Metabolomics and Lipidomics for Studying Metabolic Syndrome: Insights into Cardiovascular Diseases, Type 1 & 2 Diabetes, and Metabolic Dysfunction-Associated Steatotic Liver Disease

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Supplementary Materials

Table S1. Metabolomics and lipidomics cohort studies focused on cardiovascular disease

Table S2. Metabolomics and lipidomics cohort studies focused on type 1 and type 2 diabetes

Table S3. Metabolomics and lipidomics cohort studies focused on metabolic dysfunction-associated steatotic liver disease

Table S1. Metabolomics and lipidomics cohort studies focused on cardiovascular disease

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Discovery cohort: n = 4,824 (27.8% female) Replication cohort: n = 1,716 (56.3% female)	Participants with 15.8 years follow-up	Serum	NMR	41	Isoleucine Leucine Phenylalanine Glycerol Cholesterol Total lipid concentrations, Glycerides and other Phospholipids, Fatty acids, Fatty acids ratios — see the original paper.	• Association with adherence to dietary recommendations provided by the Alternative Healthy Eating Index	[1]
European cohort: n = 352 USA cohort: n = 1,777	European participants (100% Caucasian) with either abdominal aortic aneurysm or sub-aneurysmal aortic dilations, and healthy non-aneurysm subjects US participants (96% Caucasian) with abdominal aortic diameter of 3.0 cm or greater, and subjects with history of dilated aorta with measurements of abdominal aortic diameter less than 3 cm or no prior aortic aneurysm, and no MI, stroke or death over the following 3 years	Plasma	LC-MS/MS	3	Choline Trimethylamine *N*-oxide Trimethylamine	• Association of elevated TMAO with increased abdominal aortic aneurysm incidence	[2]
Low-risk cohort: n = 620 Borderline-risk cohort: n = 110 Intermediate-risk cohort: n = 225 Highrisk cohort: n = 147 (53.3% female)	Participants with LDL levels less than 190 mg/dl and no pre-existing coronary artery disease or myocardial infarction	Plasma	LC-MS/MS	50	Alanine Arginine Aspartic acid CAR 4:0-DC CAR 8:1 CAR 16:0-OH Citrulline Glutamic acid Glutamine Glycine Histidine Phenylalanine Threonine Tryptophan	• Association with the 10-year ASCVD risk score • Identification of metabolic pathways associated with the development of 10-year ASCVD events	[3]
EPIC-Potsdam Study cohort: Common reference subcohort: n = 1262 T2D subcohort: n = 1886 (775 incident cases) CVD subcohort: n = 1671 (551 incident cases) **DIVAS study cohort:** CVD risk subcohort: n = 113 (on 3 different isoenergic diets)	General population Patients with estimated moderate CVD risk	Plasma	DMS-MS/MS	282	CE 20:3 DG 16:0 DG 18:0 FA 15:0 FA 20:4 LPC 18:2 MG 15:0 MG 20:4 PC 20:3 PE 20:3 TG 16:0 TG 18:0 TG 18:2 TG 18:3 TG 22:1	• Association with cardiometabolic disease risk and T2D risk • Dietary fat intervention as a potential tool for primary disease prevention	[4]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Discovery cohort: n = 1,162 (36.3% female) Validation cohorts: n = 2,331 (US, 33.2% female), n = 832 (European, 29.9% female)	Stable participants undergoing elective diagnostic cardiac evaluation	Plasma	LC-MS/MS	5	N^1-Methyl-2-pyridone-5-carboxamide N^1-Methyl-4-pyridone-3-carboxamide Phenylacetylglutamine Trimethylamine *N*-oxide Trimethyllysine	• Association of terminal breakdown products of excess niacin with residual CVD risk	[5]
