medium LDL
M.LDL.FC%	Free cholesterol to total lipids ratio in medium LDL
M.LDL.PL%	Phospholipids to total lipids ratio in medium LDL
M.LDL.C%	Total cholesterol to total lipids ratio in medium LDL
M.LDL.TG%	Triglycerides to total lipids ratio in medium LDL
S.LDL.CE%	Cholesterol esters to total lipids ratio in small LDL

S.LDL.FC%	Free cholesterol to total lipids ratio in small LDL
S.LDL.PL%	Phospholipids to total lipids ratio in small LDL
S.LDL.C%	Total cholesterol to total lipids ratio in small LDL
S.LDL.TG%	Triglycerides to total lipids ratio in small LDL
XL.HDL.CE%	Cholesterol esters to total lipids ratio in very large HDL
XL.HDL.FC%	Free cholesterol to total lipids ratio in very large HDL
XL.HDL.PL%	Phospholipids to total lipids ratio in very large HDL
XL.HDL.C%	Total cholesterol to total lipids ratio in very large HDL
XL.HDL.TG%	Triglycerides to total lipids ratio in very large HDL
L.HDL.CE%	Cholesterol esters to total lipids ratio in large HDL
L.HDL.FC%	Free cholesterol to total lipids ratio in large HDL
L.HDL.PL%	Phospholipids to total lipids ratio in large HDL
L.HDL.C%	Total cholesterol to total lipids ratio in large HDL
L.HDL.TG%	Triglycerides to total lipids ratio in large HDL
M.HDL.CE%	Cholesterol esters to total lipids ratio in medium HDL
M.HDL.FC%	Free cholesterol to total lipids ratio in medium HDL
M.HDL.PL%	Phospholipids to total lipids ratio in medium HDL
M.HDL.C%	Total cholesterol to total lipids ratio in medium HDL
M.HDL.TG%	Triglycerides to total lipids ratio in medium HDL
S.HDL.CE%	Cholesterol esters to total lipids ratio in small HDL
S.HDL.FC%	Free cholesterol to total lipids ratio in small HDL
S.HDL.PL%	Phospholipids to total lipids ratio in small HDL
S.HDL.C%	Total cholesterol to total lipids ratio in small HDL
S.HDL.TG%	Triglycerides to total lipids ratio in small HDL
ApoB.ApoA1%	Ratio of apolipoprotein B to apolipoprotein A-I
DAG.TG%	Ratio of diglycerides to triglycerides
TG.PG%	Ratio of triglycerides to phosphoglycerides
SFA.FA%	Ratio of saturated fatty acids to total fatty acids
MUFA.FA%	Ratio of monounsaturated fatty acids to total fatty acids
PUFA.FA%	Ratio of polyunsaturated fatty acids to total fatty acids
FAw6.FA%	Ratio of omega-6 fatty acids to total fatty acids
FAw3.FA%	Ratio of omega-3 fatty acids to total fatty acids
LA.FA%	Ratio of 18:2 linoleic acid to total fatty acids
CLA.FA%	Conjugated linoleic acids (CLAs) to total FAs % ratio
DHA.FA%	Ratio of 22:6 docosahexaenoic acid to total fatty acids

Descriptions of studies and measurements

Cohort on Diabetes and Atherosclerosis Maastricht (CODAM)

Cohort description

The CODAM (Cohort on Diabetes and Atherosclerosis Maastricht) study is a cohort study including 574 subjects with an elevated risk of type 2 diabetes mellitus and cardiovascular disease.(1) This study was initiated in 1999. In short, participants were selected from a large population-based cohort.(2) Inclusion criteria were a Caucasian descent and age > 40 years and, in addition, at least one of the following: body mass index (BMI) >25 kg/m^2; a positive family history of type 2 diabetes mellitus; a history of gestational diabetes and ⁄ or glucosuria; and use of anti-hypertensive medication. Study participants were extensively characterized with regard to their metabolic, cardiovascular and lifestyle profiles during two visits to the University's research unit. The CODAM study was approved by the Medical Ethical Committee of the Maastricht University Medical Center, and all participants gave written informed consent.

Depression

During the first visit through the research unit, participants underwent an Oral Glucose Tolerance Test (OGTT) during which they completed several questionnaires, including a questionnaire on depression. Depressive symptoms were measured with the Center for Epidemiologic Studies Depression Scale (CES-D),(3) a 20-item self-report scale designed to measure depressive symptomatology in the general population (score range 0-60, with higher scores indicating higher severity of depression. A score of ≥16 is used to identify individuals at risk for clinical depression, with good sensitivity and specificity and high internal consistency.(4) This cut-off was used to distinguish persons with and without depression for the present study.

Blood sampling

Blood samples were taken during the same day as the depression questionnaire. Subjects were asked to stop their lipid-lowering medication 14 days before the first visit and all other medication was stopped the day before the visit (>80% adherence). Blood samples were obtained by venipuncture. EDTA plasma aliquots were stored at -80°C until use. In the current study, two subcohorts were defined: CODAMDM (type 2 diabetes) and CODAMnoDM (no type 2 diabetes, with central obesity), because the metabolites of these subgroups were measured at two separate occasions (November 2014 and May 2015, respectively) at Brainshake Ltd./Nightingale Health. In CODAM, a total of 112 participants had a depression (CODAMDM N=34, CODAMnoDM N=78, whereas 443 participants had no depression (CODAMDM N=105, CODAMnoDM N=338).

The Maastricht Study (TMS)

Cohort description

The Maastricht Study (5) is a prospective observational population-based cohort study focusing on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus. In this study individuals with type 2 diabetes mellitus were oversampled. In brief, eligible participants were selected based on age (between 40 and 75 years) and place of residence (the Southern part of the Netherlands). Recruitment proceeded through mass media campaigns, municipal registries and the regional Diabetes Patient Registry via mailings.

Depression

Depressive disorder was assessed by the Mini-International Neuropsychiatric Interview (MINI).(6) The MINI is a short diagnostic structured interview, used to assess the presence of current major depressive disorder (MDD) in lifetime and in the preceding 2 weeks according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition). For the present study, persons with depression were defined as having a MDD in lifetime. Controls were defined as persons without a depressive disorder according to the MINI.

Blood sampling

After overnight fasting, EDTA plasma was collected in EDTA tubes on ice, separated after centrifugation, and stored at -80°C until the assays were performed. The time between collection and storage was less than 2 hours. Samples were collected from April 2010 until April 2013. In the current study, two subcohorts were defined: TMSDM (type 2 diabetes) and TMSnoDM (no type 2 diabetes, with central obesity), because the metabolites of these subgroups were measured at two separate occasions (November 2014 and May 2015, respectively) at Brainshake Ltd./Nightingale Health. The study was approved by the institutional medical ethical committee. All participants gave written informed consent. EDTA plasma samples were available for 515 persons with depression (TMSDM N=272, TMSMnoDM N=243) and 1002 non-depressed controls (TMSDM N=503, TMSnoDM N=480).

Erasmus Rucphen Family (ERF) study

Cohort description

The Erasmus Rucphen Family (ERF) study is a large family-based cohort study aiming to identify genetic risk factors in the development of complex disorders, in a young genetically isolated population in the Southwest of The Netherlands.(7) Possible participants were selected based on genealogical background. Eligible participants included three generations of living descendants, of twenty couples with at least six children living in the community in the 19th century. Genealogical information on this population was reconstructed using church and municipality records. All living descendants of these families aged 18 years and older, as well as their spouses, were invited to attend a series of clinical examinations. Data were collected between June 2002 and February 2005.The medical ethical committee of the Erasmus Medical Center, Rotterdam approved the study and informed consent was obtained from all participants.

Depression

Symptoms of depression were assessed using the HADS(8) and the CES-D(3) in 2385 participants. The CES-D is a 20-item self-report scale designed to measure depressive symptomatology in the general population (score range 0-60, with higher scores indicating higher severity of depression. A score of ≥16 is used to identify individuals at risk for clinical depression, with good sensitivity and specificity and high internal consistency.(4) The HADS consists of a 14-item self-report questionnaire incorporating seven questions for anxiety and seven others for depression. [8] Each item is scored 0 to 3, and a total score of 8 or greater on the HADS-depression (HADS-D) and HADS-anxiety (HADS-A) subscale indicates the presence of an depressive or anxiety disorder, respectively. For the current study, cases with depression were defined as having a CES-D≥16 and/or HADS-D≥8. Controls were defined as having a CES-D<16 and HADS-D<8 and never reporting any depressive or psychiatric disorder during one of the measurements.

Blood sampling

EDTA plasma samples were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein. After centrifugation, plasma was aliquoted and stored at -80°C. Samples were not thawed prior to shipment to Brainshake Ltd./Nightingale Health for analysis. Samples were measured in April 2014 at Brainshake Ltd./Nightingale Health. This was done for 153 participants with depression and 193 controls.

Leiden University Migraine Neuro-Analysis (LUMINA)

Cohort description

Participants of the Leiden University Migraine Neuro-Analysis (LUMINA)(9) were recruited through a nationwide website inviting migraine patients and non-migraine controls to participate in migraine research. In addition, patients attending the Leiden University Medical Centre (LUMC) dedicated headache clinic were invited to participate. Hence, persons with migraine are oversampled.

Depression

Symptoms of depression were assessed using the HADS(8) and the CES-D.(3) The CES-D is a 20-item self-report scale designed to measure depressive symptomatology in the general population. A score of ≥16 is used to identify individuals at risk for clinical depression, with good sensitivity and specificity and high internal consistency.(4) The HADS consists of a 14-item self-report questionnaire incorporating seven questions for anxiety and seven others for depression. Each item is scored 0 to 3, and a total score of 8 or greater on the HADS-depression (HADS-D) and HADS-anxiety (HADS-A) subscale indicates the presence of an depressive or anxiety disorder, respectively. For the current study, cases with depression were defined as having a CES-D≥16 and/or HADS-D≥8. Controls were defined as having a CES-D<16 and HADS-D<8.

Blood sampling

After an overnight fast, EDTA plasma samples were collected. Blood samples were drawn by venipuncture from the median cubital vein, after centrifugation on room temperature; plasma was aliquoted and stored at -80°C. Samples from 59 persons with depression and in 172 controls were sent to Brainshake Ltd./Nightingale Health for analysis; samples were measured in April 2014.

Netherlands Epidemiology of Obesity Study (NEO)

Cohort description

The NEO Study was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. Participants were recruited via general practitioners, advertisements and through municipalities in the West of the Netherlands. Data collection took place between September 2008 and September 2012. During the study visit, participants underwent an extensive physical examination and blood sampling. Prior to the visit, various questionnaires were completed. Participants are currently being followed for the incidence of obesity-related diseases and mortality.

Depression

Prior to the study visit, the 30-item self-report inventory of depressive symptomatology (IDS-SR) was used to measure depression symptoms.(10) Scores between 0 and 84 can be obtained and higher scores reflect more depressive symptoms. This IDS-SR has good psychometric properties.(11) The established cut-off of 14 (for mild depression) was used to distinguish persons with depression from controls.

Blood sampling

Participants were invited to come to the study center for the baseline study visit after an overnight fast of at least 10 hours. Fasting blood was collected, and subsequently separated into plasma and serum, and aliquots were stored at -80°C for future analyses. Samples of 1934 persons with depression and 4620 controls were send to Brainshake Ltd./Nightingale Health for analysis; samples were measured in April 2014.

Netherlands Study of Depression and Anxiety (NESDA)

Cohort description

The Netherlands Study of Depression and Anxiety (NESDA) is an observational longitudinal cohort study on the long term course and consequences of depressive and anxiety disorders.(12) In total 2,981 participants aged 18 to 65 years were recruited between 2004 and 2007 through different settings: community, primary care and specialized mental health clinics in order to obtain a representative sample of persons with and without depressive and anxiety disorders.

Depression

During the baseline assessment, the presence of depressive disorders (MDD and dysthymia) and anxiety disorders (panic disorder, social phobia, generalized anxiety disorder and/or agoraphobia) was ascertained with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSMIV)-based Composite Interview Diagnostic Instrument (CIDI, version 2.1, World Health Organization, 1997) by specially trained research staff. The CIDI has a high reliability and validity for the assessment of depressive and anxiety disorders.(13) Persons with depression were defined as having a MDD in lifetime, whereas controls were defined as having no depressive or anxiety disorder in lifetime.

Blood sampling

After an overnight fast, EDTA plasma samples were collected and stored in aliquots at -80°C until further analysis. Blood samples were analyzed in 2 batches (April 2014 and December 2014) by Brainshake Ltd./Nightingale Health.

Statistical analysis

To account for potential batch difference, batch was added as an additional covariate in the analysis with NESDA data.

Netherlands Twin Registry (NTR)

Cohort description

The Netherlands Twin Registry (NTR) collects data from Dutch twins, other siblings, their parents and partners. Participants were recruited either at birth or when already older at start of NTR, through city councils in 1990-1991 and through additional efforts in later years. Participants are invited every 2 to 3 years to complete a survey that contains questions about health, lifestyle, personality and psychopathology. A detailed description of NTR can be found elsewhere.(14)

Depression

In a subsample of NTR participants, the lifetime version of the Composite Interview Diagnostic Instrument (CIDI) was administered to measure the presence of MDD. The CIDI has a high reliability and validity for the assessment of depressive disorders.(13) In a larger number of NTR participants, depression questionnaires were used. Cases with depression were defined as having a diagnosis of MDD in lifetime. Controls were defined as persons that scored below established cut-offs on depressive symptom questionnaires in every wave.

Blood sampling

Samples were drawn by venipuncture from the medial cubital vein during a home-visit, after centrifugation back at the laboratory at 4°C, plasma was aliquoted and stored at -20°C. Samples were collected in two biobanking initiatives (the first between June 2004 and July 2008 and the second between January 2011 and December 2011) prior to shipment to Brainshake Ltd./Nightingale Health for analysis. Samples were measured in April 2014 at Brainshake Ltd./Nightingale Health.

Statistical analysis

To account for family relatedness in the data, logistic generalized estimating equations were used in this cohort.

Rotterdam Study (RS)

Cohort description

The Rotterdam study (RS) is a large prospective population based cohort study, among people 55 years of age or older living in the well-defined Ommoord district in Rotterdam (The Netherlands) in 1990. Later the RS was expanded in 2000 and 2006 to include people 45 years of age or older living in the Ommoord district.(15)

Depression

Symptoms of depression were assessed with the CES-D.(3) The CES-D is a 20-item self-report scale designed to measure depressive symptomatology in the general population. A score of ≥16 is used to identify individuals at risk for clinical depression, with good sensitivity and specificity and high internal consistency.(4) Cases with depression were defined as having a CES-D score ≥16 during one of the measurement waves. Controls were defined as having a CES-D score <16 in all of the measurement waves.

Blood sampling

Samples were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, after centrifugation back on site; plasma was aliquoted and stored at -80°C. Samples were not freeze-thawed prior to shipment to Brainshake Ltd./Nightingale Health for analysis. Samples were measured in April 2014 at Brainshake Ltd./Nightingale Health.

LifeLines-DEEP Study (LLD)

Cohort description

LLD is a sub-cohort of the LifeLines population cohort (167,729 subjects), which aims to assess the biomedical, socio-demographic, behavioral, physical and psychological factors that contribute to health and disease in a general Dutch population.(16) A subset of approximately 1,500 LifeLines participants make up LLD. These subjects gave additional biological materials, including blood samples for metabolite profiling.(17)-(18) For the current study we focused on the 1024 LLD individuals for whom plasma metabolism and information on depression and confounders were available.

Depression

Depression mood was accessed by the MINI questionnaire, that was answered by all participants within 2 months prior to plasma sample collection.

Blood sampling

Samples were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, after centrifugation back on site; plasma was aliquoted and stored at -80°C. Samples were not freeze-thawed prior to shipment to Brainshake Ltd./Nightingale Health for analysis. Samples were measured in April 2014 at Brainshake Ltd./Nightingale Health.

Classification of depressed cases and controls

Controls were those with a negative diagnostic interview for lifetime depression, or had a score on the depression questionnaires below established cut-off scores (i.e. CES-D<16, HADS-D<8 and/or IDS-SR30<14). If multiple self-reports of depressive symptoms before blood sampling were available, controls needed to score below the established cut-offs during all these assessments. When diagnostic data on other psychiatric disorders were available (e.g. anxiety disorders), persons with other psychiatric disorders were excluded from the controls.

Metabolomics assessment

A total of 230 metabolites or metabolite ratios were reliably quantified from Ethylenediaminetetraacetic acid (EDTA) plasma samples using targeted high-throughput proton Nuclear Magnetic Resonance (^{1}H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland) (19). This platform provides simultaneous quantification of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular-weight metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites in molar concentration units. This metabolomics platform has been extensively used and described in numerous studies (see https://nightingalehealth.com/publications for an overview), including large-scaled epidemiological studies in the field of type 2 diabetes (20), cardiovascular disease (21), mortality (22), and lifestyle factors such as alcohol intake (23). Details of the experimentation and applications of the ^{1}H-NMR metabolomics platform have been extensively described previously (19, 24)(25).

The entire process from sample handling to data processing is highly standardized and fully automated. Samples were prepared irrespective of depression status, because depression cases and controls entered each study at random order (i.e. unrelated to depression status), and the laboratory analyzing the samples was unaware of depression cases vs. control status when preparing the samples. Automated liquid handlers mixed 260 µL buffer (75 mM Na_2HPO_4 in 80%/20% H_2O/D_2O, pH 7.4; 4.64

mM sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4, and 6.15 mM sodium azide) with the plasma in 1:1 ratio and moved the prepared samples to 96-format racks of NMR tubes, which were subsequently moved to the robotic sample changer, cooled to refrigerator temperature. Each rack contained 2 quality control samples: 1 serum mimic and a mixture of 2 low-molecular-weight metabolites. For the native plasma samples, the lipoprotein (80k data points after 4 dummy scans using 8 transients, 90° pulse) and low-molecular-weight metabolites (64k data points, using 24 (or 16) transients acquired after 4 steady state scans, T2-relaxation-filtered pulse sequence) data were automatically collected at 310.1K either with the 500 MHz or the 600 MHz Bruker AVANCE IIIHD NMR spectrometer, with a relaxation delay of 3.0 seconds.(19)(25)

The NMR spectra are converted to absolute concentrations via Bayesian modeling performed via advanced proprietary software and integrates quality control checks. Several of the metabolic biomarkers have already been 'validated' with other techniques (i.e. routine clinical chemistry assays, gas chromatography, an enzymatic method, and/or mass spectrometry).(21, 24, 26–28) Furthermore, genetic studies(29–31) performed on the same metabolomics platform showed that the labels applied to the metabolites are coherent and linked with biologically relevant and plausible genes.

The 14 lipoprotein subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses, IDL, three LDL subclasses and four HDL subclasses. The following components of the lipoprotein subclasses were quantified: phospholipids (PL), TG, cholesterol (C), free cholesterol (FC), and cholesteryl esters (CE). The mean size for VLDL, LDL and HDL particles was calculated by weighting the corresponding subclass diameters with their particle concentrations.

NMR spectroscopy provides highly consistent biomarker quantification. This is due to the inherently reproducible nature of the technology; the samples never come into contact with the radiofrequency detector in the NMR spectrometer. Biomarker quantification directly from plasma, without any sample extraction procedures, further contributes to the high reproducibility (24). Representative coefficients of variations (CVs) for the metabolic biomarkers are published as

Supplementary Data 3 in Kettunen et al.(30) with the CVs determined for 9,600 samples. Values ranged between 0.3 and 19.5 (mean 4.5%), and most values are comparable to routinely used assays in clinical chemistry.

Covariates

To be largely in line with previous metabolomics meta-analytic studies,(23), we adjusted analyses for the following potentially confounding variables: age (in years), gender, fasting status (yes/no), use of lipid modifying medication (yes/no), and current smoking (yes/no). The lipid modifying drugs were defined according to the related Anatomical Therapeutic Chemical Classification System (ATC) code C10 (Lipid modifying agents) in order to capture all the medications falling under this category, including the use of single agents (C10A - Lipid modifying agents, plain: C10AA HMG CoA reductase inhibitors; C10AB Fibrates; C10AC Bile acid sequestrants; C10AD Nicotinic acid and derivatives; C10AX Other lipid modifying agents) and all their potential combinations (C10B - Lipid modifying agents, combination: C10BA HMG CoA reductase inhibitors in combination with other lipid modifying agents; C10BX HMG CoA reductase inhibitors, other combinations). The antidepressant medications selected for the sensitivity analyses included all classes listed under the ATC code N06A (N06AA Non-selective monoamine reuptake inhibitors, N06AB Selective serotonin reuptake inhibitors, N06AF Monoamine oxidase inhibitors, non-selective, N06AG Monoamine oxidase A inhibitors, N06AX Other antidepressants). Given the bidirectional relationship between depression and obesity and their shared biological processes (including genes, endocrine and immuno-inflammatory mechanisms),(32) the role of obesity was explored in greater detail in sensitivity analysis (see Statistical analyses). Body mass index (BMI) was calculated as measured weight (kg)/length $(m)^2$, and divided into normal weight (BMI=18.50-24.99), overweight (BMI=25.00-29.99) and obesity (BMI≥30).

Assessment of potential bias due to metabolites data transformation

According to the standardized protocol of data processing applied in the present study a constant of 1 was added to the metabolite values before log-transformation. This common practice, adopted also in several other studies also from the same BBMRI-NL Metabolomics Consortium,(33) aims to achieve normalization of the distribution also for metabolites with initial values equaling zero. Nevertheless, it is important to acknowledge that this transformation may have had introduced some bias due to the high variability in the normal range of different metabolite. In the present analyses we aimed to estimate the potential degree of bias introduced by comparing the results of the metabolites-depression associations obtained applying three different transformation before log-transformation: A) adding a constant of 1; B) adding the value of the 10^{th} percentile of the distribution (excluding 0 values) of each metabolite, a value therefore within the normal range of the original metabolite; C) excluding all 0 values, a more conservative approach.

Analyses were performed in the NESDA sample (N=2,509), the most representative dataset for the trait under study, which involves subjects well phenotyped in psychiatric terms including healthy controls and depressed patients from various settings and developmental stages of psychopathology. Furthermore, analyses focused on the 51 metabolites classified in the cluster of "lipids, fatty acids and various low-molecular-weight metabolites".

Ridge plots in Figure S13 shows the distribution (per SD increase) of the (log)values of the metabolites after the three different transformation. The three sets of values were used in logistic regression analyses estimating the association between metabolites and lifetime depression, adjusting for gender, age, smoking, lipid modifying drugs and fasting status. Results were highly similar across the three transformations. In Figure S14, the estimates obtained used the original transformation A were plotted against estimates obtained with transformation B (panel 1), and against those obtained with transformation C (panel 2). In both instances the correlation between association effect sizes equaled 1 as the estimates were substantially identical across transformation (coefficient from regressing estimates of transformation A on those from transformation B = 1.02, se=0.01; coefficient from regressing estimates

of transformation A on those from transformation C = 1.00, se=0.02). Overall, these results suggest that the degree of bias potentially introduced by the transformation applied in original analyses is minimal and negligible.

