

SUPPLEMENTARY TABLE 1: Mean (SD) concentrations of metabolic biomarker measures from serum samples collected in 2011 in absolute concentration units, and their correlations with the categorical fatty liver score.

	Normal liver (n=1,630)		Fatty liver (n=372)		Spearman correlation ¹
	Mean	SD	Mean	SD	r
LIPOPROTEIN PARTICLES					
<i>VLDL</i>					
Total VLDL particle concentration (pmol/L)	82	36	117	46	0.300*
Extremely large VLDL particles (pmol/L)	0.07	0.15	0.20	0.27	0.298*
Very large VLDL particles (pmol/L)	0.37	0.85	1.23	1.50	0.327*
Large VLDL particles (pmol/L)	3.5	4.8	8.5	7.6	0.316*
Medium VLDL particles (pmol/L)	14.5	11.6	26.7	17.0	0.310*
Small VLDL particles (pmol/L)	26.9	12.8	39.3	15.8	0.306*
Very small VLDL particles (pmol/L)	36.1	10.3	40.4	11.8	0.162*
<i>IDL and LDL</i>					
Total IDL+LDL particle concentration (pmol/L)	557	134	618	159	0.175*
IDL particles (pmol/L)	99	24	105	29	0.114*
Large LDL particles (pmol/L)	168	40	183	48	0.154*
Medium LDL particles (pmol/L)	137	35	154	40	0.189*
Small LDL particles (pmol/L)	153	39	175	47	0.204*
<i>HDL</i>					
Total HDL particle concentration (nmol/L)	8.1	1.1	7.9	1.1	-0.114*
Very large HDL particles (nmol/L)	0.34	0.21	0.18	0.16	-0.287*
Large HDL particles (nmol/L)	1.16	0.50	0.77	0.45	-0.300*
Medium HDL particles (nmol/L)	1.98	0.37	1.99	0.40	-0.040
Small HDL particles (nmol/L)	4.62	0.45	4.94	0.46	0.218*
<i>PARTICLE SIZE</i>					
VLDL particle size (nm)	36.1	1.4	37.7	1.8	0.301*
LDL particle size (nm)	23.6	0.2	23.5	0.2	-0.167*
HDL particle size (nm)	9.9	0.3	9.7	0.2	-0.319*
TRIGLYCERIDES					
Serum triglycerides (mmol/L)	1.24	0.67	1.96	1.00	0.318*
Extremely large VLDL triglycerides (mmol/L)	0.009	0.023	0.030	0.044	0.342*
VLDL triglycerides (mmol/L)	0.75	0.60	1.39	0.90	0.319*
IDL triglycerides (mmol/L)	0.135	0.037	0.153	0.044	0.178*
LDL triglycerides (mmol/L)	0.201	0.075	0.246	0.117	0.199*
HDL triglycerides (mmol/L)	0.152	0.038	0.169	0.043	0.143*
CHOLESTEROL AND APOLIPOPROTEINS					
Serum cholesterol (mmol/L)	5.03	0.93	5.26	1.11	0.106*
VLDL cholesterol (mmol/L)	0.64	0.27	0.86	0.33	0.268*
Non-HDL cholesterol (mmol/L)	3.37	0.91	3.84	1.05	0.199*
IDL cholesterol (mmol/L)	0.75	0.18	0.78	0.22	0.091*
LDL cholesterol (mmol/L)	1.98	0.56	2.20	0.65	0.162*
HDL cholesterol (mmol/L)	1.65	0.38	1.41	0.37	-0.248*