Phase I: Discovery cohort: n = 3,613 Validation cohorts: n = 121,733 **Phase II:** n = 118,120	UK Biobank participants have undergone a wide range of physical measures, provided information on their lifestyle and medical history (follow-up)	Plasma	NMR	111	Multiple markers – see the original paper.	• Association with a healthy lifestyle • Association of healthy lifestyle-associated metabolites with coronary artery disease (CAD)	[6]
Discovery cohort: n = 1,028 Validation cohort: n = 1,670	Discovery cohort: Participants free of coronary heart disease (10 years follow-up)	Plasma	LC-MS/MS	32	LPC 18:1 LPC 18:2 MG 18:2 SM d28:1	• Association of MG 18:2 with coronary heart disease • Association of LPCs with body mass index, C-reactive protein and with less evidence of subclinical CVD	[7]
Discovery cohort: n = 1,833 (57% female) Validation cohorts: n = 1,522 Low walnut intake subcohort: n = 691 High walnut intake subcohort: n = 467	Participants at high cardiovascular risk	Plasma	LC-MS	385	4-Hydroxy-3-methylacetophenone Cyclohexylamine Guanine Isocitric acid *N*-Acetylaspartic acid Piperine Serine Sorbitol Succinic acid Bilirubin Biliverdin CAR 10:2 LPC 14:0 LPC 16:1 MG 22:1 PC 36:4 PE 36:5 PS 40:6 TG 54:6	• Association of walnut consumption with a lower risk of incident T2D and CVD in a Mediterranean population at high cardiovascular risk	[8]
Study cohort: n = 1,057	Participants with symptomatic coronary artery disease	Blood-platelets	LC-MS/MS	767	CAR 10:0 CAR 14:0 CAR 14:1 CAR 16:0 CAR 16:1 FA 18:1 FA 18:2 FA 18:2;2O LPE 18:1|LPE 0:0/18:1 LPE 18:1|LPE 18:1/0:0 LPE 18:2|LPE 0:0/18:2 LPE 18:2|LPE 18:2/0:0 LPE 20:1|LPE 20:1/0:0 LPE 20:3|LPE 0:0/20:3 LPE 20:3|LPE 20:3/0:0 LPE 20:4 LPE 20:5 LPE 22:4|LPE 0:0/22:4 LPE 22:4|LPE 22:4/0:0 LPE 22:5	• Association of adverse cardiovascular events with alterations in the platelet lipidome	[9]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
					LPE 22:6 LPS 18:1|LPS 0:0/18:1 PC 34:2;O PE 34:3|PE 16:1_18:2;O PI 36:4|PI 16:0_20:4 PI 38:5|PI 18:1_20:4 TG 48:1|TG 14:0_16:0_18:1 TG 48:2|TG 16:0_14:1_18:1		
Study cohort: n = 1,021 (48.3% female)	Participants with T2D and were followed up for CVD over the subsequent 10 years	Serum	NMR	228	3-Hydroxybutyric acid Acetic acid Creatinine Glycine Lactic acid Leucine Phenylalanine	• Association with 10-year cardiovascular risk in people with type 2 diabetes • Metabolite-based risk score created	[10]
Malmo Diet and Cancer-Cardiovascular cohort: n = 4,067	General population followed up to 23 years and stratified into risk groups	Plasma	DI-MS/MS	184	*Sum of lipid subclasses:* Ceramide Cholesteryl ester Cholesterol Diacylglycerol Ether-phosphatidylcholine Ether-phosphatidylethanolamine Lysophosphatidylcholine Lysophosphatidylethanolamine Phosphatidylcholine Phosphatidylethanolamine Phosphatidylinositol	• Possible identification of lipidomic risk before disease incidence (CVD and T2D)	[11]
Discovery cohort 1: n = 99 Discovery cohort 2: n = 1,162 Validation cohort: n = 2,140	Sequential stable subjects without evidence of acute coronary syndrome undergoing elective diagnostic coronary angiography for evaluation of CAD with longitudinal (3–5 years) follow-up	Plasma	HILIC-MS/MS LC-MS/MS		Trimethyllysine Trimethylamine *N*-oxide	• Association with CVD risks	[12]
Study cohort: n = 2,278 (50% female)	Participants were followed up for CVD incident (almost 10 years)	Plasma	LC-MS/MS	790 (37)	Dimethylglycine *N*-Acetylmethionine (top findings)	• Association with CVD risks	[13]