Table S1. Overview of depression instruments and definitions per study

	Depression		Current depression*		Controls	
	definition	n	definition	n	definition	n
CODAM DM	CES-D ≥16 at time of blood sampling	34	CES-D ≥16 at time of blood sampling	34	CES-D <16 at time of blood sampling	105
CODAM noDM	CES-D ≥16 at time of blood sampling	78	CES-D ≥16 at time of blood sampling	78	CES-D <16 at time of blood sampling	338
TMS DM	MINI lifetime MDD	272	MINI current MDD (past two weeks)	46	MINI no depressive disorder	503
TMS noDM	MINI lifetime MDD	243	MINI current MDD (past two weeks)	24	MINI no depressive disorder	480
ERF	HADS-D ≥8 OR CES-D ≥16 (ever during any of the longitudinal ERF assessments)	153	HADS-D ≥8 OR CES-D ≥16 within month of blood sampling	25	HADS-D <8 AND CES-D <16 AND never reported depression or psychiatric problem during any of the longitudinal ERF assessments	193
LUMINA	HADS-D ≥8 OR CES-D ≥16 (ever during any of the longitudinal LUMINA assessments)	59	HADS-D ≥8 OR CES-D ≥16 within month of blood sampling	14	HADS-D <8 AND CES-D <16 within month of blood sampling	172
NEO	IDS-SR ≥14 within month of blood sampling	1,934	IDS-SR ≥14 within month of blood sampling	1,934	IDS-SR <14 within month of blood sampling	4,620
NESDA	CIDI MDD lifetime	1,875	CIDI current MDD (past month)	782	CIDI no lifetime depressive or anxiety disorder	634
NTR	CIDI MDD lifetime	170	n.a.	n.a.	Hypercontrols (negative screening for depressive symptoms in multiple questionnaires)	1,353
RS	CES-D ≥16 (at least 1 out of the longitudinal 3 RS waves)	451	CES-D ≥16 at time of blood sampling	314	CES-D <16 at all three RS waves	737

	Depression		Current depression*		Controls	
	definition	n	definition	n	definition	n
LLD		14		14		1,010
Total		**5,283**		**3,265**		**10,145**

Abbreviations: CODAM DM = Cohort on Diabetes and Atherosclerosis Maastricht, subgroup with type 2 diabetes mellitus, CODAM DM = Cohort on Diabetes and Atherosclerosis Maastricht, subgroup without diabetes mellitus, TMS DM = The Maastricht Study, subgroup with type 2 diabetes mellitus, TMS noDM = The Maastricht Study, subgroup 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 Registry, RS = Rotterdam Study.

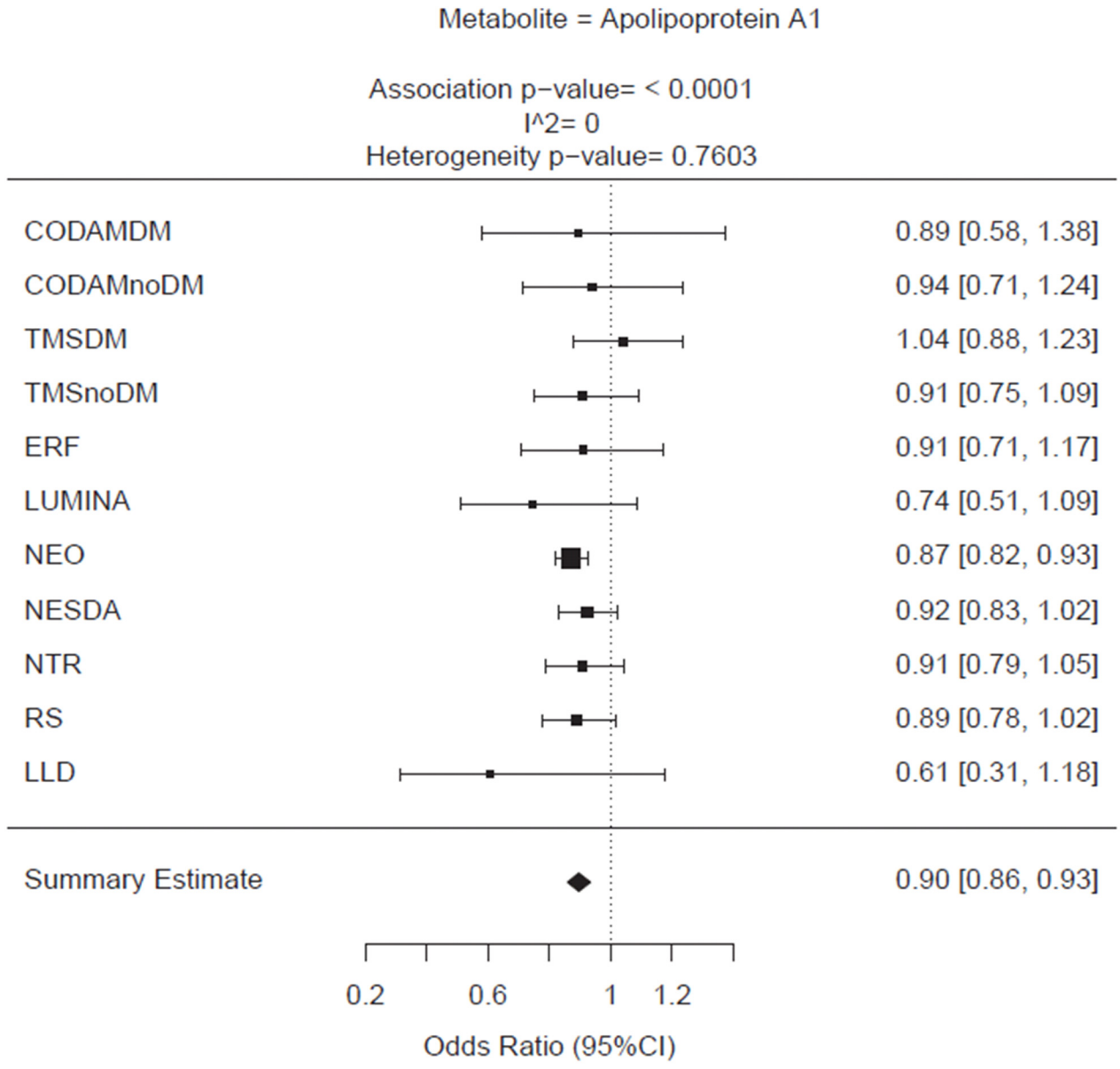
CES-D = Center for Epidemiologic Studies Depression scale, CIDI = Composite International Diagnostic Interview, HADS-D = Hospital Anxiety and Depression Scale Depression subscale, IDS-SR = Inventory of Depressive Symptomatology self-report, MINI = Mini International Neuropsychiatric Interview. n.a. = not applicable. MDD = Major Depressive Disorder

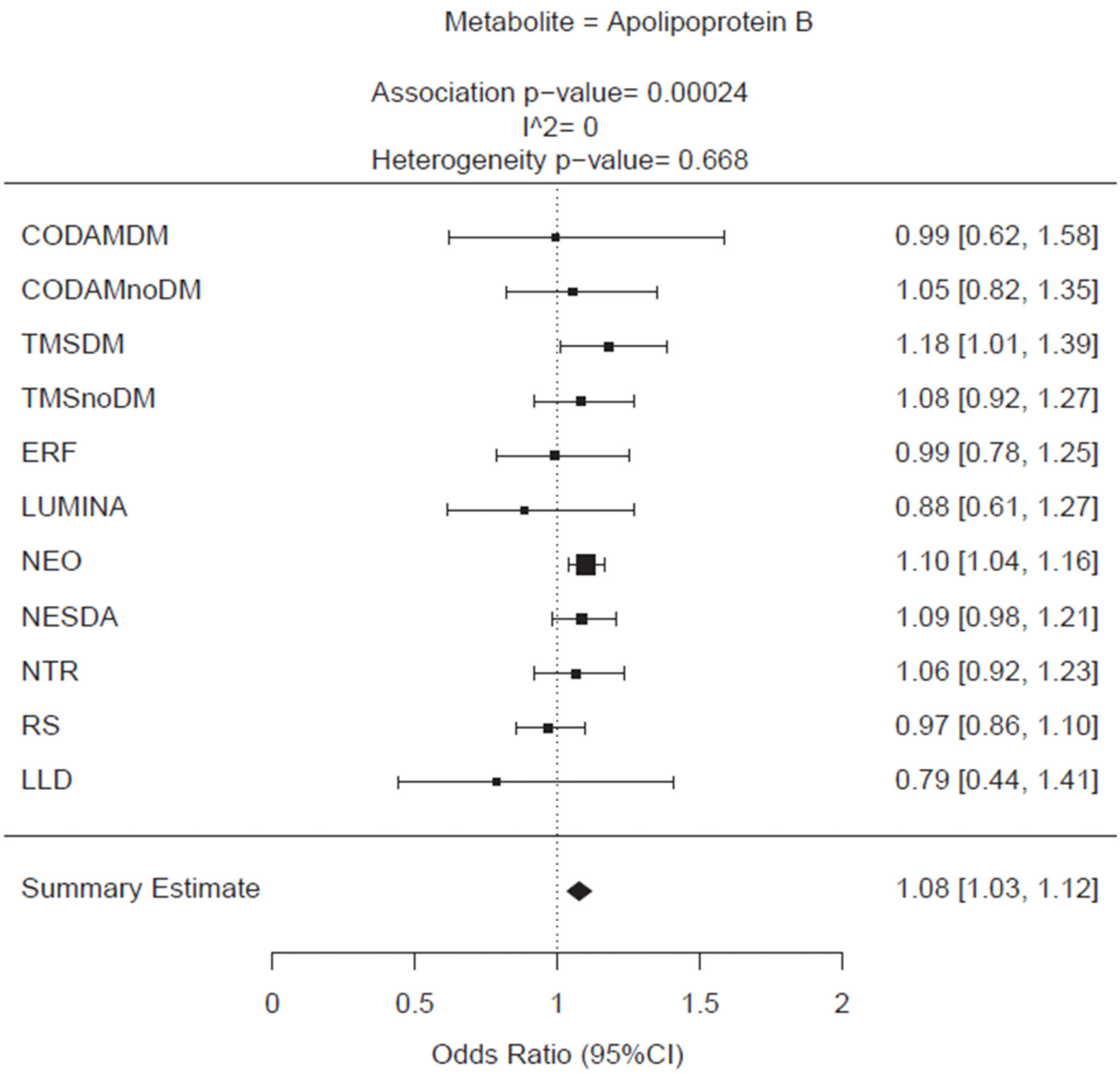
*subgroup of people with depression that experienced depression within one month before or after the metabolomics blood sampling.

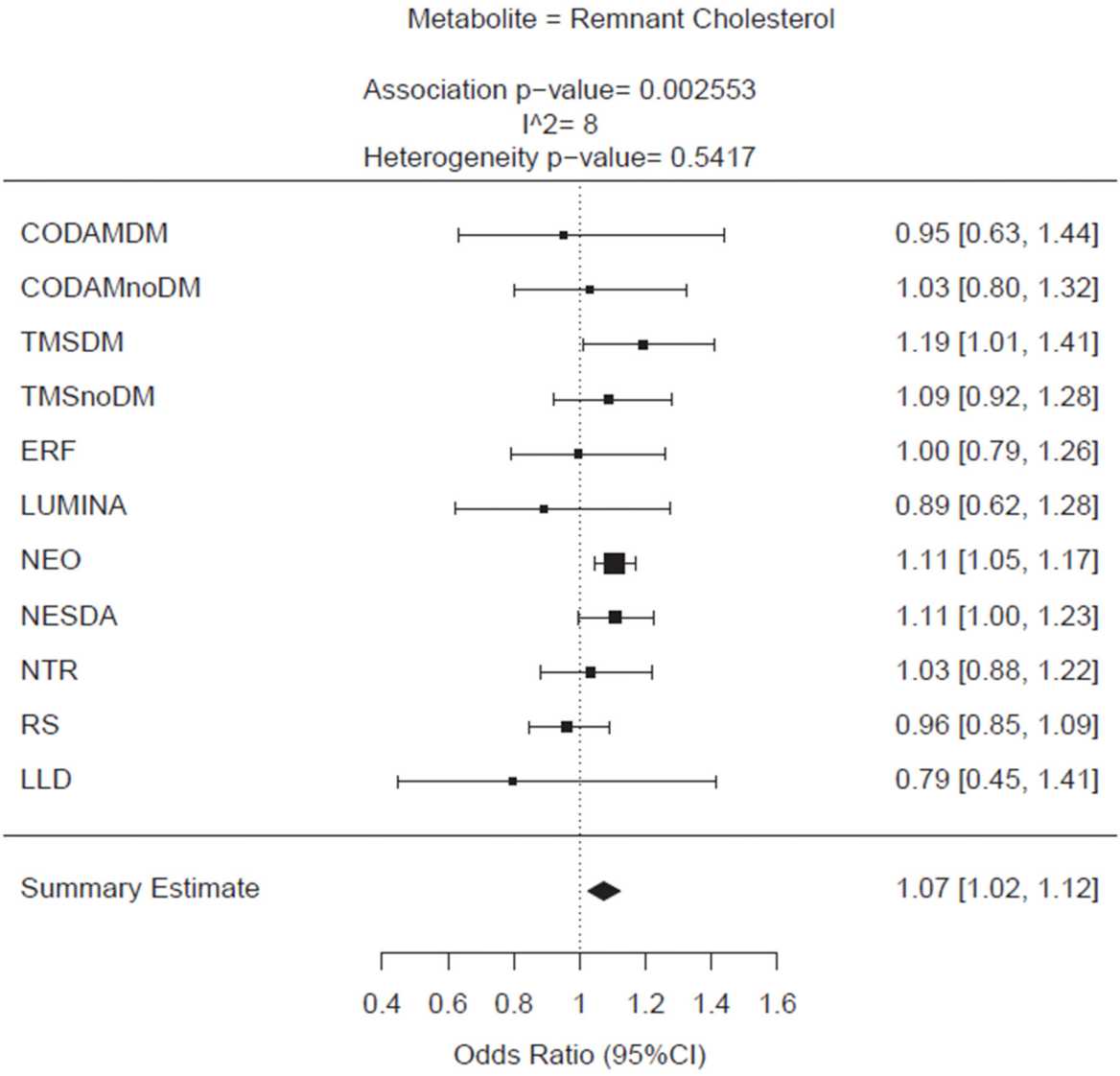
Table S2. Results of pooled analyses for depression for each metabolite

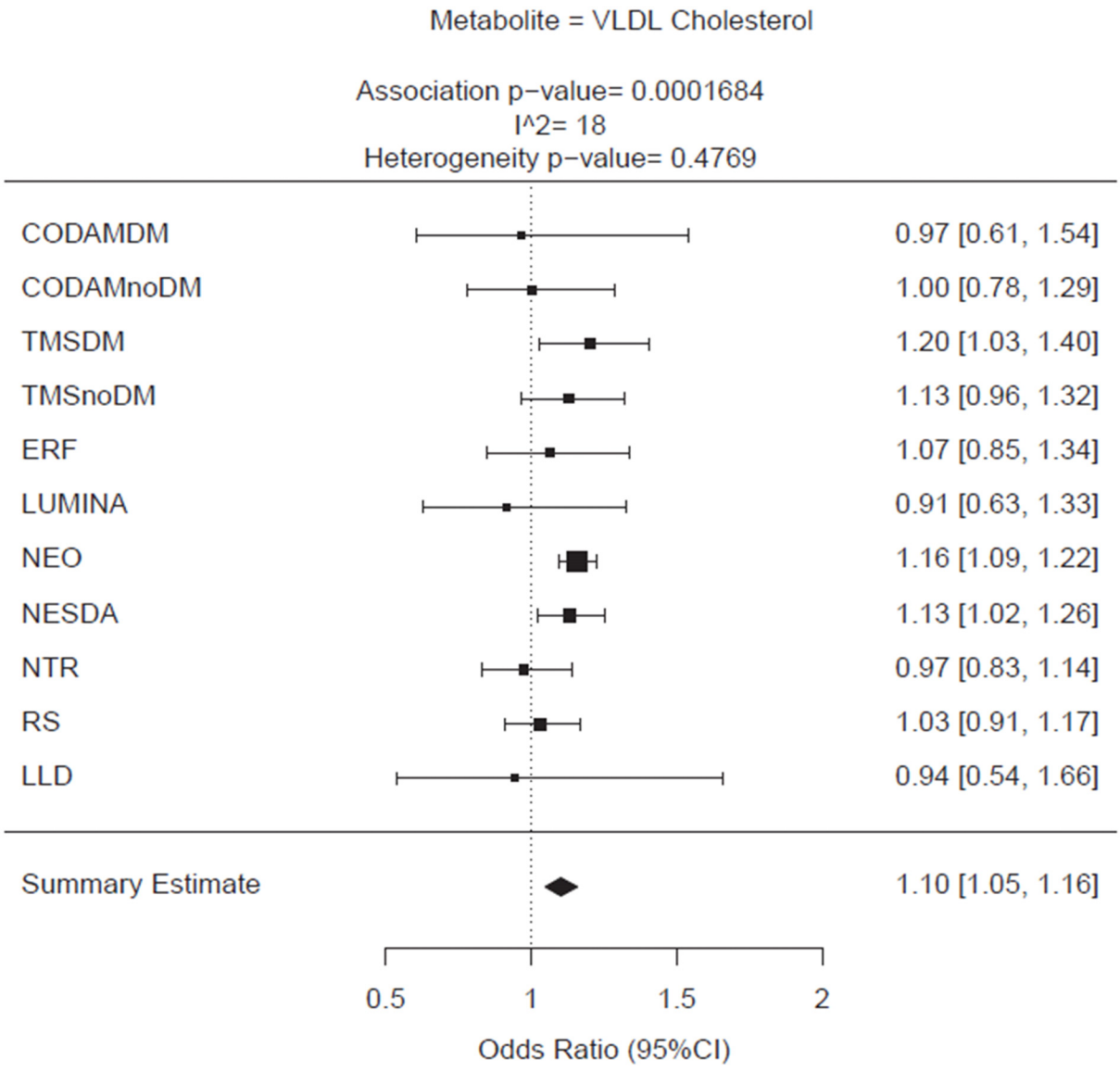
See Supplement 2,Excel file.

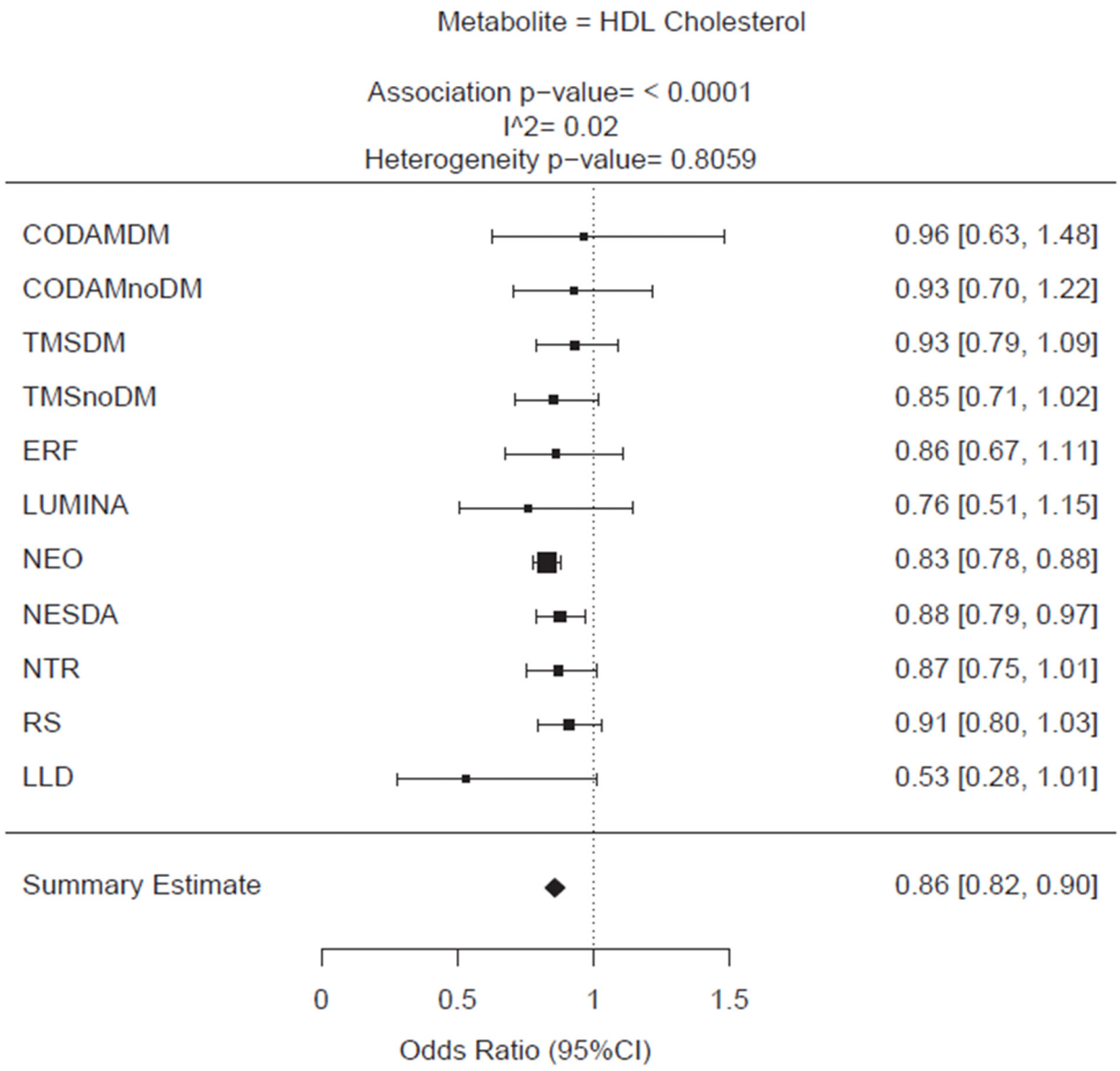
Figure S1. Forest plots of the 21 lipids, fatty acids and various low-molecular-weight metabolites significantly associated with depression (FDR<0.05)