Study cohort: n = 5,072	Participants with diabetes	Plasma	NMR	44	3-Hydroxybutyric acid Acetic acid Acetoacetatic acid Acetone Alanine Citric acid Creatinine Glucose Glutamine Glycine Histidine Isoleucine Lactic acid Leucine Phenylalanine Pyruvic acid Tyrosine Valine	• Association of multiple healthy lifestyle factors with improved circulating metabolites from different pathways	[14]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Discovery cohort: n = 1,833 (57.6% female) Validation subcohort: n = 1,522	Participants at high risk of CVD (1-year follow-up)	Plasma	LC-MS/MS	382	1-Methylguanine γ-Aminobutyric acid (GABA) Aminoisobutyric acid Asparagine Cortisol Creatine Cytosine Glycodeoxycholic acid Hippuric acid Homoarginine Hypoxanthine Lactic acid Lysine N^1-Acetylspermidine *N*-Acetylaspartic acid *N*-Acetylornithine *N*-Carbamoyl-β-alanine Piperine Pyroglutamic acid Sorbitol Sucrose Trimethylbenzene CAR 7:0 CAR 18:2 CAR 18:0 DG 34:3 DG 36:0 LPC 16:1 MG 22:1 PC 34:3 PC 36:4 PC 38:4 PE 32:0 PE 38:6 PE 40:7 SM d34:2|SM d18:1/16:1 TG 50:3 TG 50:4 TG 55:2 TG 56:2	• Association of legume consumption with T2D incidence, but not with CVD incidence risk	[15]
Study cohort: n_1 = 5,991; n_2 = 3,779 (38.9% female)	Participants with an 8-year follow-up	Plasma	LC-MS/MS	342	CE 24:0 LPI 18:2 PC 38:5 PC O-34:2 PC O-36:1 PC P-40:6 PE 38:6 PI 38:3 SM d42:1	• Association with future cardiovascular events and cardiovascular death	[16]
Discovery cohort: n = 1,162 Validation cohort: n = 4,000	Sequential stable subjects undergoing elective diagnostic cardiac evaluation with longitudinal (3 years) follow-up	Plasma	HILIC-MS LC-MS/MS	5 (top-ranked)	Phenylacetylglutamine	• Association with cardiovascular disease and death in humans	[17]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Discovery cohort: n = 1,149 Validation cohort: n = 3,954	Participants with preserved kidney function undergoing elective diagnostic cardiac evaluation with longitudinal follow-up (5 years)	Plasma	GC-MS LC-MS/MS	N/A	*p*-Cresol sulfate Indoxyl sulfate	• Association with CVD risk and overall mortality	[18]
Study cohort: n = 2,627	Participants were invited to attend a health examination for additional tests and collection of 8–12 h fasting blood samples (mean 12.9 years follow-up)	Plasma	HILIC-MS LC-MS/MS	79	Hex2Cer d34:2|Hex2Cer d18:2/16:0 HexCer d36:1|HexCer d18:1/18:0 HexCer d34:1|HexCer d18:1/16:0 HexCer d42:2|HexCer d18:2/24:0 SM d34:1|SM d18:1/16:0 SM d36:1|SM d18:1/18:0 SM d36:2|SM d18:2/18:0 SM d42:1|SM d18:1/24:0	• Association with higher CVD risk	[19]
Study cohort: n_1=50; n_2=4,007	Healthy participants before and after the suppression of intestinal microbiota with oral broad-spectrum antibiotics underwent phosphatidylcholine challenge (ingestion of two hard-boiled eggs and deuterium [d_9]-labeled phosphatidylcholine) Participants undergoing elective diagnostic cardiac catheterization with no history of acute coronary syndrome	Plasma	LC-MS/MS	3	Betaine Choline Trimethylamine *N*-oxide	• Association among intestinal microbiota-dependent metabolism of dietary phosphatidylcholine, TMAO levels, and adverse CVD events	[20]
Discovery cohort: n = 3,867 Validation cohort: n = 3,569	Participants were free of known CVD at baseline	Serum	NMR	N/A	1,5-Anhydrosorbitol 1-Methylhistidine 3-Hydroxybutyric acid 5-Oxoproline Acetaminophen + glucuronide Alanine Aspartic acid Citratic acid Glucose Glutamatic acid Glutamine Glycerol Glycine Histidine Lactic acid Lysine Mannose Methionine myo-Inositol Dimethylglycine Phenylalanine Glyceryl groups of lipids Lipids (CH2-CO) Lipids (CH2-CH2-C=, CH2-CH2-CO) Lipids (CH2-CH2-CH=CH) Lipids (CH3-CH2-R, (CH2)n) Lipids (CH3-CH2-R, CH3-CH2-C=)	• Association with atherosclerosis and incident CVD	[21]
Discovery cohort: n = 50 Validation cohort: n = 25	Stable patients undergoing elective cardiac evaluation who subsequently experienced a heart attack, stroke or death over the ensuing three-year period vs. age- and gender-matched subjects who did not	Plasma	LC-MS/MS	18	Betaine Choline Trimethylamine *N*-oxide	• Identification of markers as predictors of CVD risk • Discovery of a relationship between gut-flora-dependent metabolism of dietary	[22]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
						phosphatidylcholine and CVD pathogenesis	
Discovery cohort: n = 1,157 Validation cohorts: n_1 = 2,149; n_2 = 833	Stable subjects undergoing cardiac risk assessment Healthy volunteers (n = 8)	Plasma	GC-MS LC-MS/MS	N/A	Creatinine Erythritol Xylitol	• Association with major adverse cardiovascular event	[23]
Discovery cohort: n = 1,157 Validation cohort: n = 2,149	Stable subjects undergoing elective diagnostic cardiac evaluations Healthy volunteers (n = 10)	Plasma	GC-MS LC-MS/MS	N/A	Creatinine Erythritol Xylitol	• Association with major adverse cardiovascular event	[24]
Discovery cohort: n = 7,256 Validation cohorts: n_1 = 2,622; n_2 = 3,563	Participants were followed up for CVD incident (15 years)	Serum	NMR	68	3-Hydroxybutyric acid Acetic acid Acetoacetatic acid Alanine Citratic acid Glucose Glutamine Glycerol Glycine Histidine Isoleucine Lactic acid Leucine Phenylalanine Pyruvic acid Tyrosine Valine Docosahexaenoic acid (FA 22:6) Linoleic acid (FA 18:2) Monounsaturated FA Omega-3 FA Omega-6 FA Polyunsaturated FA Saturated FA	• Association with incident CVD	[25]
Study cohort: n = 4,007	Participants undergoing elective diagnostic cardiac catheterization with no history of an acute coronary syndrome	Plasma	LC-MS/MS	18	Choline Trimethylamine Trimethylamine *N*-oxide	• Discovery of increased levels of TMAO as a predictor of incident risk for thrombotic events • Association between specific dietary nutrients, gut microbes, platelet function, and thrombosis risk	[26]

Table S2. Metabolomics and lipidomics cohort studies focused on type 1 and type 2 diabetes

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Study cohort: n = 170 (48% female)	Children with high genetic risk for T1D	Plasma	GC-MS LC-MS/MS	91	γ-Aminobutyric acid (GABA) Glycine Tagatose Arabitol myo-Inositol Adipic acid Cer d38:1 Cer d39:1 LPC 18:3 LPC 20:3 LPC 20:5 SM d41:2	• Utilization of multi-omics data for the modeling of complex, multifactorial diseases, like T1D	[27]
Study cohort: n = 152 (47.4% female)	Children with T1D (n=76) and healthy control children (n=76)	Cord blood serum	LC-MS/MS	106	PC 32:1 PC 36:4 PC 38:4 PC 38:5 PC 38:5 PC 38:6 PC 40:4 PC 40:5 PC 40:5 PC 40:7 PC 40:8 PC sum PE 38:4 PE 38:4 PE 40:4	• Cord-blood metabolic patterns may be a valuable measure of type 1 diabetes risk	[28]
Study cohort: n = 101 (37.6% female)	Children who progressed to T1D (PT1D; n = 30), children who developed at least one islet autoantibody but did not progress to T1D during the follow-up (P1Ab; n = 33), and their age-matched controls (CTR; n = 38)	Cord blood plasma	LC-MS/MS	232 lipid species	CE 18:2 TG 46:2 TG 46:2 TG 48:1 TG 51:3	• Identification of lipids that can be predictive of the risk of progression to T1D • Comparison of lipidomic profiles of all subcohorts	[29]
Study cohort: n = 120	Children progressed to T1D; children developed at least a single islet autoantibody but did not progress to T1D during the follow-up; matched controls	Plasma	LC-MS/MS	45	CE 20:5 PC 33:0 TG 54:4|TG 18:2_18:1_18:1 TG 56:5	• Children who progress to T1D in the follow-up tend to have a distinct and persistently dysregulated lipid profile as compared to those who later progress to islet autoimmunity but not to T1D	[30]