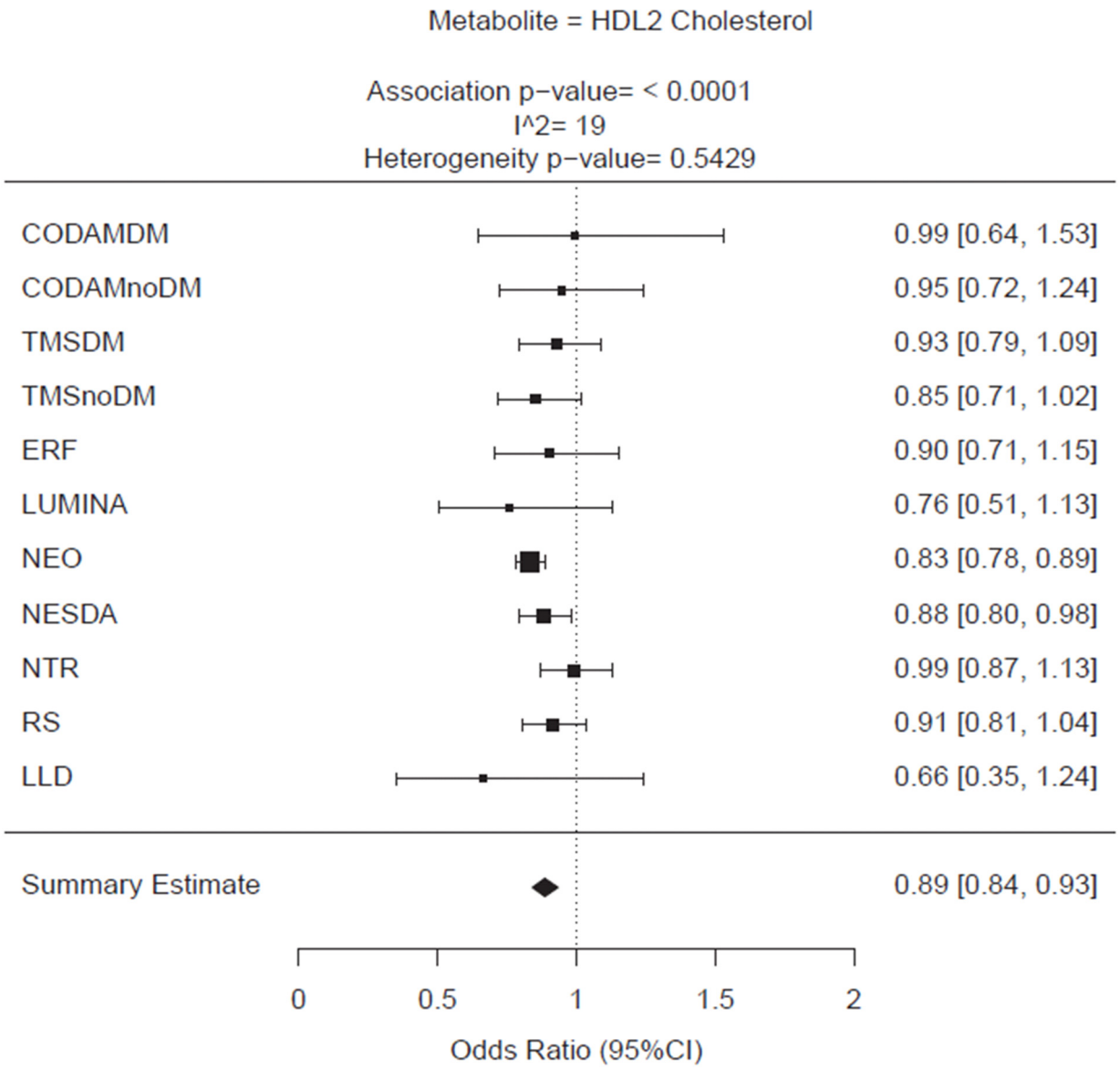


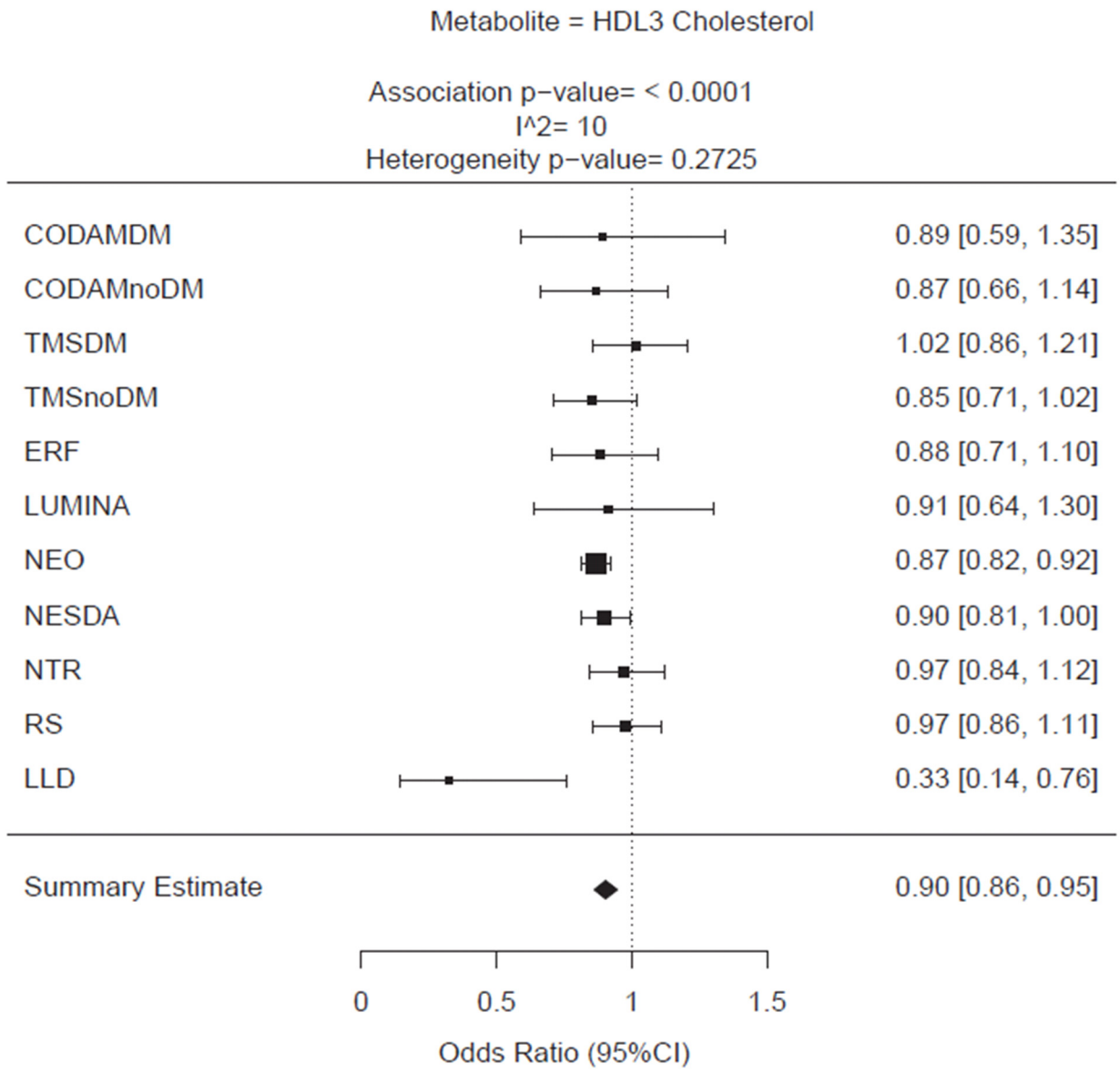

Metabolite = Apolipoprotein B
Association p−value= 0.00024
I^2= 0
Heterogeneity p−value= 0.668
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.99 [0.62, 1.58]
1.05 [0.82, 1.35]
1.18 [1.01, 1.39]
1.08 [0.92, 1.27]
0.99 [0.78, 1.25]
0.88 [0.61, 1.27]
1.10 [1.04, 1.16]
1.09 [0.98, 1.21]
1.06 [0.92, 1.23]
0.97 [0.86, 1.10]
0.79 [0.44, 1.41]
Summary Estimate
1.08 [1.03, 1.12]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

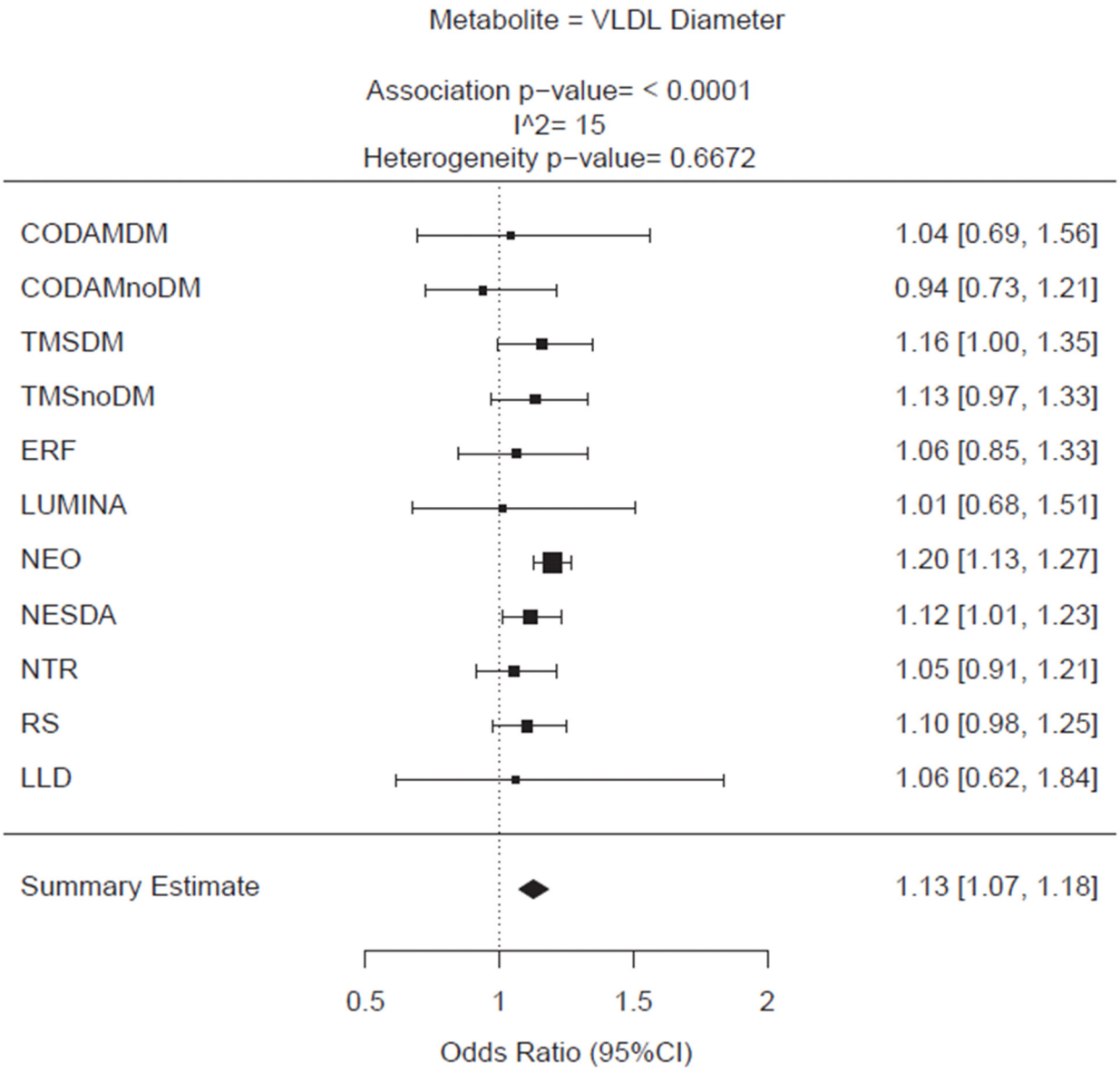

Metabolite = Remnant Cholesterol
Association p−value= 0.002553
I^2= 8
Heterogeneity p−value= 0.5417
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.95 [0.63, 1.44]
1.03 [0.80, 1.32]
1.19 [1.01, 1.41]
1.09 [0.92, 1.28]
1.00 [0.79, 1.26]
0.89 [0.62, 1.28]
1.11 [1.05, 1.17]
1.11 [1.00, 1.23]
1.03 [0.88, 1.22]
0.96 [0.85, 1.09]
0.79 [0.45, 1.41]
Summary Estimate
1.07 [1.02, 1.12]
0.4 0.6 0.8 1 1.2 1.4 1.6
Odds Ratio (95%CI)

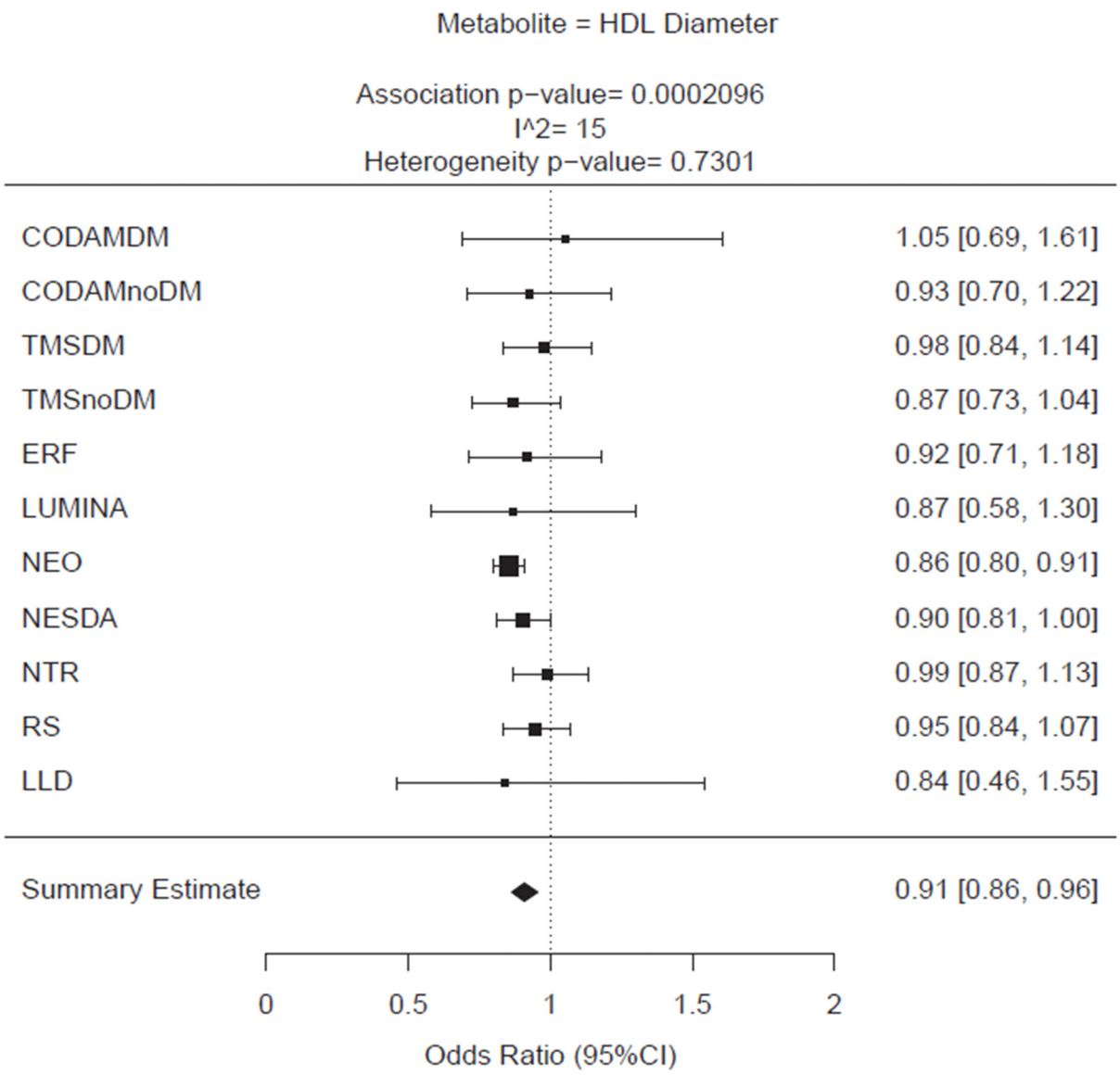

Metabolite = VLDL Cholesterol
Association p−value= 0.0001684
I^2= 18
Heterogeneity p−value= 0.4769
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
Summary Estimate
0.97 [0.61, 1.54]
1.00 [0.78, 1.29]
1.20 [1.03, 1.40]
1.13 [0.96, 1.32]
1.07 [0.85, 1.34]
0.91 [0.63, 1.33]
1.16 [1.09, 1.22]
1.13 [1.02, 1.26]
0.97 [0.83, 1.14]
1.03 [0.91, 1.17]
0.94 [0.54, 1.66]
1.10 [1.05, 1.16]
0.5
1
1.5
2
Odds Ratio (95%CI)

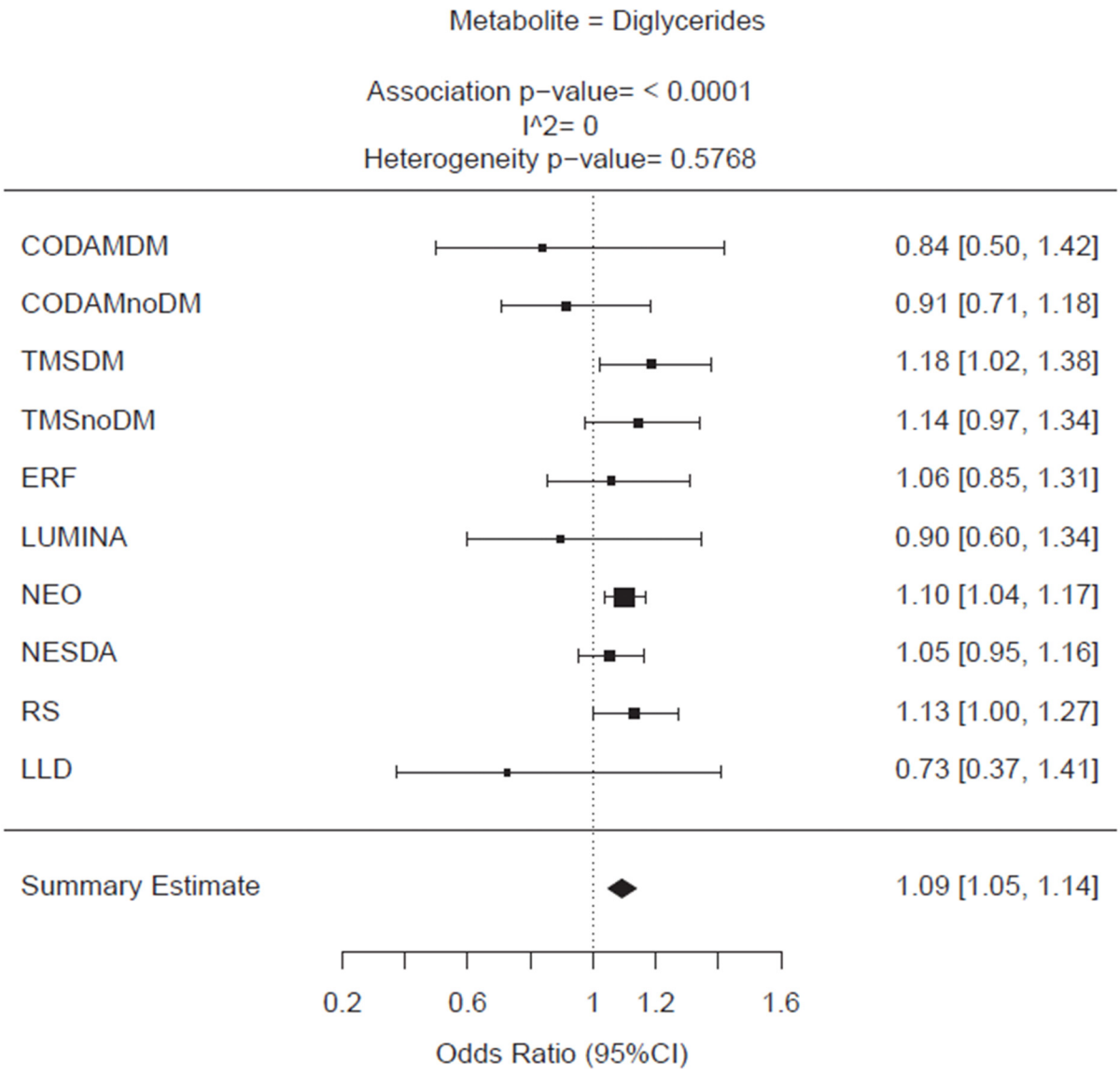

Metabolite = HDL Cholesterol
Association p−value= < 0.0001
I^2= 0.02
Heterogeneity p−value= 0.8059
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.96 [0.63, 1.48]
0.93 [0.70, 1.22]
0.93 [0.79, 1.09]
0.85 [0.71, 1.02]
0.86 [0.67, 1.11]
0.76 [0.51, 1.15]
0.83 [0.78, 0.88]
0.88 [0.79, 0.97]
0.87 [0.75, 1.01]
0.91 [0.80, 1.03]
0.53 [0.28, 1.01]
Summary Estimate
0.86 [0.82, 0.90]
0
0.5
1
1.5
Odds Ratio (95%CI)