Study cohort: n = 120	Progressors to T1D (n = 40); children tested positive for at least one antibody in a minimum of two consecutive samples but did not progress to clinical T1D during the follow-up (n = 40); control children remained islet autoantibody-negative during the follow-up (n = 40)	Plasma	GC-MS	94	2-Ketoisocaproic acid 3,4-Dihydroxybutanoic acid Aspartic acid Bisphenol A Glutamic acid Glycerol-2-phosphate Levoglucosan Malic acid Methionine Pyruvic acid	• Association of unique metabolomic profile with T1D	[31]
Study cohort: n = 2,124	Children with high genetic risk for T1D	Plasma	GC-MS LC-MS/MS	357	5-Methoxytryptamine Alanine Glutamic acid Isoleucine Leucine	• Studying autoantibodies and metabolomic markers, which are associated with the risk of progression to T1D	[32]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
					Methionine Proline Valine Vitamin E α-Ketoglutaric acid		
Study cohort: n = 166	T1D patients (n = 85) and healthy controls (n = 81). All patients had a stable dose of insulin usage for more than 3 months (dose change <10%)	Serum Urine	LC-MS/MS	54 (serum) 45 (urine)	4-(2-Aminophenyl)-2,4-dioxobutanoic acid 4-Pyridoxic acid 5-Hydroxytryptophan 5-Methoxyindole-3-acetic acid Hypoxanthine Thromboxane B3	• Identification of altered metabolic profiles in T1D individuals with different time in range (TIR)	[33]
Study cohort: n = 286	Infants later developed T1D (n=33); infants developed different numbers of islet autoantibodies during the follow-up (n=110); controls matched for sex, HLA-DQB1 genotype, city of birth, and period of birth (n=143)	Cord blood serum	LC-MS/MS	137	PC 32:0|PC 16:0_16:0 PC 32:1|PC 16:0_16:1 PC 34:1|PC 16:0_18:1 PC 34:3|PC 16:0_18:3 PC 36:1|PC 18:0_18:1 PC 38:3|PC 18:0_20:3 SM d34:1|SM d18:1/16:0 SM d36:1|SM d18:1/18:0 SM d38:0|SM d18:0/20:0 SM d38:1|SM d18:1/20:0 SM d42:1|SM d18:0/24:1 SM d42:2|SM d18:1/24:1 SM d42:2|SM d18:0/24:2 SM d42:3|SM d18:2/24:1	• Association with high risk for progression to T1D	[34]
Study cohort: n = 343	Children, who later developed type 1 diabetes (n=166), and random control children in the Norwegian Mother, Father, and Child cohort (n=177)	Cord blood plasma	LC-MS/MS	27	Aminoadipic acid Indoxyl sulfate Tryptophan	• Association with T1D	[35]
Study cohort: n = 655	Children with high genetic risk for T1D	Plasma	GC-MS	139	Ascorbic acid Piperidone	• Association with progression to T1D	[36]
Study cohort: n = 141	Children with T1D (n=76) and gender- and age-matched healthy controls (n=65)	Serum	GC-MS	70	1,5-Anhydroglucitol Adenine Fructose Glycerol-α-phosphate Inosine Levoglucosan Pyruvic acid Uridine Xylulose	• Association with T1D and with the duration of the disease	[37]
Study cohort: n = 11,896	Participants from four prospective population-based cohorts in Finland (follow-up for 7.8–15 years)	Serum	NMR	229	3-Hydroxybutyric acid Acetatic acid Acetoacetatic acid Citratic acid Creatinine Glutamine Glycerol Glycine Histidine Isoleucine Lactic acid Leucine Phenylalanine Pyruvic acid	• Association with risk of developing diabetes • Association with deterioration in post-load glucose and insulin resistance than with future fasting hyperglycemia	[38]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
					Tyrosine Valine		
Study cohort: n = 1,016	General population	Plasma	NMR	49	3-Hydroxybutyric acid Acetatic acid Alanine Citratic acid Creatine Creatine phosphate Creatinine Cysteine Glutamine CH2CH2CO- CH2N- Isobutyratic acid Isopropanol Leucine *N*-Acetylglutamine *O*-Phosphoethanolamine Phenylpropionic acid Proline Pyruvic acid	• Strong inverse association of healthy lifestyle with incident T2D	[39]
Study cohort: n = 1,138	Participants from four prospective population-based cohorts	Plasma	LC-MS/MS	70	2-Methylbutyroylcarnitine Cortisol Deoxycholic acid Tyrosine γ-Glutamyl-leucine Barogenin CerPE 38:2 LPC 20:2 MG 18:2 PC 42:7 SM d33:1 SM d34:2 SM d36:3|SM d18:2/18:1	• Association with incident T2D	[40]
Study cohorts: n_1 = 1,261; n_2 = 2,580	Clinically healthy participants (follow-up for 3 years)	Plasma	LC-MS/MS	N/A	2-Hydroxybutyric acid LPC 18:2	• Association with insulin resistance and glucose intolerance	[41]
Study cohort: n = 2,282 Incident T2D cohort: n = 800	General population	Serum	FI-MS	163	Glycine Hexose Phenylalanine LPC 18:2 PC O-34:3 PC O-40:6 PC O-42:5 PC O-44:4 PC O-44:5 PC O-32:1 PC 36:1 PC 38:3 PC 40:5 SM d34:2|SM d18:1/16:1	• Association with increased or decreased risk of T2D	[42]
Study cohort: n_1 = 1,813; n_2 = 451	1,813 participants without any signs of T2D 451 participants with newly diagnosed T2D	Serum	FI-MS LC-MS/MS	134	Alanine/glycine	• Association of analine/glycine ration with T2D	[43]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Study cohort: n = 5844 (90% female)	Female and male nurses	Plasma	LC-MS/MS	186	1-Methylnicotinamide 1-Methylguanosine Aminoisobutyric acid Caffeine CAR 2:0 CAR 5:0 CAR 5:0-DC Cortisone Dimethylglycine Guanidoacetic acid N^2,N^2-Dimethylguanosine N^4-Acetylcytidine *N*-Acetylspermidine *N*-Acetyltryptophan *N*-Carbamoyl-β-alanine Piperine Ribothymidine Tryptophan Biliverdin Cer d34:1|Cer d18:1/16:0 LPE 18:2 PC 34:2 PC P-34:4 PC P-38:4 PE 36:4 PE P-36:2 SM d38:1|SM d18:1/20:0	• Association between inflammatory and insulinemic dietary patterns, plasma inflammatory/insulin biomarkers, plasma metabolomics and risk of type 2 diabetes.	[44]
Study cohort: n = 2240	T2D participants, prediabetes participants, and normal glucose tolerance participants	Serum	FI-MS LC-MS/MS	123	Glycine CAR 16:0 LPC 18:2 PC O-36:0	• Association with incident T2D	[45]
Study cohort: n = 4,442 (61% female)	Participants without diabetes at baseline	Plasma	LC-MS/MS	6	Betaine Carnitine Choline Crotonobetaine γ-Butyrobetaine Trimethylamine *N*-oxide	• Association with incident T2D	[46]
Study cohort: n = 1571	Healthy participants (follow-up for 14 years)	Plasma	NMR LC-MS	24	1,5-Anhydroglucitol 2-Hydroxybutyric acid 2-Oxoglutaric acid Glycerol Glycine betaine Isoleucine Lactic acid Methionine Pyruvic acid Tyrosine PC 34:2;O TG 48:0 TG 48:1 TG 50:5	• Increase of the long-term prediction performance in combination with classical measurements	[47]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Discovery cohort: n = 3,821 Validation cohort: n = 14,651	Participants with normal glucose regulation	Serum	LC-MS/MS	667 (discovery cohort) 250 (validation cohort)	CE 14:0 LPI 16:1 PC 34:3 PE 38:4|PE 18:0_20:4 TG 48:1 (16:0) TG 48:1 (16:1) TG 48:2 (16:0) TG 48:2 (16:1) TG 48:2 (18:1) TG 48:3 (16:1) TG 50:0 (18:0) TG 50:1 (16:0) TG 50:1 (16:1) TG 50:1 (18:0) TG 50:2 (16:0) TG 50:2 (16:1) TG 50:2 (16:2) TG 50:2 (18:1) TG 50:3 (16:0) TG 50:3 (16:1) TG 50:3 (16:2) TG 51:0 (17:0) TG 51:2 (17:0) TG 51:3 (17:1) TG 53:2 (19:0) TG 53:3 (16:0) TG 54:3 (16:0) TG 54:3 (16:0) TG 54:4 (16:0) TG 54:4 (16:1) TG 54:5 (16:0) TG 54:5 (16:1) TG 54:6 (20:4) TG 54:7 (20:4) TG 54:7 (22:6) TG 55:6 (19:3) TG 56:5(18:1) TG 56:5(22:4) TG 56:6(22:5)	• Association of biomarkers and lipid pathway dysregulation with T2D onset	[48]