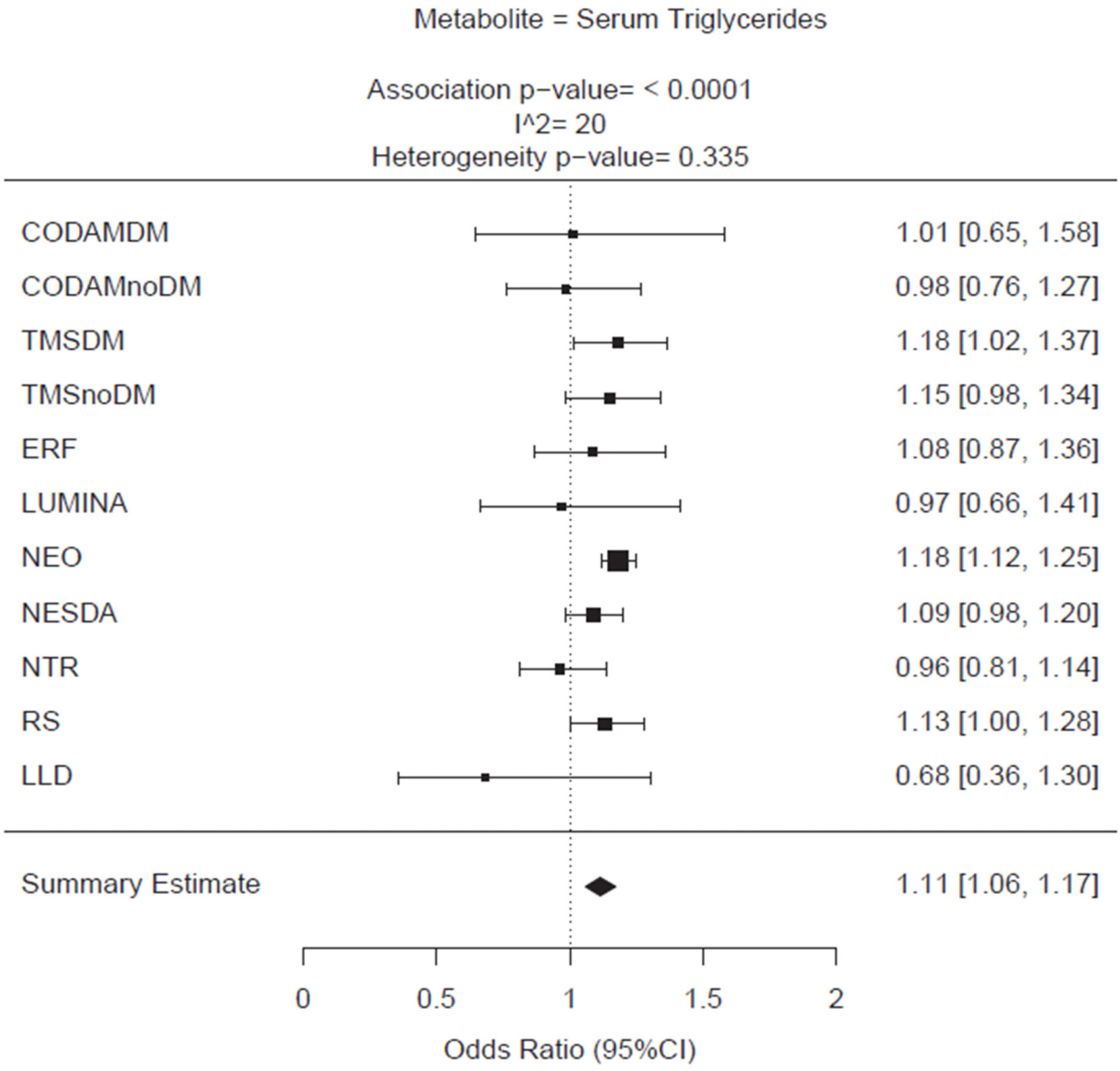

Metabolite = HDL2 Cholesterol
Association p−value= < 0.0001
I^2= 19
Heterogeneity p−value= 0.5429
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.99 [0.64, 1.53]
0.95 [0.72, 1.24]
0.93 [0.79, 1.09]
0.85 [0.71, 1.02]
0.90 [0.71, 1.15]
0.76 [0.51, 1.13]
0.83 [0.78, 0.89]
0.88 [0.80, 0.98]
0.99 [0.87, 1.13]
0.91 [0.81, 1.04]
0.66 [0.35, 1.24]
Summary Estimate
0.89 [0.84, 0.93]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

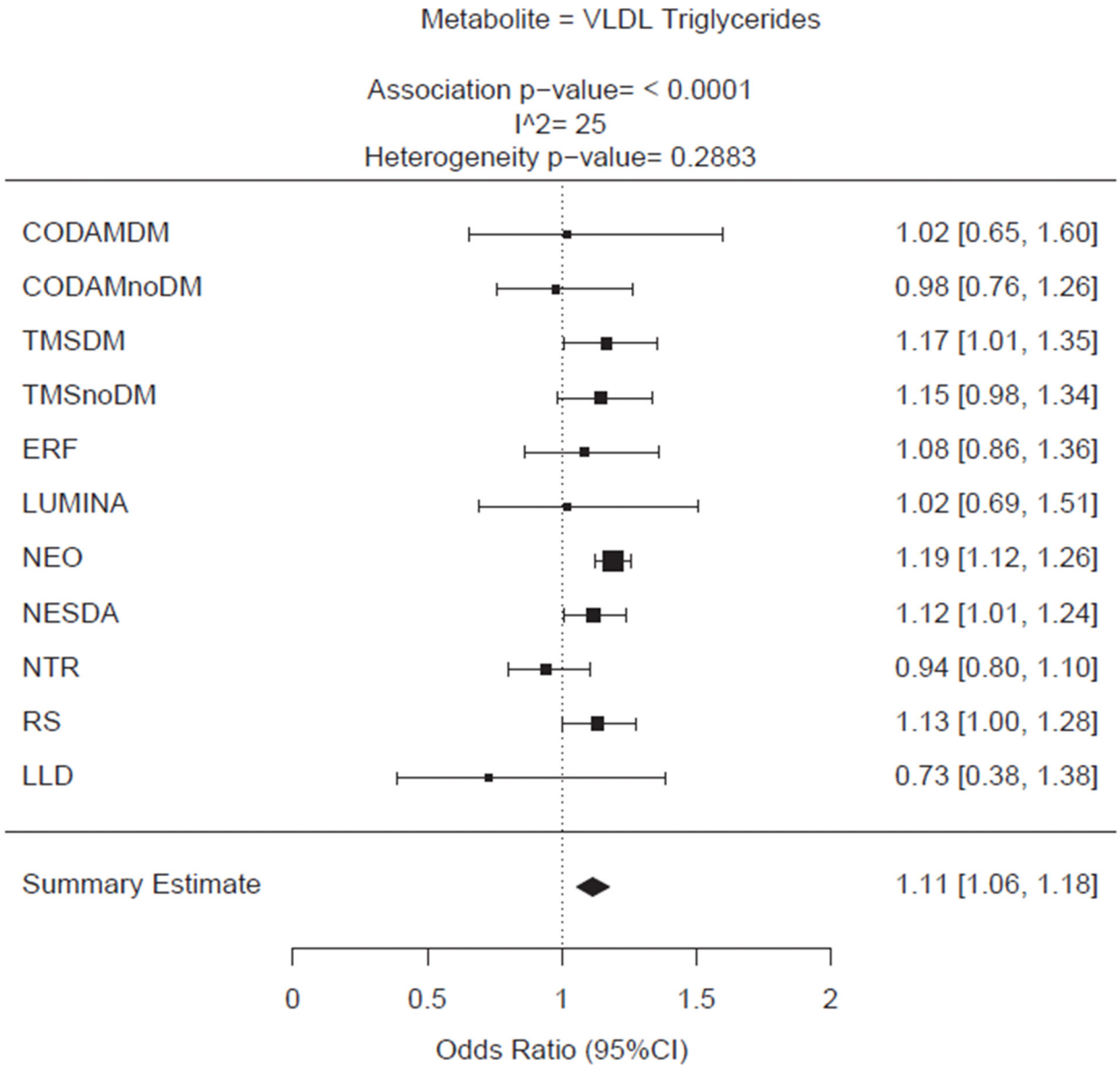

Metabolite = HDL3 Cholesterol
Association p−value= < 0.0001
I^2= 10
Heterogeneity p−value= 0.2725
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.89 [0.59, 1.35]
0.87 [0.66, 1.14]
1.02 [0.86, 1.21]
0.85 [0.71, 1.02]
0.88 [0.71, 1.10]
0.91 [0.64, 1.30]
0.87 [0.82, 0.92]
0.90 [0.81, 1.00]
0.97 [0.84, 1.12]
0.97 [0.86, 1.11]
0.33 [0.14, 0.76]
Summary Estimate
0.90 [0.86, 0.95]
0
0.5
1
1.5
Odds Ratio (95%CI)

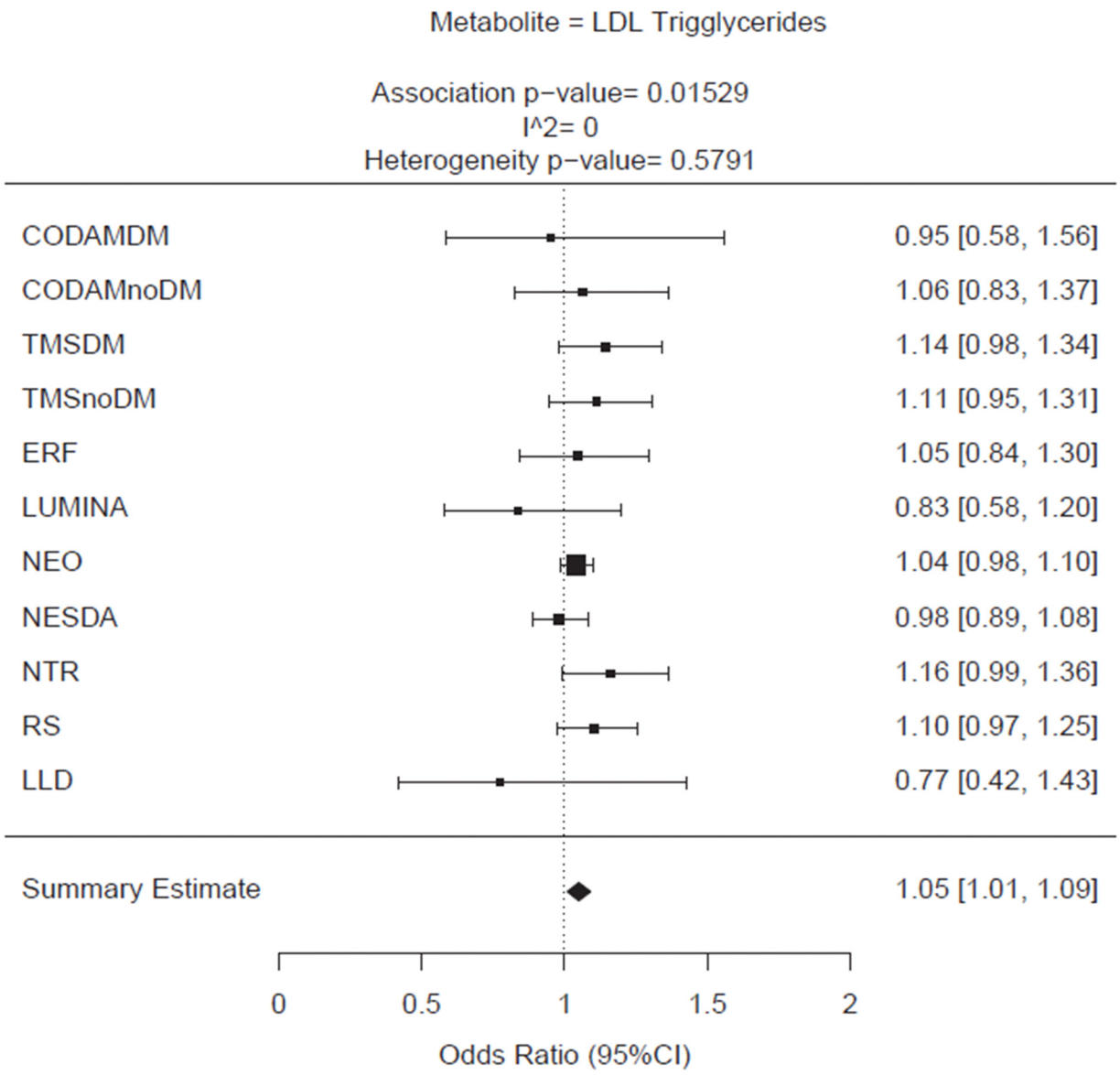

Metabolite = VLDL Diameter
Association p−value= < 0.0001
I^2= 15
Heterogeneity p−value= 0.6672
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.04 [0.69, 1.56]
0.94 [0.73, 1.21]
1.16 [1.00, 1.35]
1.13 [0.97, 1.33]
1.06 [0.85, 1.33]
1.01 [0.68, 1.51]
1.20 [1.13, 1.27]
1.12 [1.01, 1.23]
1.05 [0.91, 1.21]
1.10 [0.98, 1.25]
1.06 [0.62, 1.84]
Summary Estimate
1.13 [1.07, 1.18]
0.5
1
1.5
2
Odds Ratio (95%CI)

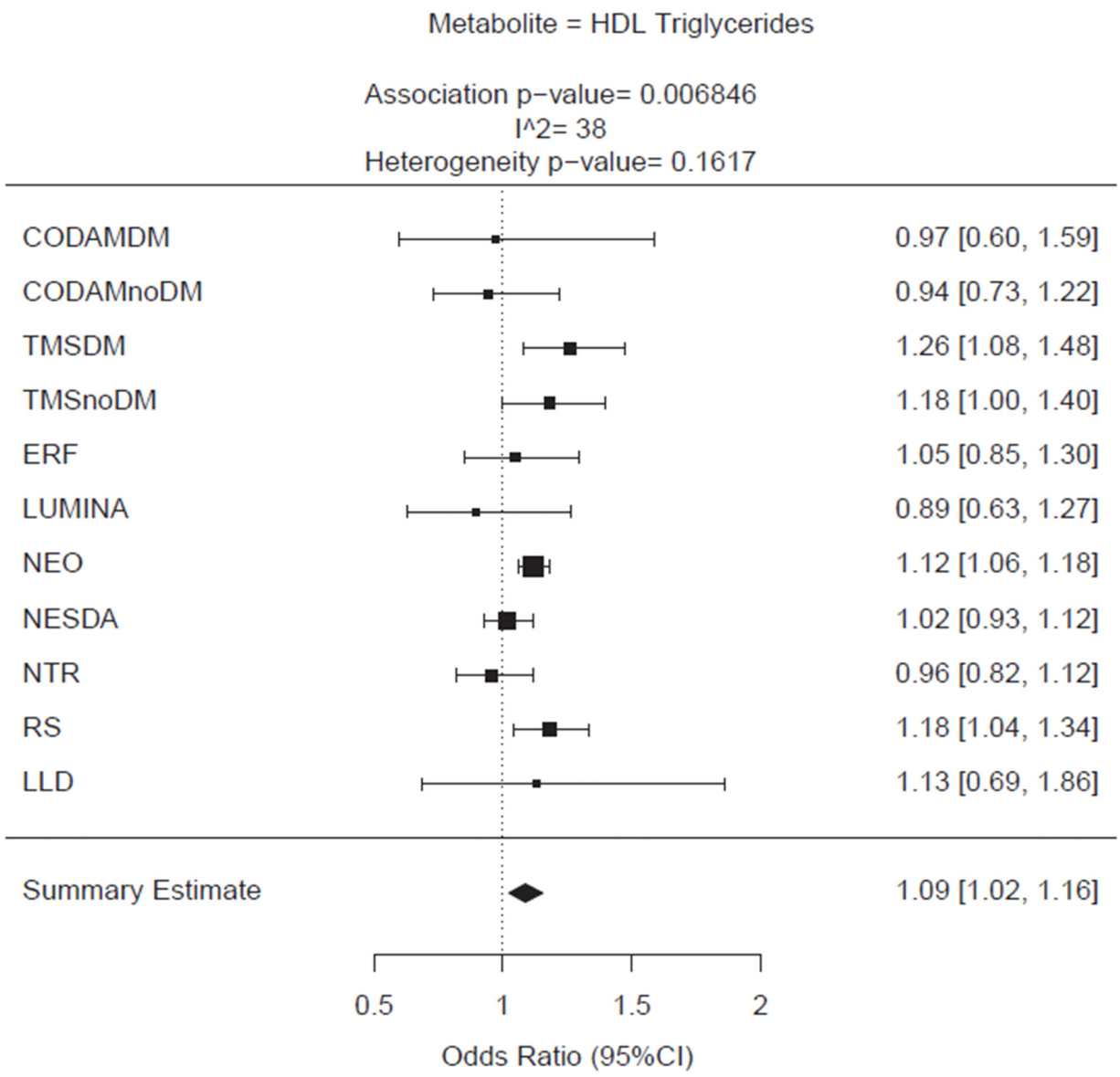

Metabolite = HDL Diameter
Association p−value= 0.0002096
I^2= 15
Heterogeneity p−value= 0.7301
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.05 [0.69, 1.61]
0.93 [0.70, 1.22]
0.98 [0.84, 1.14]
0.87 [0.73, 1.04]
0.92 [0.71, 1.18]
0.87 [0.58, 1.30]
0.86 [0.80, 0.91]
0.90 [0.81, 1.00]
0.99 [0.87, 1.13]
0.95 [0.84, 1.07]
0.84 [0.46, 1.55]
Summary Estimate
0.91 [0.86, 0.96]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

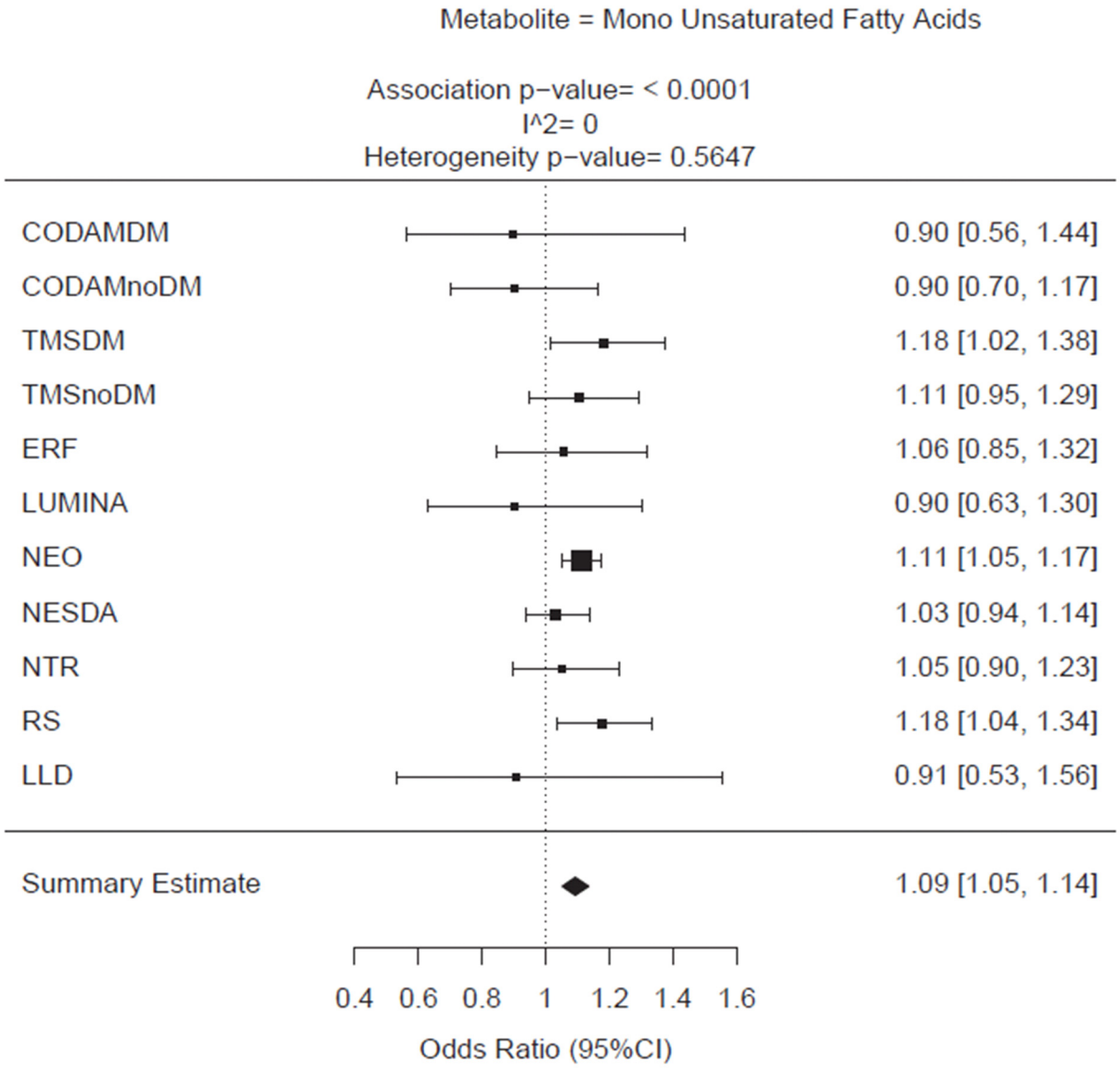

Metabolite = Diglycerides
Association p−value= < 0.0001
I^2= 0
Heterogeneity p−value= 0.5768
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
RS
LLD
0.84 [0.50, 1.42]
0.91 [0.71, 1.18]
1.18 [1.02, 1.38]
1.14 [0.97, 1.34]
1.06 [0.85, 1.31]
0.90 [0.60, 1.34]
1.10 [1.04, 1.17]
1.05 [0.95, 1.16]
1.13 [1.00, 1.27]
0.73 [0.37, 1.41]
Summary Estimate
1.09 [1.05, 1.14]
0.2
0.6
1
1.2
1.6
Odds Ratio (95%CI)

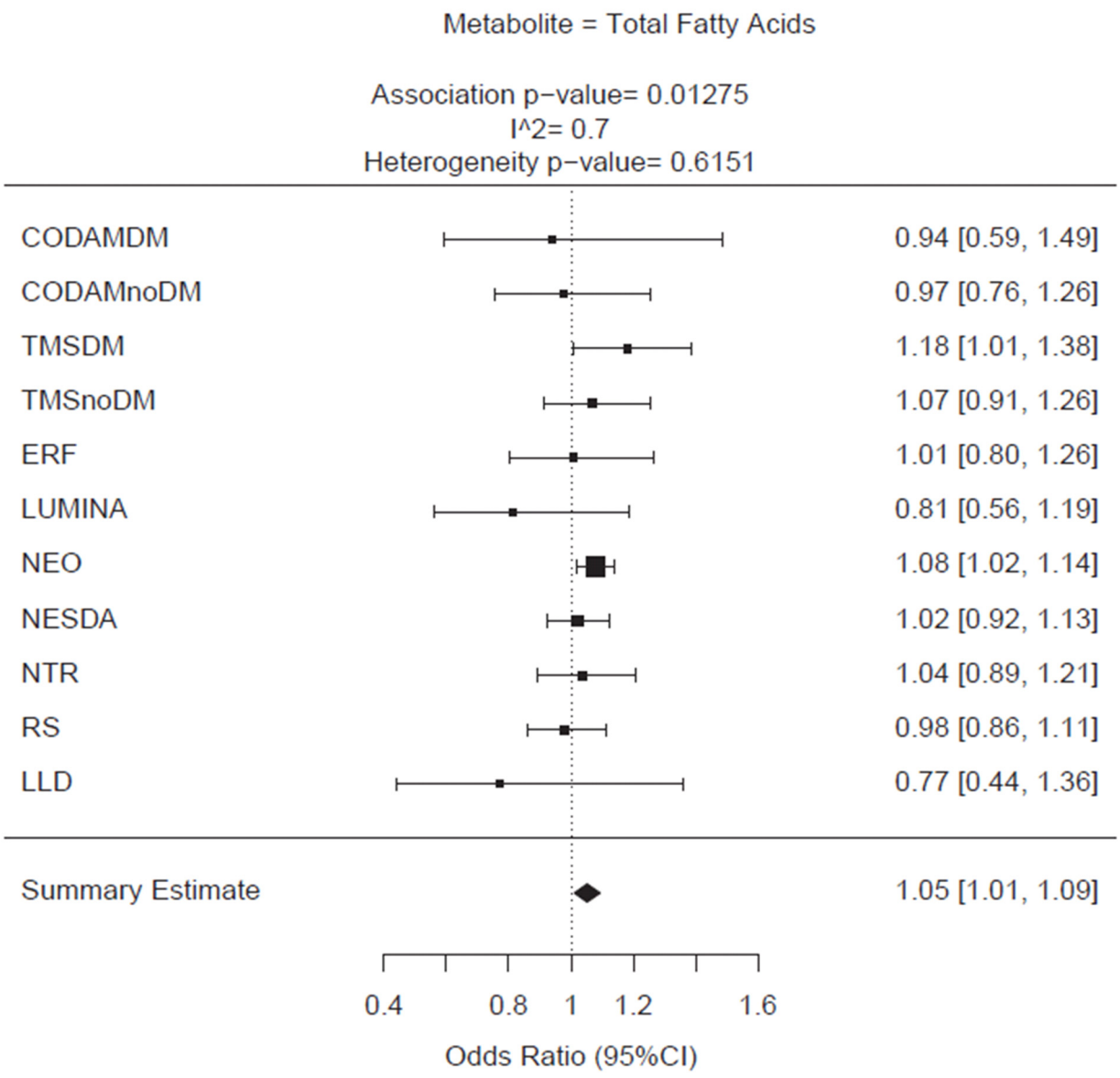

Metabolite = Serum Triglycerides
Association p−value= < 0.0001
I^2= 20
Heterogeneity p−value= 0.335
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.01 [0.65, 1.58]
0.98 [0.76, 1.27]
1.18 [1.02, 1.37]
1.15 [0.98, 1.34]
1.08 [0.87, 1.36]
0.97 [0.66, 1.41]
1.18 [1.12, 1.25]
1.09 [0.98, 1.20]
0.96 [0.81, 1.14]
1.13 [1.00, 1.28]
0.68 [0.36, 1.30]
Summary Estimate
1.11 [1.06, 1.17]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