Study cohort: n = 2,204 (100% female)	Participants with T2D or impaired fasting glucose + normoglycemic control participants	Plasma Urine	LC-MS/MS GC-MS	447	2-Hydroxybutyric acid 1,5-Anhydroglucitol Arabinose Citrulline Dimethylarginine Erythritol Fructose Glucose Isoleucine Lactic acid Leucine Malic acid Mannose *N*-Acetylglycine Octanoylcarnitine Proline	• Association with incident T2D and IFG	[49]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
					Uric acid Valine 10-Heptadecenoic acid (FA 17:1n7) 15-Methylpalmitic acid (FA iso-17:0) 3-Methyl-2-oxobutanoic acid 3-Methyl-2-oxovaleric acid 4-Methyl-2-oxopentanoic acid 5-Dodecenoic acid (FA 12:1n7) Adrenic acid (FA 22:4n6) Arachidonic acid (FA 20:4n6) Cholesterol Heptanoic acid (FA 7:0) Myristic acid (FA 14:0) Myristoleic acid (FA 14:1n5) Palmitoleic acid (FA 16:1n7) SM d34:1|SM d18:1/16:0 Pelargonic acid (FA 9:0) Pentadecanoic acid (FA 15:0)		
Study cohort: n = 1,150	Participants with normal fasting glucose (follow-up for 20 years)	Plasma	LC-MS/MS	N/A	5-Hydroxyindoleacetic acid Glucose Glycine Isocitric acid Phenylalanine Taurine 2-Aminodipic acid 3-Methyladipic acid CE 20:3 DG 36:1 LPC 18:1 LPC 18:2 PC 36:4 SM d42:1|SM d18:1/24:0 TG 48:0 TG 48:1 TG 52:1 TG 54:8 TG 58:11	• Association with improved prediction of T2D beyond conventional risk factors	[50]
Discovery cohort: n = 543 Validation cohort: n = 1,044	Non-diabetic participants (follow-up)	Serum	LC-MS/MS GC-MS	568	2-Hydroxybutyric acid Bilirubin Glucose Glutamic acid Glutamine Histidine Isoleucine Mannose Trehalose Valine α-Tocopherol	• Association with positive or negative impact on progression to T2D	[51]
Study cohort: n = 1,248	Participants with 6.5 years follow-up	Plasma	DMS-MS/MS GC-MS	N/A	*Lipid classes containing species with FA 15:0 and FA 17:0:* CE 15:0 CE 17:0 DG 15:0 FA 15:0	• Association with incident T2D	[52]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
					FA 17:0 LPC 15:0 LPC 17:0 LPE 17:0 MG 15:0 MG 17:0 PC 15:0 PC 17:0 PE 17:0 PL-OCFA (phospholipid species containing odd-chain fatty acids) TG 15:0 TG 17:0		
Study cohorts: n_1 = 1,039; n_2 = 520	Participants with mean follow-ups: 4.61 and 7.57 years	Plasma	LC-MS/MS	166	CE 16:1 LPC 15:0 LPC 18:2 PC 33:3 PC 35:3 PC 40:7 PC 43:6 PC 44:1 SM d34:2 SM d41:2 TG 46:1 (12:0) TG 48:1 (16:0) TG 48:2 (14:0) TG 49:7 (16:0) TG 50:1 (16:0) TG 50:2 (16:0) TG 50:3 (18:1) TG 51:7 (16:0) TG 52:5 (18:2) TG 52:6 (18:2) TG 54:3 (18:0) TG 54:4 (18:2) TG 54:5 (18:2) TG 54:6 (18:2) TG 54:7 (18:3) TG 56:5 (20:4)	• Association with incident T2D	[53]
Study cohort: n = 2,939	Participants without diabetes prevalence	Serum	LC-MS/MS	245	3-(4-Hydroxyphenyl)lactic acid Asparagine Erythritol Isoleucine Leucine Trehalose Valine	• Association with incident T2D (protective biomarker of diabetes risk)	[54]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Study cohort: n = 2,103	Participants with a 6-year follow-up	Plasma	LC-MS/MS	34	Carnitine 3-Dehydroxycarnitine 3-Dehydrocarnitine CAR 2:0 CAR 3:0 CAR 3:0-DC CAR 4:0 CAR 5:0 CAR 5:0-OH CAR 5:1 CAR 6:0 CAR 6:0-OH CAR 6:0-DC CAR 7:0-DC CAR 8:0 CAR 8:1 CAR 10:0 CAR 10:0-DC CAR 12:0 CAR 12:0-OH CAR 12:1 CAR 12:0-DC CAR 14:0 CAR 14:0-OH CAR 14:1-OH CAR 16:0 CAR 16:1 CAR 16:2 CAR 18:0 CAR 18:0-OH CAR 18:1 CAR 18:2 CAR 20:0 CAR 20:4	• Association with improved predictive ability for type 2 diabetes beyond conventional risk factors	[55]
Study cohort: n = 3,234	Participants were assigned to 1) intensive lifestyle, 2) metformin, or 3) placebo (all followed up for 3.2 years)	Plasma	HILIC-MS/MS	84	Betaine Methionine sulfoxide Serine	• Association with incident T2D	[56]

Table S3. Metabolomics and lipidomics cohort studies focused on metabolic dysfunction-associated steatotic liver disease

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Study cohort: n = 121,032	Participants with a mean 12.6-year follow-up	Plasma	NMR	170	3-Hydroxybutyric acid Acetic acid Acetoacetatic acid Acetone Alanine Citric acid Creatinine Glucose Glutamine Glycine Histidine Isoleucine Lactic acid Leucine Phenylalanine Pyruvic acid Tyrosine Valine Docosahexaenoic acid (FA 22:6) Linoleic acid (FA 18:2) Omega-3 FA Omega-6 FA	• Positive and negative association with MASLD	[57]
Study cohort: n = 10,809	Participants with and without MASLD	Plasma	NMR	123	Tyrosine	• Association with MASLD	[58]
Study cohort: n = 3,048	Participants have been followed up since birth, including questionnaires and clinical assessments starting from age 7 years	Plasma	NMR	154	3-Hydroxybutyric acid Acetic acid Acetoacetatic acid Alanine Creatinine Glutamine Histidine Isoleucine Leucine Phenylalanine Tyrosine Valine	• Association with incident MASLD	[59]
Study cohort: n = 928 (67% female)	Participants with and without MASLD	Plasma	CE-MS	94	4-Methyl-2-oxopentanoic acid Alanine Glutamic acid Isoleucine Leucine Proline Tryptophan Tyrosine Valine Glycerophosphorylcholine	• Association with both MASLD and cardio-ankle vascular index (CAVI)	[60]
Study cohort: n = 1,479 Study subcohort: n = 447 (known age)	Participants were not treated for cancer or infectious disease or had undergone surgery in the previous year, and they had no history of cancer or an infectious disease.	Serum	LC-MS/MS	N/A	Oleic acid-hydroxy oleic acid (OAHOA) Sphingosine Uric acid	• Association with MASLD	[61]
Study cohort: n = 997 (53% female)	Participants free of prevalent myocardial infarction or congestive heart failure at the first examination cycle	Plasma	HILIC-MS/MS	179	Anandamide	• Association with MASLD severity, the presence of nonalcoholic steatohepatitis, and fibrosis	[62]

Subjects (n)	Cohort	Matrix	Platforms	Reported metabolites (n)	Markers	Outcomes	Ref.
Study cohort: n = 559	Participants with and without MASLD	Plasma	LC-MS/MS	11	Dihydrothymine Serine Tryptophan LPC 18:1 LPE 20:0	• Screening tool for MASLD	[63]
Study cohort: n = 1,154 (50% female) Control cohort: n = 350	Participants with biopsy-proven MASLD and participants from the general population with similar gender and age to the cohort of patients with MASLD	Serum	LC-MS NMR	105	PC 32:0|PC 16:0_16:0 PC 32:2|PC 14:0_18:2 PC 34:2|PC 16:0_18:2 PC 36:1|PC 18:0_18:1 PC 36:3 PC 36:6|PC 18:3_18:3 PC 37:5 PC 38:2|PC 20:0_18:2 PC 38:3|PC 18:0_20:3 SM d32:1 SM d39:1 TG 48:3	• Identification of three MASLD subgroups, independent of histological disease severity	[64]
Study cohort: n = 627	Histologically characterized participants. Participants include the full spectrum of disease, from histologically normal liver tissue through NAFL to NASH-F4 (cirrhosis)	Serum	LC-MS/MS GC-MS/MS	211	*Markers of fibrosis 0–1 vs. 2–4:* 2-Hydroxybutyric acid 3-Hydroxybutyric acid LPC O-16:0 LPC P-16:0 LPC 18:2 LPC 20:4 Oleic acid PC 32:0|PC 16:0/16:0 PC 32:1 PC 37:4 PC O-34:2 PC O-34:3 PE 16:0/18:1 PE 34:2 PE 38:6 SM d42:1|SM d18:1/24:0 SM d36:0 SM d41:1 TG 56:4 TG 58:6	• Identification of a key metabolic 'watershed' in the progression of liver damage, separating severe disease from mild	[65]
Discovery cohort: n = 1,546 Internal validation cohort: n = 377 Prospective validation cohort: n = 749	Participants with and without MASLD (4 years follow-up	Feces	LC-MS/MS	198	Taurocholic acid	• Positive association with both a higher microbiome risk score and MASLD risk	[66]

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