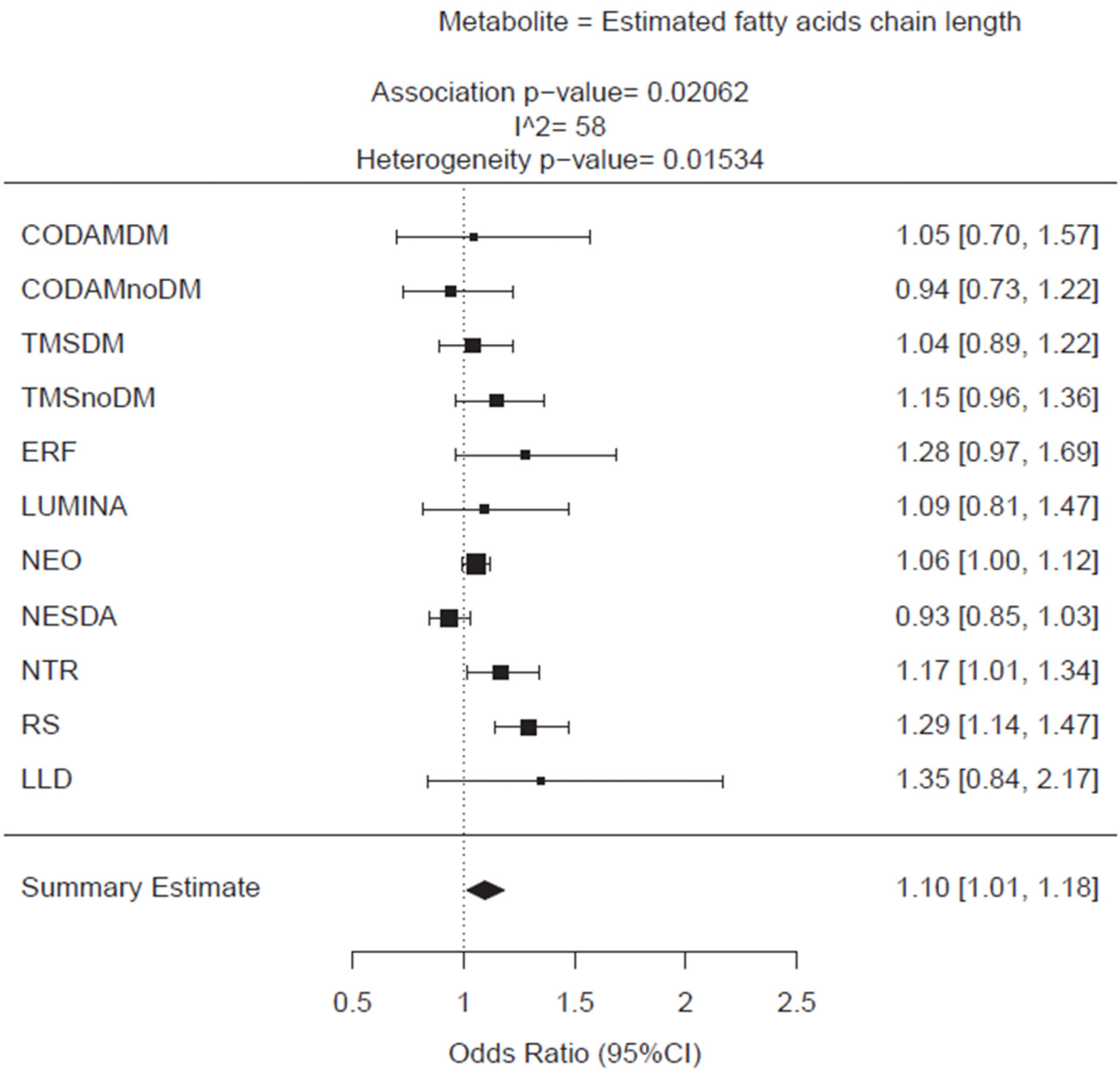

Metabolite = VLDL Triglycerides
Association p−value= < 0.0001
I^2= 25
Heterogeneity p−value= 0.2883
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.02 [0.65, 1.60]
0.98 [0.76, 1.26]
1.17 [1.01, 1.35]
1.15 [0.98, 1.34]
1.08 [0.86, 1.36]
1.02 [0.69, 1.51]
1.19 [1.12, 1.26]
1.12 [1.01, 1.24]
0.94 [0.80, 1.10]
1.13 [1.00, 1.28]
0.73 [0.38, 1.38]
Summary Estimate
1.11 [1.06, 1.18]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

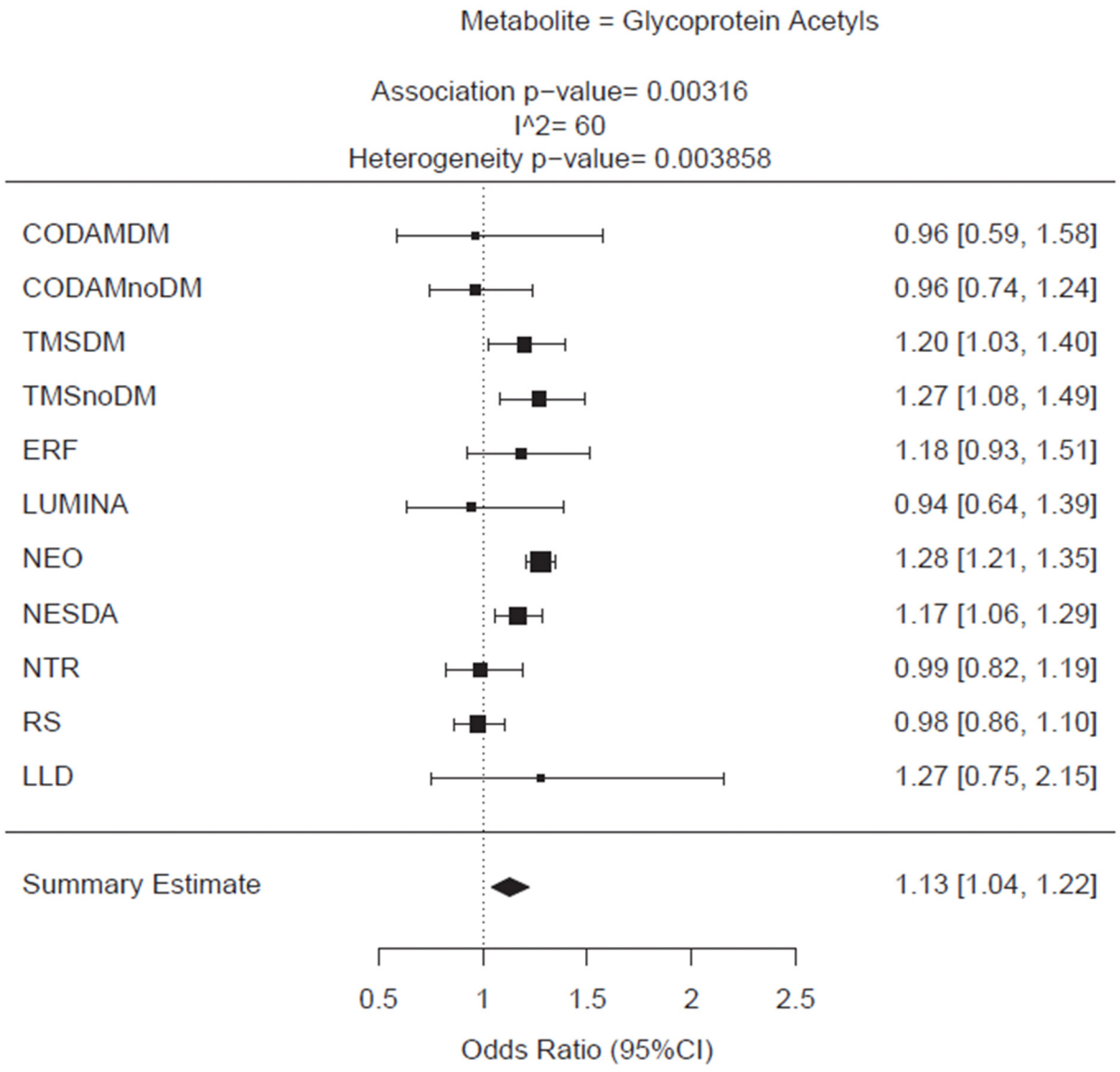

Metabolite = LDL Trigglycerides
Association p−value= 0.01529
I^2= 0
Heterogeneity p−value= 0.5791
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.95 [0.58, 1.56]
1.06 [0.83, 1.37]
1.14 [0.98, 1.34]
1.11 [0.95, 1.31]
1.05 [0.84, 1.30]
0.83 [0.58, 1.20]
1.04 [0.98, 1.10]
0.98 [0.89, 1.08]
1.16 [0.99, 1.36]
1.10 [0.97, 1.25]
0.77 [0.42, 1.43]
Summary Estimate
1.05 [1.01, 1.09]
0
0.5
1
1.5
2
Odds Ratio (95%CI)

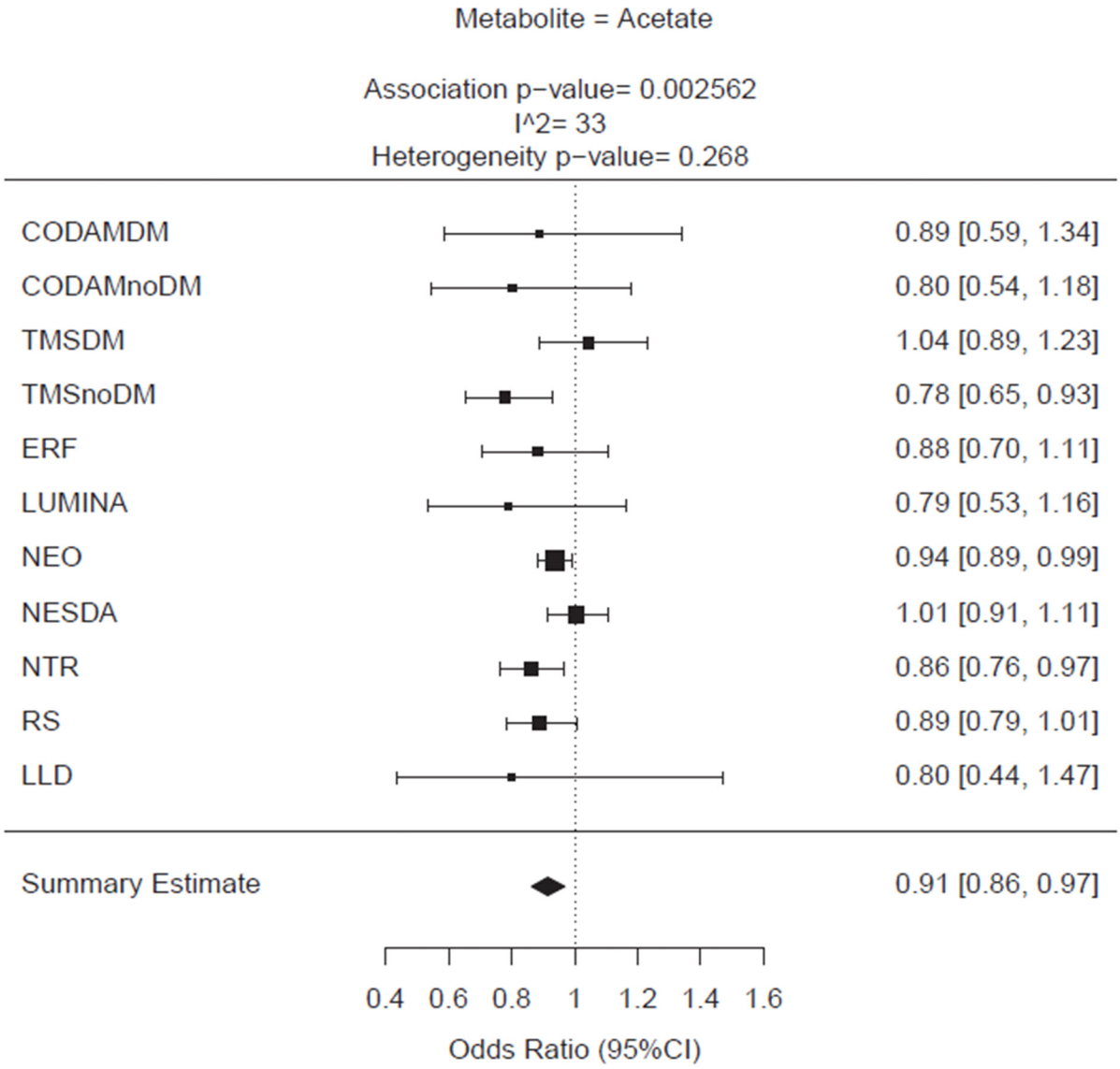

Metabolite = HDL Triglycerides
Association p−value= 0.006846
I^2= 38
Heterogeneity p−value= 0.1617
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.97 [0.60, 1.59]
0.94 [0.73, 1.22]
1.26 [1.08, 1.48]
1.18 [1.00, 1.40]
1.05 [0.85, 1.30]
0.89 [0.63, 1.27]
1.12 [1.06, 1.18]
1.02 [0.93, 1.12]
0.96 [0.82, 1.12]
1.18 [1.04, 1.34]
1.13 [0.69, 1.86]
Summary Estimate
1.09 [1.02, 1.16]
0.5
1
1.5
2
Odds Ratio (95%CI)

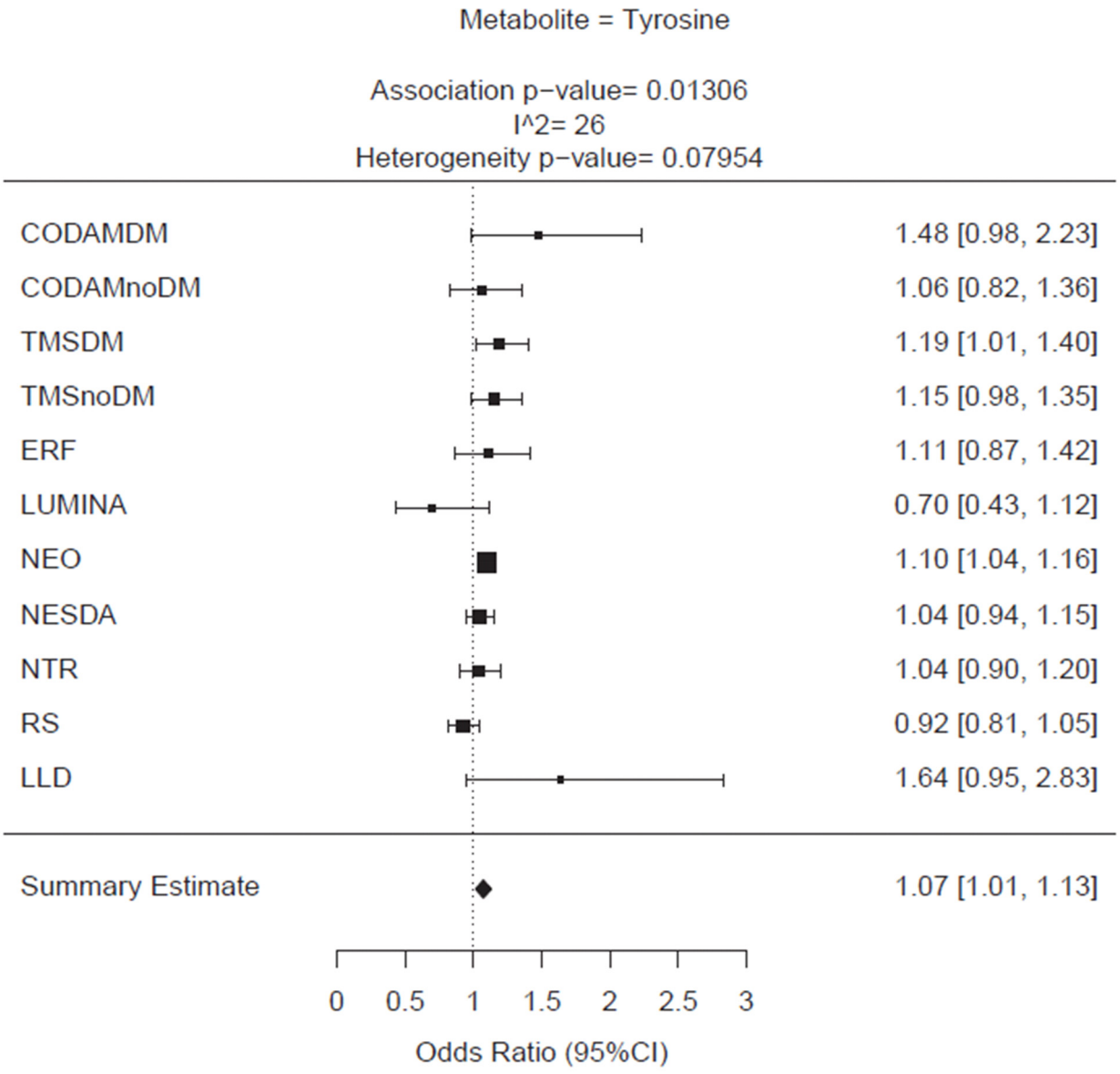

Metabolite = Mono Unsaturated Fatty Acids
Association p−value= < 0.0001
I^2= 0
Heterogeneity p−value= 0.5647
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.90 [0.56, 1.44]
0.90 [0.70, 1.17]
1.18 [1.02, 1.38]
1.11 [0.95, 1.29]
1.06 [0.85, 1.32]
0.90 [0.63, 1.30]
1.11 [1.05, 1.17]
1.03 [0.94, 1.14]
1.05 [0.90, 1.23]
1.18 [1.04, 1.34]
0.91 [0.53, 1.56]
Summary Estimate
1.09 [1.05, 1.14]
0.4 0.6 0.8 1 1.2 1.4 1.6
Odds Ratio (95%CI)


Metabolite = Total Fatty Acids
Association p−value= 0.01275
I^2= 0.7
Heterogeneity p−value= 0.6151
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.94 [0.59, 1.49]
0.97 [0.76, 1.26]
1.18 [1.01, 1.38]
1.07 [0.91, 1.26]
1.01 [0.80, 1.26]
0.81 [0.56, 1.19]
1.08 [1.02, 1.14]
1.02 [0.92, 1.13]
1.04 [0.89, 1.21]
0.98 [0.86, 1.11]
0.77 [0.44, 1.36]
Summary Estimate
1.05 [1.01, 1.09]
0.4
0.8
1
1.2
1.6
Odds Ratio (95%CI)


Metabolite = Estimated fatty acids chain length
Association p−value= 0.02062
I^2= 58
Heterogeneity p−value= 0.01534
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.05 [0.70, 1.57]
0.94 [0.73, 1.22]
1.04 [0.89, 1.22]
1.15 [0.96, 1.36]
1.28 [0.97, 1.69]
1.09 [0.81, 1.47]
1.06 [1.00, 1.12]
0.93 [0.85, 1.03]
1.17 [1.01, 1.34]
1.29 [1.14, 1.47]
1.35 [0.84, 2.17]
Summary Estimate
1.10 [1.01, 1.18]
0.5
1
1.5
2
2.5
Odds Ratio (95%CI)


Metabolite = Glycoprotein Acetyls
Association p−value= 0.00316
I^2= 60
Heterogeneity p−value= 0.003858
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.96 [0.59, 1.58]
0.96 [0.74, 1.24]
1.20 [1.03, 1.40]
1.27 [1.08, 1.49]
1.18 [0.93, 1.51]
0.94 [0.64, 1.39]
1.28 [1.21, 1.35]
1.17 [1.06, 1.29]
0.99 [0.82, 1.19]
0.98 [0.86, 1.10]
1.27 [0.75, 2.15]
Summary Estimate
1.13 [1.04, 1.22]
0.5
1
1.5
2
2.5
Odds Ratio (95%CI)


Metabolite = Acetate
Association p−value= 0.002562
I^2= 33
Heterogeneity p−value= 0.268
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
0.89 [0.59, 1.34]
0.80 [0.54, 1.18]
1.04 [0.89, 1.23]
0.78 [0.65, 0.93]
0.88 [0.70, 1.11]
0.79 [0.53, 1.16]
0.94 [0.89, 0.99]
1.01 [0.91, 1.11]
0.86 [0.76, 0.97]
0.89 [0.79, 1.01]
0.80 [0.44, 1.47]
Summary Estimate
0.91 [0.86, 0.97]
0.4 0.6 0.8 1 1.2 1.4 1.6
Odds Ratio (95%CI)


Metabolite = Tyrosine
Association p−value= 0.01306
I^2= 26
Heterogeneity p−value= 0.07954
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.48 [0.98, 2.23]
1.06 [0.82, 1.36]
1.19 [1.01, 1.40]
1.15 [0.98, 1.35]
1.11 [0.87, 1.42]
0.70 [0.43, 1.12]
1.10 [1.04, 1.16]
1.04 [0.94, 1.15]
1.04 [0.90, 1.20]
0.92 [0.81, 1.05]
1.64 [0.95, 2.83]
Summary Estimate
1.07 [1.01, 1.13]
0
0.5
1
1.5
2
2.5
3
Odds Ratio (95%CI)

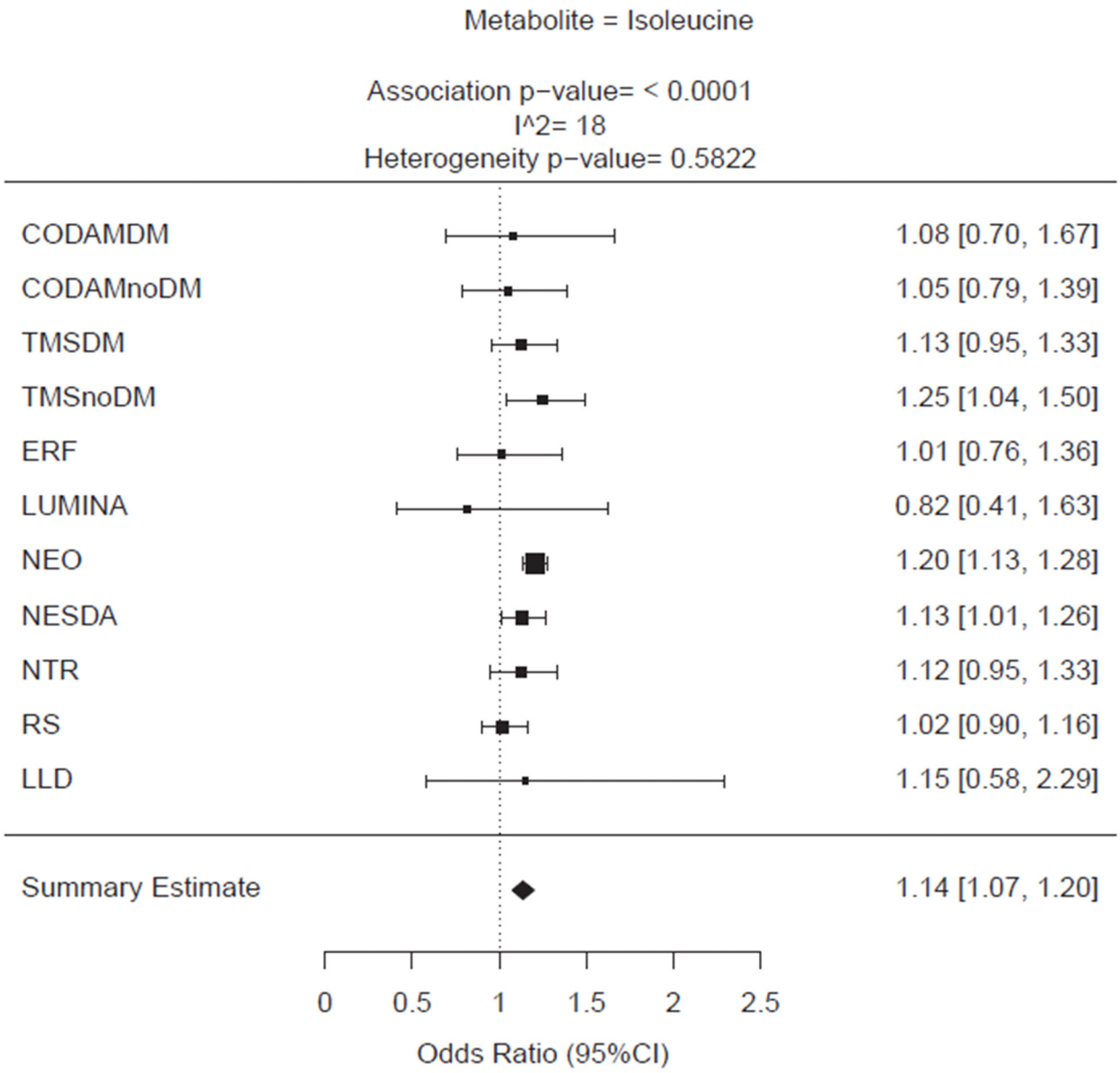

Metabolite = Isoleucine
Association p−value= < 0.0001
I^2= 18
Heterogeneity p−value= 0.5822
CODAMDM
CODAMnoDM
TMSDM
TMSnoDM
ERF
LUMINA
NEO
NESDA
NTR
RS
LLD
1.08 [0.70, 1.67]
1.05 [0.79, 1.39]
1.13 [0.95, 1.33]
1.25 [1.04, 1.50]
1.01 [0.76, 1.36]
0.82 [0.41, 1.63]
1.20 [1.13, 1.28]
1.13 [1.01, 1.26]
1.12 [0.95, 1.33]
1.02 [0.90, 1.16]
1.15 [0.58, 2.29]
Summary Estimate
1.14 [1.07, 1.20]
0
0.5
1
1.5
2
2.5
Odds Ratio (95%CI)

Figure S2. Pooled odds ratio and 95% confidence intervals for the association of the 81 metabolite ratios with depression

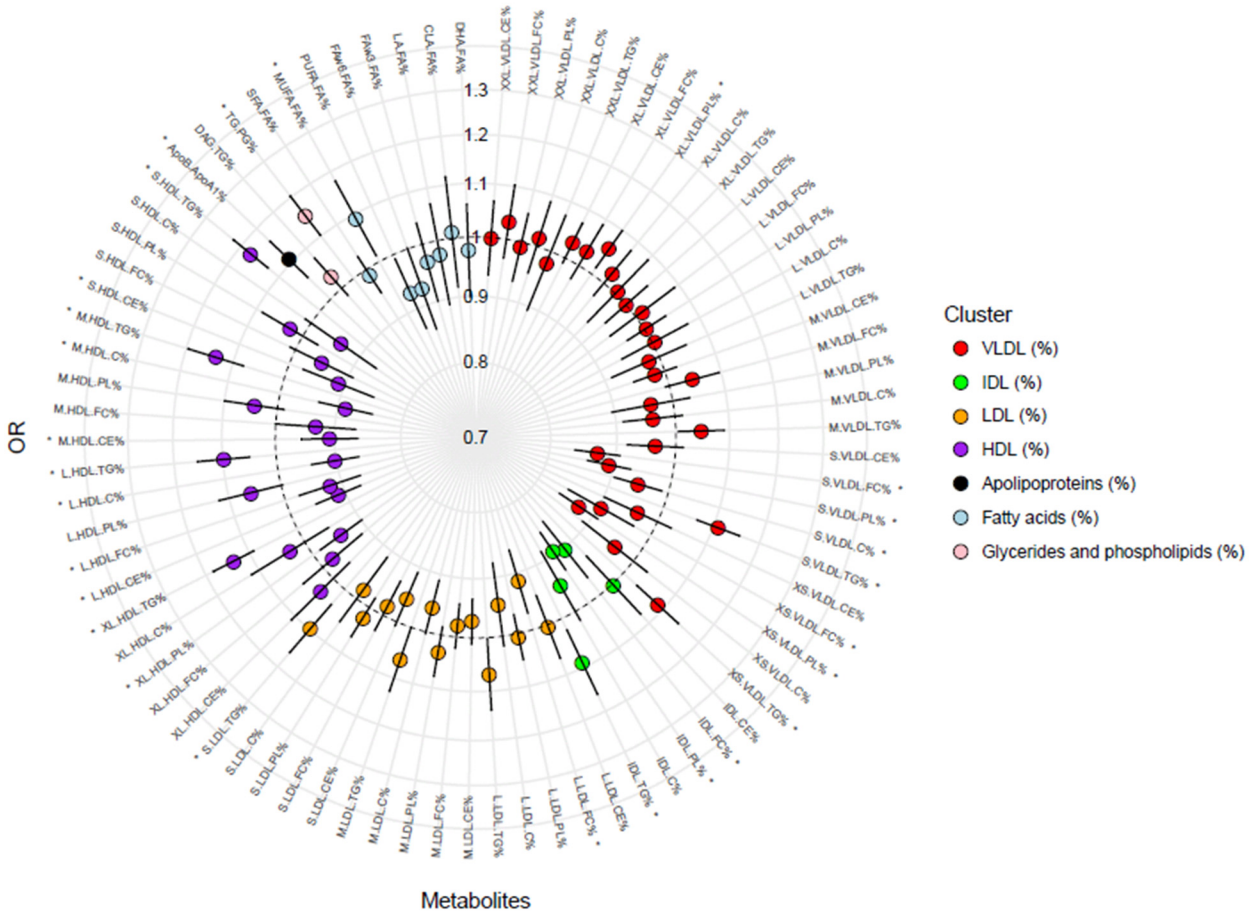
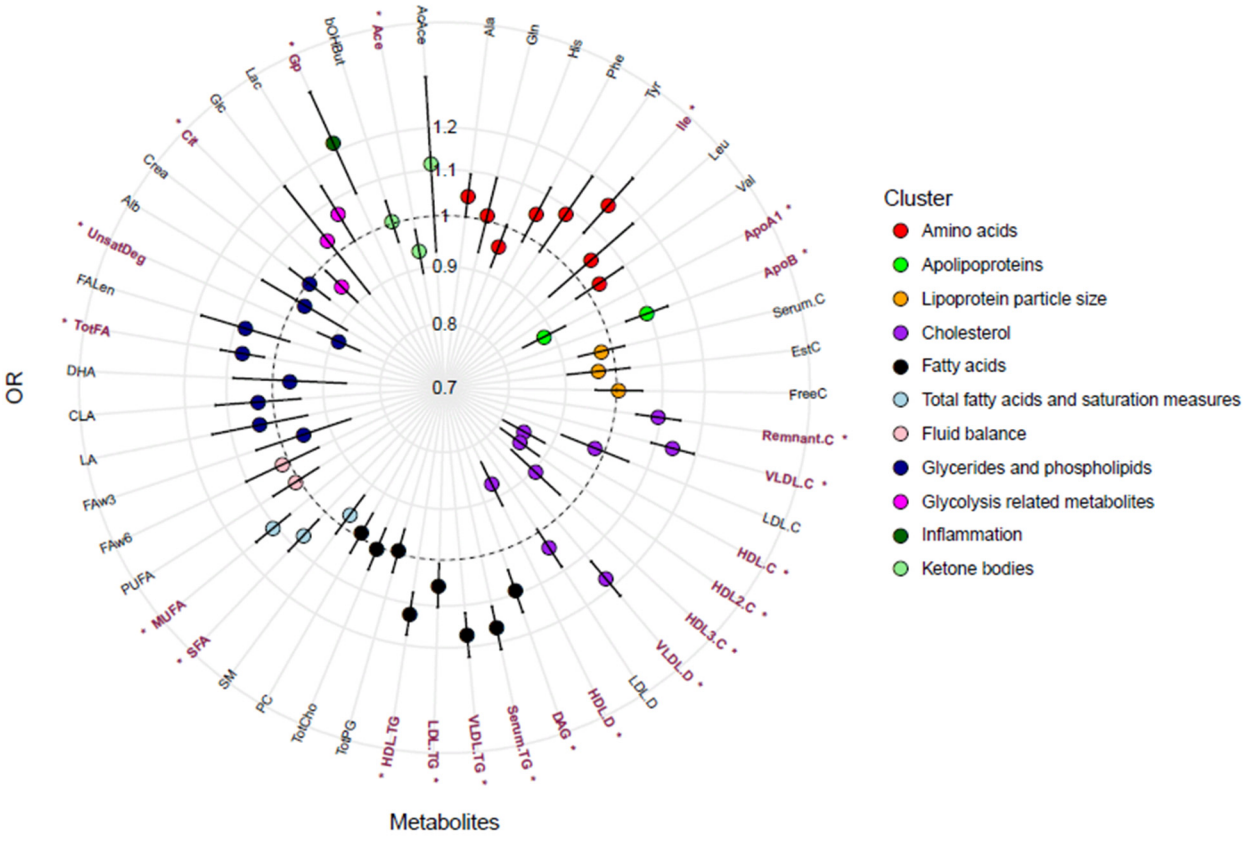


Figure S3. Pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with current depression[a]



[a] Nineteen of the 22 significant metabolites were previously identified with the broader defined depression. In addition to these 19 metabolites, saturated fatty acid level was related to increased odds of current depression, whereas degree of unsaturation and citrate level were associated to a decreased odds of current depression. Tyrosine, by contrast, was associated with overall depression, but not with the more restricted current depression.

Figure S4. Pooled odds ratio and 95% confidence intervals for the association of the 98 lipid measures of lipoprotein subclasses with current depression

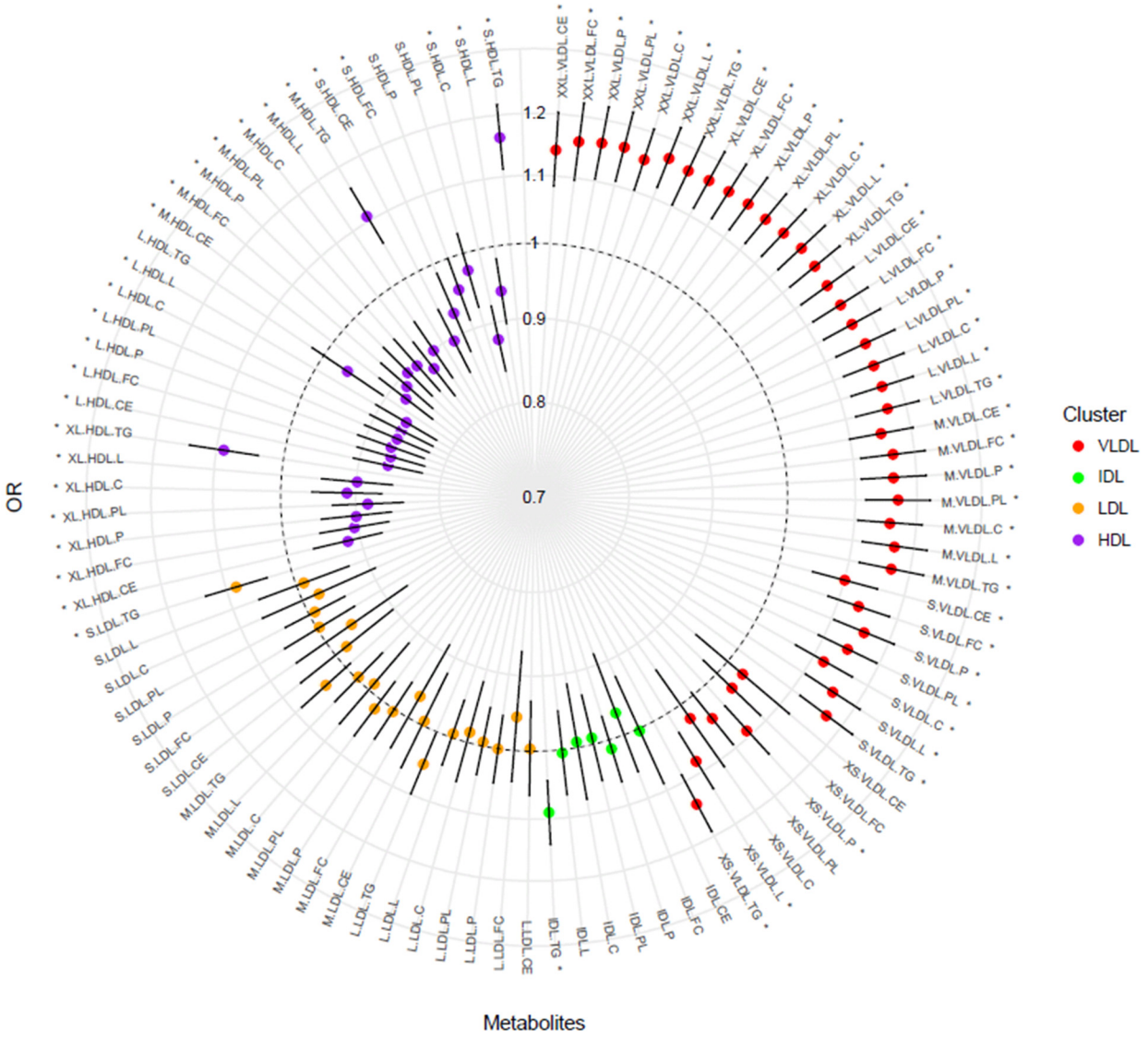


Figure S5. Pooled odds ratio and 95% confidence intervals for the association of the 81 metabolite ratios with current depression

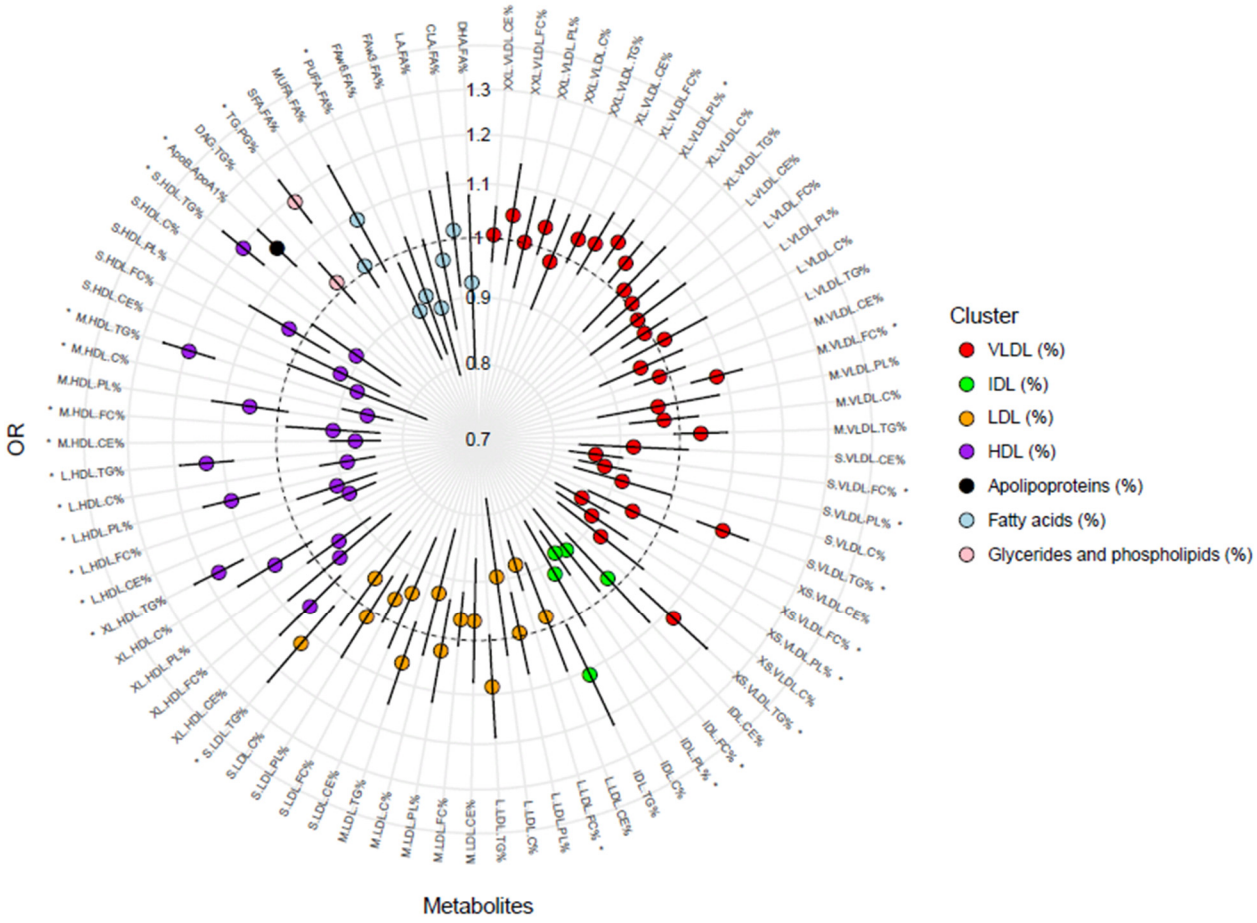


Figure S6. Pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with depression after excluding antidepressant users

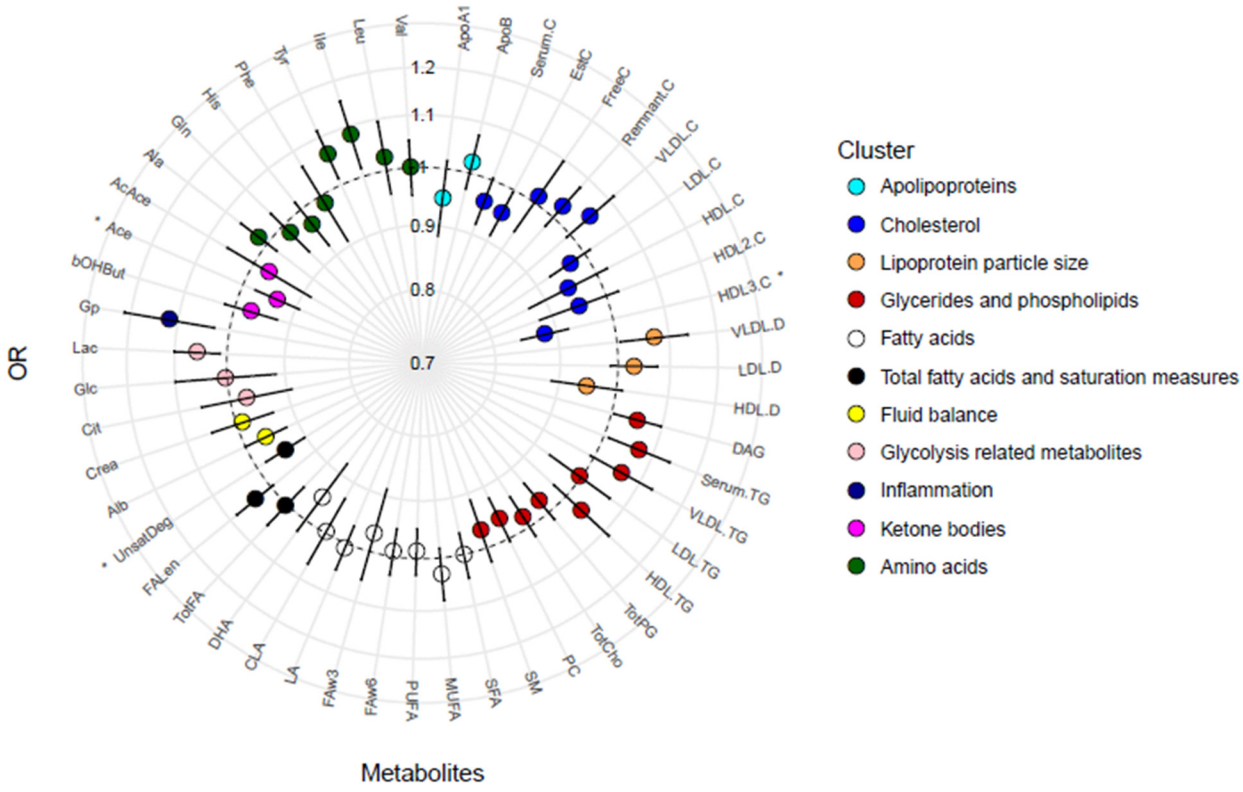


Figure S7. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids and various low-molecular-weight metabolites and depression stratified by diagnosis vs. self-reported depression subgroups

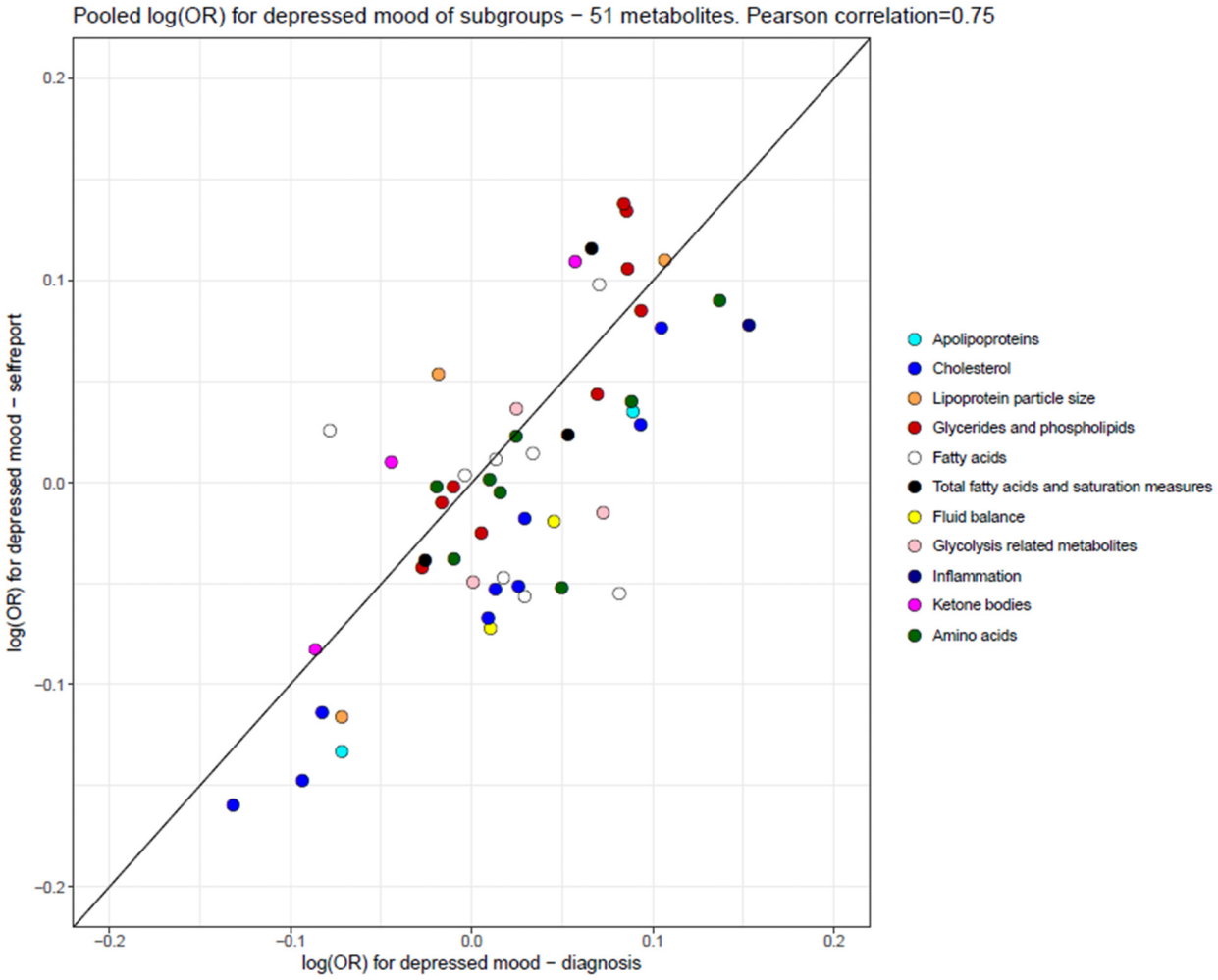


Figure S8. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by men vs. women subgroups

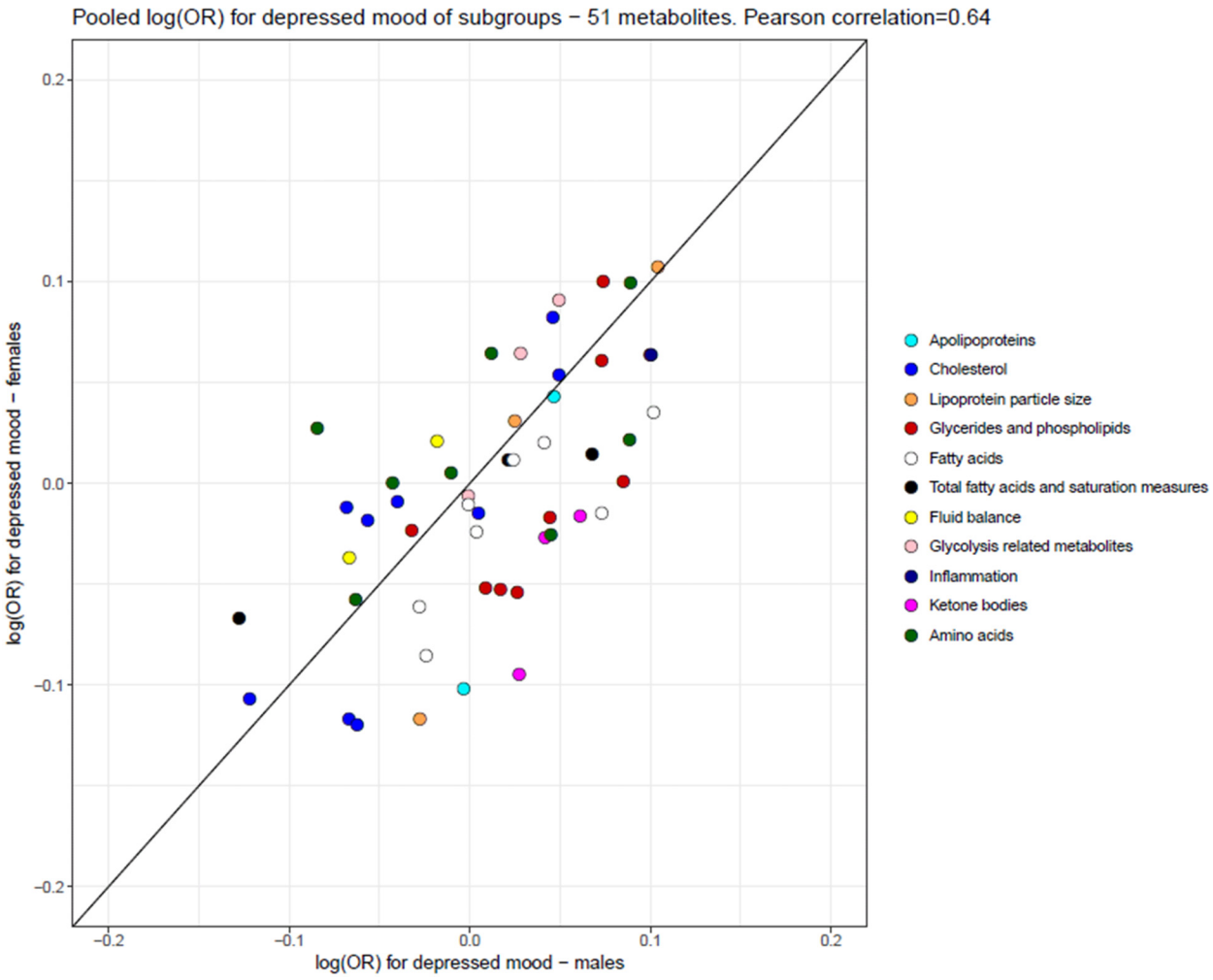


Figure S9. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by age<50 vs. age>=50y subgroups

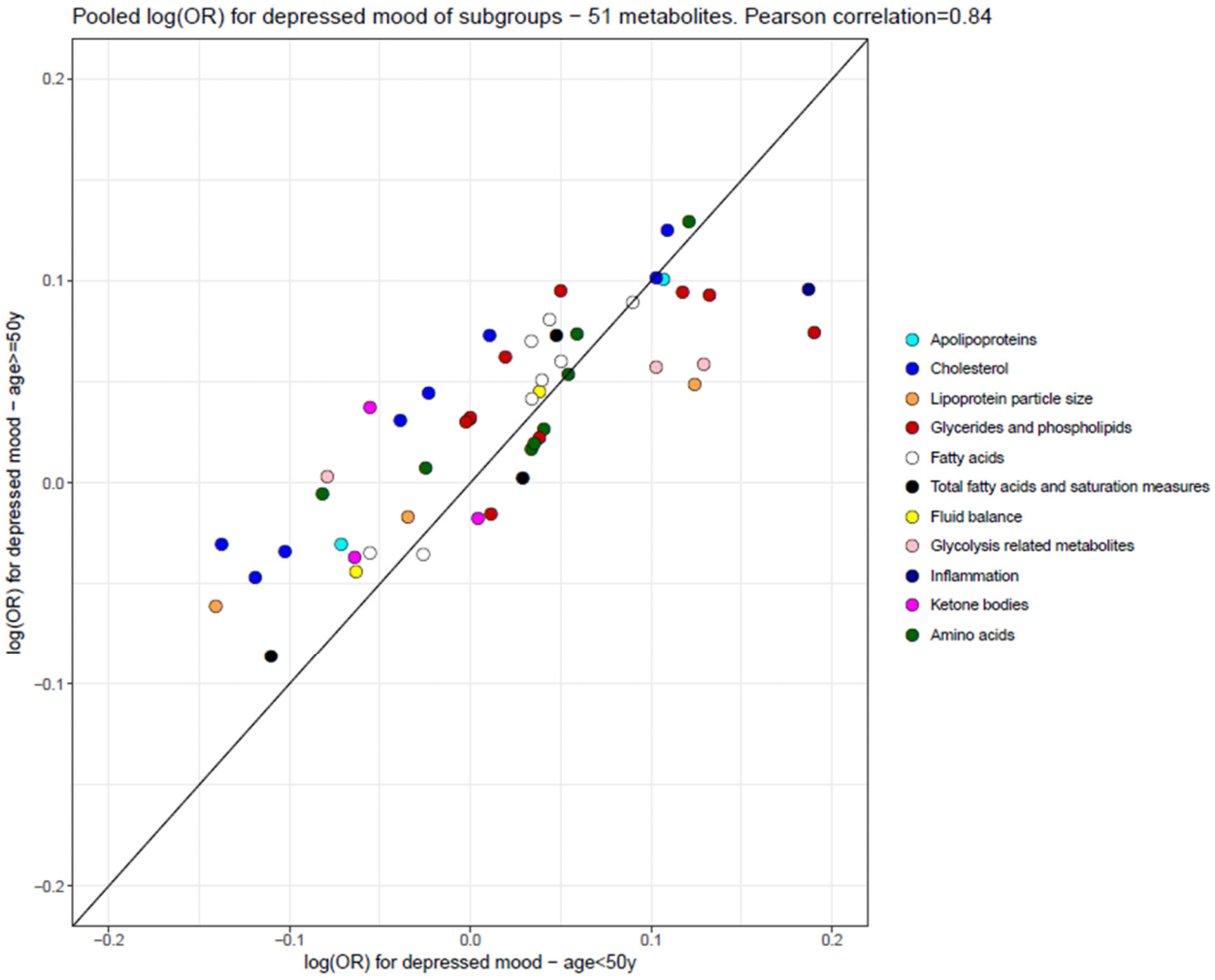


Figure S10. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI<25 vs. BMI 25-29.9

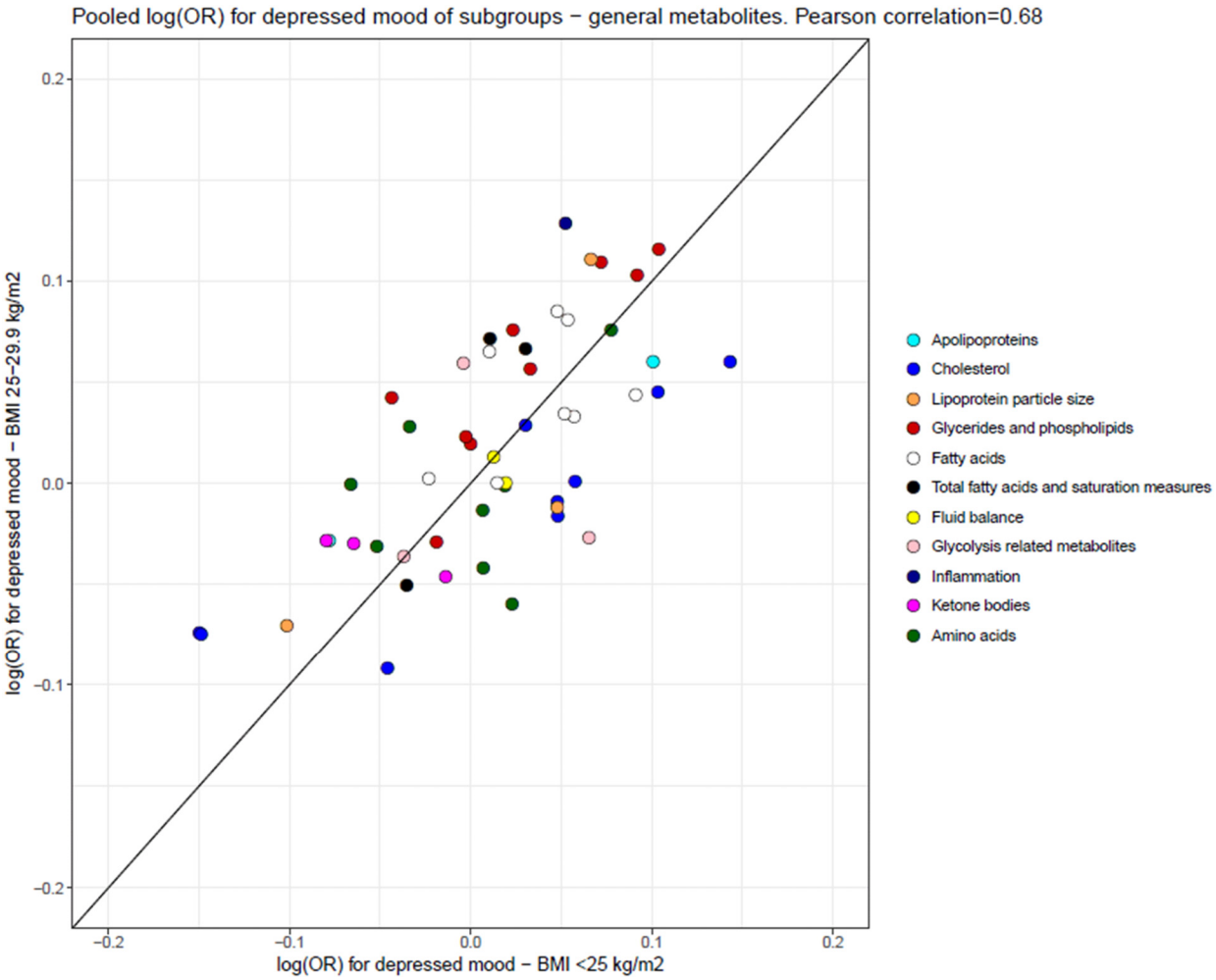


Figure S11. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI<25 vs. BMI>=30

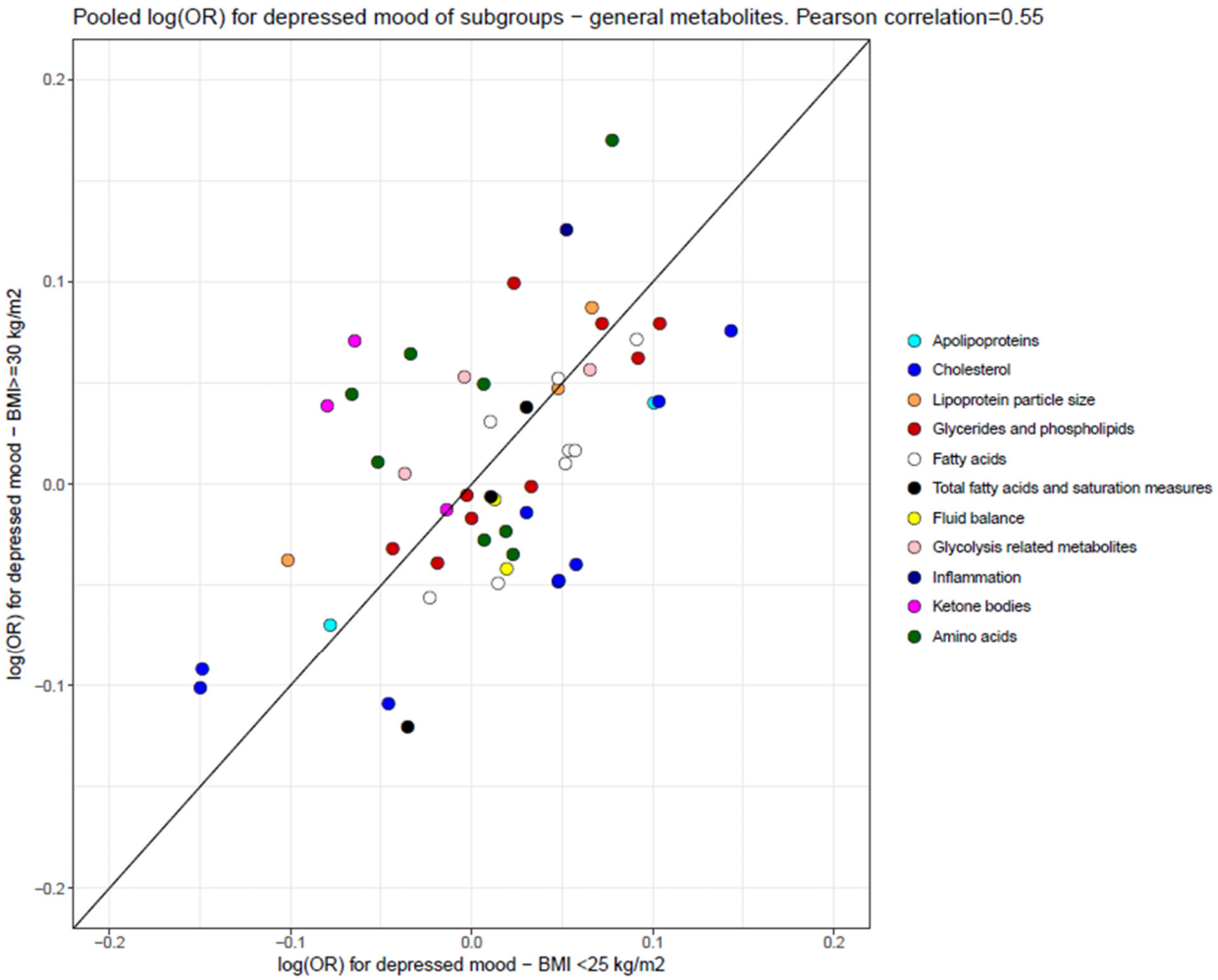


Figure S12. Plot of log(OR) from the pooled associations between the 51 lipids, fatty acids, and various low-molecular-weight metabolites and depression stratified by BMI 25-29.9 vs. BMI>=30

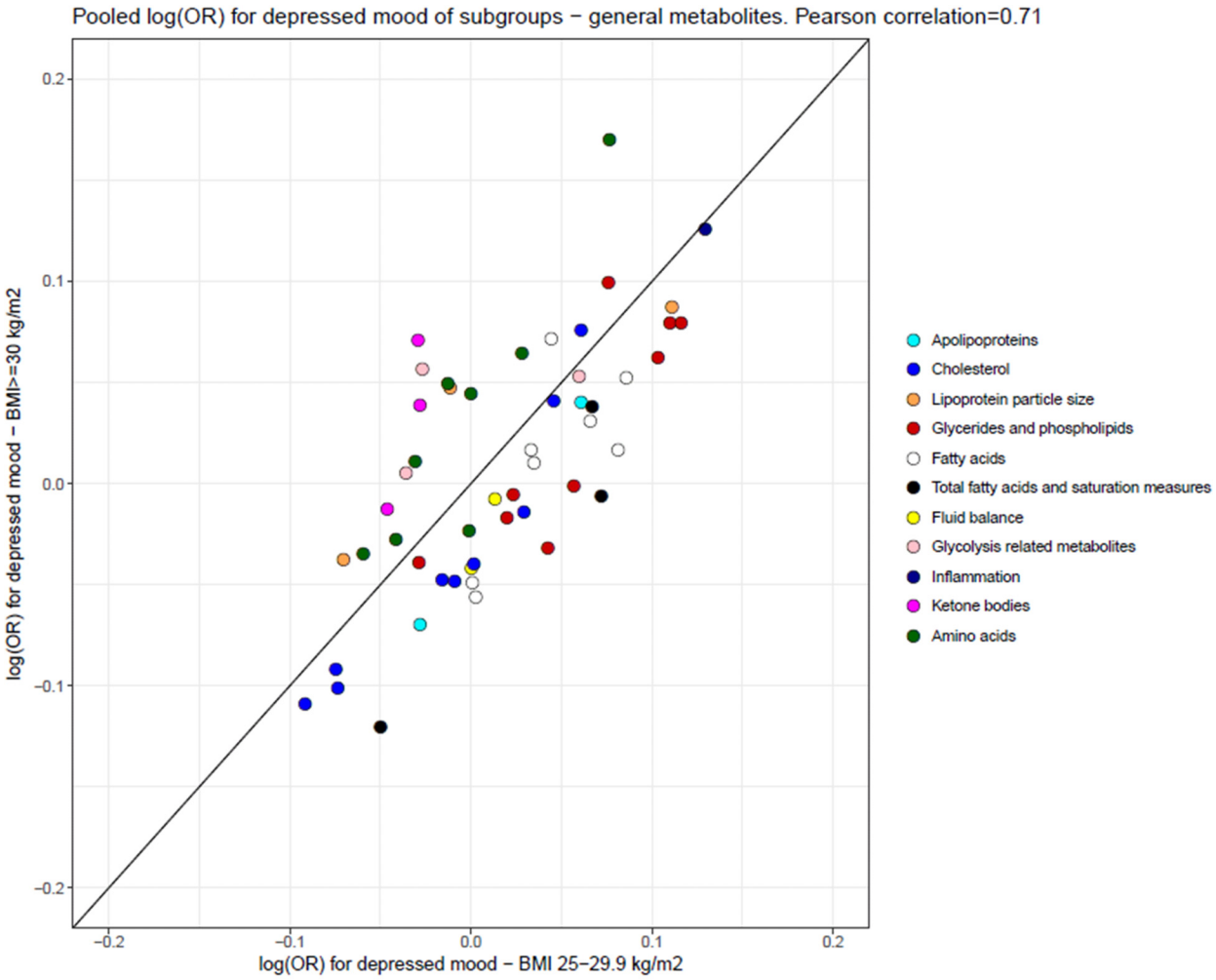


Table S3. Overview of the 21 lipids, fatty acids and various low-molecular-weight metabolites that are significantly related to depression in the pooled analysis at FDR q<0.05 after exclusion of datasets including patients with diabetes

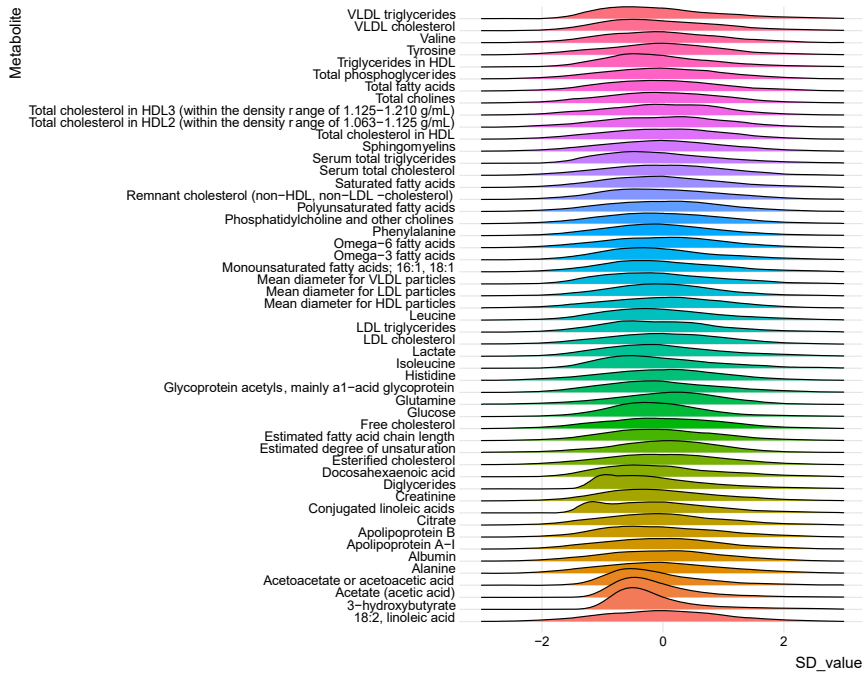
Metabolite	Pooled OR	p-value	FDR q-value
Apolipoproteins			
Apolipoprotein A1	0.89	6.33×10^{-8}	7.67×10^{-7}
Apolipoprotein B	1.07	4.61×10^{-3}	1.18×10^{-2}
Cholesterol			
Remnant cholesterol	1.06	2.19×10^{-2}	4.67×10^{-2}
VLDL cholesterol	1.09	2.71×10^{-3}	7.32×10^{-3}
HDL cholesterol	0.85	9.59×10^{-13}	1.10×10^{-10}
HDL_2 cholesterol	0.88	8.52×10^{-6}	5.44×10^{-5}
HDL_3 cholesterol	0.89	5.49×10^{-7}	5.05×10^{-6}
Mean diameter of VLDL	1.12	3.04×10^{-5}	1.63×10^{-4}
Mean diameter of HDL	0.90	5.21×10^{-5}	2.44×10^{-4}
Di- and triglycerides			
Diglycerides	1.09	1.36×10^{-4}	5.38×10^{-4}
Serum total TG	1.10	8.70×10^{-4}	1.15×10^{-4}
VLDL TG	1.10	1.76×10^{-3}	5.00×10^{-3}
LDL TG	1.04	3.64×10^{-2}	7.42×10^{-2}
HDL TG	1.07	2.09×10^{-2}	4.49×10^{-2}
Fatty acids			
Mono Unsaturated FA	1.09	8.91×10^{-5}	3.73×10^{-4}
Total FA	1.04	8.04×10^{-2}	8.04×10^{-1}
Estimated FA chain length	1.11	2.63×10^{-2}	5.44×10^{-2}
Inflammation			
Glycoprotein acetyls	1.12	1.39×10^{-2}	3.10×10^{-2}
Ketone bodies			
Acetate	0.90	1.26×10^{-3}	3.76×10^{-2}
Amino acids			
Tyrosine	1.05	6.36×10^{-2}	1.23×10^{-1}
Isoleucine	1.13	7.31×10^{-5}	3.17×10^{-4}

Data of 914 subjects with diabetes from CODAM-DM and TMS-DM datasets were removed

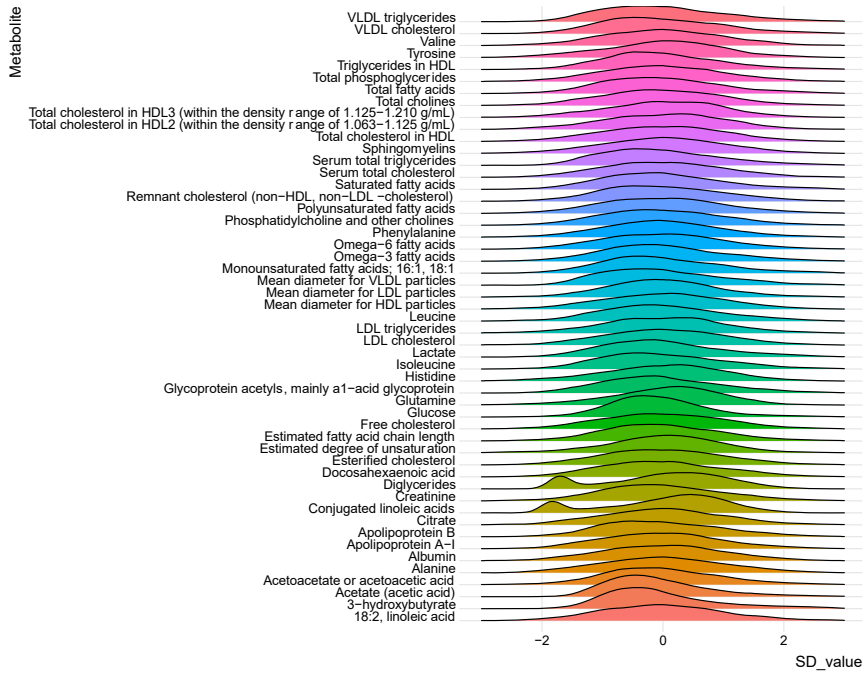
Adjusted for gender, age, smoking, lipid modifying drugs, fasting status.

Figure S13. Standardized distribution of the (log)values of the metabolites after different data transformations.

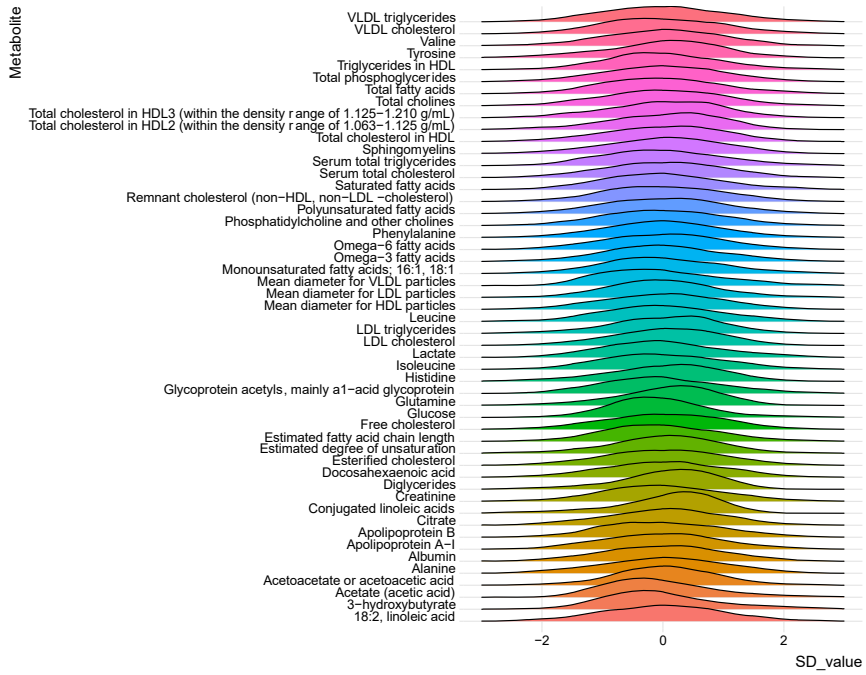
A) Adding a constant of 1



B) Adding the 10th percentile of the distribution (excluding 0 values)



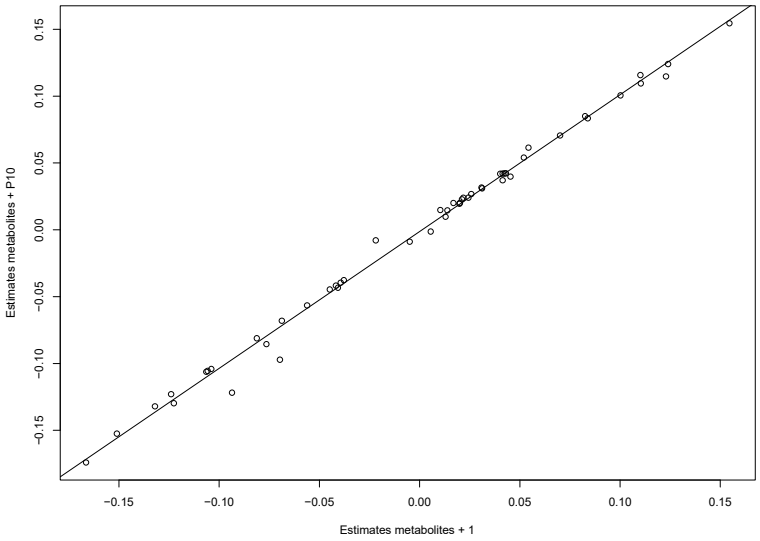
C) Excluding all zero values



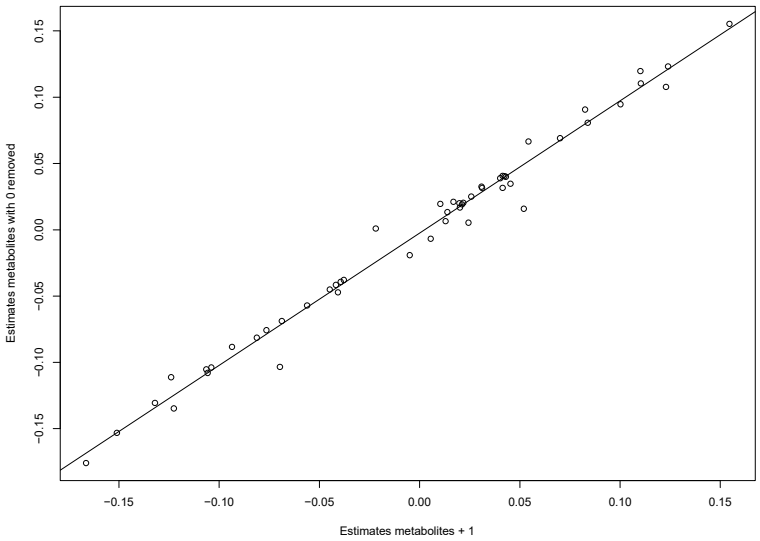
Data from the NESDA cohort (N=2,509)

Figure S14. Comparisons between estimates of the metabolites-depression association obtained after different data transformations.

1) Transformations A vs B



2) Transformations A vs C



Data from the NESDA cohort (N=2,509)

Estimates from logistic regression adjusted for gender, age, smoking, lipid modifying drugs, fasting status.

Transformations: A) adding 1; B) Adding the 10^{th} percentile of the distribution (excluding 0 values); C) excluding all 0 values

BBMRI-NL Metabolomics Consortium

Cohort collection and sample management group:

M. Beekman[1], H.E.D. Suchiman[1], J. Deelen[1,6], N. Amin[7], J.W. Beulens[8,9], J.A. van der Bom[10-13], N. Bomer[14], A. Demirkan[7], J.A. van Hilten[15], J.M.T.A. Meessen[16], R. Pool[17], M.H. Moed[1], J. Fu[18,19], G.L.J. Onderwater[20], F. Rutters[8], C. So-Osman[10], W.M. van der Flier[8,21], A.A.W.A. van der Heijden[22], A. van der Spek[7], F.W. Asselbergs[23], E. Boersma[24], P.M. Elders[25,26], J.M. Geleijnse[27], M.A. Ikram[7,29,30], M. Kloppenburg[13,31], I. Meulenbelt[1], S.P. Mooijaart[4], R.G.H.H. Nelissen[32], M.G. Netea[33,34], B.W.J.H. Penninx[26,35], C.D.A. Stehouwer[36,37], C.E. Teunissen[38], G.M. Terwindt[20], L.M. 't Hart[1,8,26,39,40], A.M.J.M. van den Maagdenberg[41], P. van der Harst[14], I.C.C. van der Horst[42], C.J.H. van der Kallen[36,37], M.M.J. van Greevenbroek[36,37], W.E. van Spil[43], C. Wijmenga[18], A.H. Zwinderman[44], A. Zhernikova[18], J.W. Jukema[5], N. Sattar[45]

Database & Catalogue: J.J.H. Barkey Wolf[1], M. Beekman[1], D. Cats[1], H. Mei[1,46], M. Slofstra[18], M. Swertz[18]

Steering committee: D.I. Boomsma[26,47], C.M. van Duijn[7], P.E. Slagboom[1]

Affiliations

1. Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands
2. Leiden Computational Biology Center, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Pattern Recognition and Bioinformatics, Delft University of Technology, Delft, The Netherlands
4. Department of Internal Medicine, Division of Gerontology and Geriatrics, Leiden University Medical Centre, Leiden, The Netherlands
5. Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands
6. Max Planck Institute for Biology of Ageing, Cologne, Germany
7. Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, The Netherlands
8. Department of Epidemiology and Biostatistics, Amsterdam University Medical Center, Amsterdam, the Netherlands
9. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands.
10. Centre for Clinical Transfusion Research, Sanquin Research, Leiden, The Netherlands.

11. Jon J van Rood Centre for Clinical Transfusion Research, Leiden University Medical Centre, Leiden, The Netherlands.
12. TIAS, Tilburg University, Tilburg, The Netherlands.
13. Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands.
14. Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.
15. Center for Clinical Transfusion Research, Sanquin Research, Leiden, the Netherlands.
16. Department of Orthopedics, Leiden University Medical Centre, Leiden, The Netherlands.
17. Department of Biological Psychology, Vrije Universiteit, Amsterdam, the Netherlands.
18. Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
19. Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
20. Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands.
21. Department of Neurology and Alzheimer Center, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands.
22. Department of General Practice, The EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands.
23. Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands.
24. Thorax centre, Erasmus Medical Centre, Rotterdam, the Netherlands
25. Department of General Practice and Elderly Care Medicine, VU University Medical Center, Amsterdam, The Netherlands
26. Amsterdam Public Health research institute, VU University Medical Center, Amsterdam, The Netherlands
27. Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands

28. Department of Epidemiology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
29. Department of Radiology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
30. Department of Neurology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
31. Department of Rheumatology, Leiden University Medical Center, The Netherlands
32. Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
33. Department of Internal Medicine, Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, Netherlands
34. Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany
35. Department of Psychiatry, VU University Medical Center, Amsterdam, The Netherlands.
36. Department of Internal Medicine, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands
37. School for Cardiovascular Diseases (CARIM), Maastricht University, Maastricht, the Netherlands
38. Neurochemistry Laboratory, Clinical Chemistry Department, Amsterdam University Medical Center, Amsterdam Neuroscience, the Netherlands
39. Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the Netherlands
40. Department of General practice, Amsterdam University Medical Center, Amsterdam, the Netherlands
41. Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
42. Department of Critical Care, University Medical Center Groningen, Groningen, The Netherlands.
43. UMC Utrecht, Department of Rheumatology & Clinical Immunology, Utrecht, The Netherlands
44. Department of Clinical Epidemiology, Biostatistics, and Bioinformatics, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands
45. Institute of Cardiovascular and Medical Sciences, Cardiovascular Research Centre, University of Glasgow, Glasgow, UK
46. Sequencing Analysis Support Core, Leiden University Medical Center, Leiden, The Netherlands

47. Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands

Supplemental References

1. van Greevenbroek MMJ, Jacobs M, van der Kallen CJH, Vermeulen VMMJ, Jansen EHJM, Schalkwijk CG, *et al.* (2011): The cross-sectional association between insulin resistance and circulating complement C3 is partly explained by plasma alanine aminotransferase, independent of central obesity and general inflammation (the CODAM study). *Eur J Clin Invest*. 41: 372–379.

2. Van Dam RM, Boer JMA, Feskens EJM, Seidell JC (2001): Parental history off diabetes modifies the association between abdominal adiposity and hyperglycemia. *Diabetes Care*. 24: 1454–1459.

3. Radloff LS (1977): A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas*. 1: 385–401.

4. Lewinsohn PM, Seeley JR, Roberts RE, Allen NB (1997): Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychol Aging*. 12: 277–87.

5. Schram MT, Sep SJS, Van Der Kallen CJ, Dagnelie PC, Koster A, Schaper N, *et al.* (2014): The Maastricht Study: An extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. *Eur J Epidemiol*. 29: 439–451.

6. Sheehan D V, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, *et al.* (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 59 Suppl 2: 22-33;quiz 34-57.

7. Sayed-Tabatabaei FA, Van Rijn MJE, Schut AFC, Aulchenko YS, Croes EA, Zillikens MC, *et al.* (2005): Heritability of the function and structure of the arterial wall: Findings of the Erasmus Rucphen Family (ERF) study. *Stroke*. 36: 2351–2356.

8. Zigmond AS, Snaith RP (1983): The hospital anxiety and depression scale. *Acta Psychiatr Scandinivica*. 67: 361–370.

9. van Oosterhout W, Weller C, Stam A, Bakels F, Stijnen T, Ferrari M, Terwindt G (2011): Validation of the web-based LUMINA questionnaire for recruiting large cohorts of migraineurs. *Cephalalgia*. 31: 1359–1367.

10. Rush A, Giles DE, Schlesser MA, Fulton CL, Weissenburger J, Burns C (1986): The inventory for depressive symptomatology (IDS): Preliminary findings. *Psychiatry Res*. 18: 65–87.

11. Rush A, Gullion C, Basco M, Jarrett R, Trivedi M (1996): The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychol Med*. 26: 477–86.

12. Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, *et al.* (2008): The Netherlands Study of Depression and Anxiety (NESDA): Rationale, objectives and methods. *Int J Methods Psychiatr Res*. 17: 121–140.

13. Wittchen HU (1994): Reliability and validity studies of the WHO--Composite International Diagnostic Interview (CIDI): a critical review. *J Psychiatr Res*. 28: 57–84.

14. Boomsma DI, Geus EJC de, Vink JM, Stubbe JH, Distel MA, Hottenga J-J, *et al.* (2006): Netherlands Twin Register: From Twins to Twin Families. *Twin Res Hum Genet*. 9: 849–857.

15. Hofman A, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebure A, *et al.* (2015): The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol*. 30: 661–708.

16. Scholtens S, Smidt N, Swertz MA, Bakker SJL, Dotinga A, Vonk JM, *et al.* (2015): Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 44: 1172–1180.

17. Tigchelaar EF, Bonder MJ, Jankipersadsing SA, Fu J, Wijmenga C, Zhernakova A (2016): Gut microbiota composition associated with stool consistency. *Gut*. 65.

18. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, *et al.* (2016): Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science (80-)*. 352: 565–569.

19. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M (2015): Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet*. 8: 192–206.

20. Würtz P, Mäkinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, *et al.* (2012): Metabolic signatures of insulin resistance in 7,098 young adults. *Diabetes*. 61: 1372–1380.

21. Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, *et al.* (2015): Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. *Circulation*. 131: 774–785.

22. Fischer K, Kettunen J, Würtz P, Haller T, Havulinna AS, Kangas AJ, *et al.* (2014): Biomarker Profiling by Nuclear Magnetic Resonance Spectroscopy for the Prediction of All-Cause Mortality: An Observational Study of 17,345 Persons. *PLoS Med*. 11. doi: 10.1371/journal.pmed.1001606.

23. Würtz P, Cook S, Wang Q, Tiainen M, Tynkkynen T, Kangas AJ, *et al.* (2016): Metabolic profiling of alcohol consumption in 9778 young adults. *Int J Epidemiol*. 45: 1493–1506.

24. Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M (2017): Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am J Epidemiol*. 186: 1084–1096.

25. Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, *et al.* (2009): High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst*. . doi: 10.1039/b910205a.

26. Tynkkynen J, Chouraki V, van der Lee SJ, Hernesniemi J, Yang Q, Li S, *et al.* (2018): Association of branched-chain amino acids and other circulating metabolites with risk of incident dementia and Alzheimer's disease: A prospective study in eight cohorts. *Alzheimer's Dement*. 14: 723–733.

27. Holmes M V., Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, *et al.* (2018): Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *J Am Coll Cardiol*. 71: 620–632.

28. Ritchie SC, Würtz P, Nath AP, Abraham G, Havulinna AS, Fearnley LG, *et al.* (2015): The Biomarker

GlycA is Associated with Chronic Inflammation and Predicts Long-Term Risk of Severe Infection. *Cell Syst*. 1: 293–301.

29. Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikäinen LP, *et al.* (2012): Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet*. 44: 269–276.

30. Kettunen J, Demirkan A, Würtz P, Draisma HHMM, Haller T, Rawal R, *et al.* (2016): Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun*. 7: 11122.

31. Tukiainen T, Kettunen J, Kangas AJ, Lyytik̈inen LP, Soininen P, Sarin AP, *et al.* (2012): Detailed metabolic and genetic characterization reveals new associations for 30 known lipid loci. *Hum Mol Genet*. 21: 1444–1455.

32. Milaneschi Y, Penninx BW, Simmons WK, van Rossum EFC (2018): Depression and obesity: evidence of shared biological mechanisms. *Mol Psychiatry*. .

33. Onderwater GLJ, Ligthart L, Bot M, Demirkan A, Fu J, Kallen CJH van der, *et al.* (2019): Large-scale plasma metabolome analysis reveals alterations in HDL metabolism in migraine. *Neurology*. 0: 10.1212/WNL.0000000000007313.