HDL ₂ cholesterol (mmol/L)	1.13	0.38	0.88	0.37	-0.270*
HDL ₃ cholesterol (mmol/L)	0.53	0.04	0.54	0.06	0.071
Apolipoprotein B (g/L)	0.93	0.23	1.12	0.27	0.270*
Apolipoprotein A-I (g/L)	1.71	0.23	1.63	0.24	-0.129*
Apo B/Apo A-I	0.55	0.15	0.70	0.16	0.311*
FATTY ACIDS					
Total FA concentration (mmol/L)	10.9	2.5	13.2	3.4	0.242*
PUFA (%), relative to total fatty acids	38.2	3.8	33.8	4.5	-0.266*
n-3 (%), relative to total fatty acids	3.86	1.01	3.55	0.85	-0.130*
DHA (%), relative to total fatty acids	1.61	0.54	1.38	0.46	-0.135*
n-6 (%), relative to total fatty acids	34.4	3.7	30.2	4.2	-0.251*
Linoleic acid (%), relative to total fatty acids	29.0	3.7	25.0	3.8	-0.265*
MUFA (%), relative to total fatty acids	28.0	3.4	31.1	3.9	0.257*
SFA (%), relative to total fatty acids	33.8	2.2	35.1	2.4	0.113*
n-6 /n-3	9.47	2.55	8.91	2.24	-0.022
PUFA/SFA	1.14	0.16	0.97	0.17	-0.237*
Double bonds per fatty acid	1.27	0.08	1.19	0.09	-0.209*
AMINO ACIDS					
Isoleucine (mmol/L)	0.051	0.015	0.069	0.019	0.351*
Leucine (mmol/L)	0.080	0.016	0.098	0.019	0.334*
Valine (mmol/L)	0.199	0.038	0.230	0.044	0.270*
Phenylalanine (mmol/L)	0.074	0.010	0.084	0.011	0.305*
Tyrosine (mmol/L)	0.050	0.010	0.059	0.012	0.281*
Alanine (mmol/L)	0.399	0.059	0.441	0.064	0.239*
Glutamine (mmol/L)	0.595	0.068	0.581	0.078	-0.069
Histidine (mmol/L)	0.065	0.009	0.066	0.009	0.071
Glycine (mmol/L)	0.296	0.063	0.276	0.053	-0.086*
GLYCOLYSIS-RELATED METABOLITES					
Glycerol (mmol/L)	0.098	0.037	0.114	0.039	0.138*
Glucose (mmol/L)	4.64	0.67	5.12	1.22	0.231*
Lactate (mmol/L)	1.42	0.35	1.62	0.44	0.140*
Pyruvate (mmol/L)	0.077	0.022	0.091	0.027	0.196*
KETONE BODIES					
Acetoacetate (mmol/L)	0.058	0.042	0.057	0.047	-0.013
3-hydroxybuturate (mmol/L)	0.10	0.11	0.10	0.14	-0.024
MISCELLANEOUS					
Citrate (mmol/L)	0.104	0.018	0.104	0.017	-0.001
Acetate (mmol/L)	0.046	0.015	0.042	0.010	-0.103*
Glycoprotein acetyls (mmol/L)	1.52	0.21	1.72	0.26	0.284*
C-reactive protein (mg/L) ²	1.70	2.70	3.15	4.17	0.253*
Creatinine (mmol/L)	0.063	0.013	0.064	0.012	0.070
Albumin (signal area)	0.105	0.009	0.105	0.009	0.040
Alanine aminotransferase (U/L) ²	14.8	9.7	31.7	36.4	0.336*
Gamma-glutamyl transferase (U/L) ²	27.6	27.7	62.5	70.8	0.356*
HOMA-insulin resistance index ²	1.9	4.0	9.7	87.5	0.389*

The number of participants with both metabolite data and fatty liver measurements was n=2,002. Since some metabolic measures were not determined in a small number of study participants, for some measures, the concentrations are reported for n=1,939–2,002.

Abbreviations: DHA, docosahexaenoic acid; HDL, high-density lipoprotein; HOMA, homeostatic model; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; VLDL, very-low-density lipoprotein.

¹Spearman correlation coefficients between absolute metabolite concentrations and the categorical fatty liver score (n=1,859–1,919). The fatty liver score was calculated by summarizing the values of the five widely accepted criteria for fatty liver: 1) the liver-to-kidney contrast (no=0 or clear contrast=1), 2) parenchymal brightness (normal=0, mild=1, intermediate=2 or severe brightness=3), 3) deep beam attenuation (clear=0, attenuated=1 or no visible=2 line of diaphragm) 4) bright vessel walls (normal=0, partly visible=1 or no visible=2 vessel walls) and 5) visibility of the neck of the gallbladder (normal=0, partly visible=1, not visible=2). The final score included in values ranging from 0 to 9.

²Measure not quantified by the NMR metabolomics platform.

* Statistical significance at $P < 0.0007$.

SUPPLEMENTARY TABLE 2: Comparison of BMI and waist circumference as anthropometric covariates in the cross-sectional (metabolites in 2011 vs. fatty liver status in 2011) and prospective (metabolites in 2001 vs. fatty liver status in 2011) associations. Odds ratios and their 95% confidence intervals are per 1-SD increment in the metabolic measures, and shown with adjustment for sex, age, BMI or waist circumference, alcohol intake, leisure-time physical activity and smoking.

	CROSS-SECTIONAL ASSOCIATIONS (2011)								PROSPECTIVE ASSOCIATIONS (2001->2011)							
	BMI as covariate				WAIST as covariate				BMI as covariate				WAIST as covariate			
	OR	95%CI Low High		P	OR	95%CI Low High		P	OR	95%CI Low High		P	OR	95%CI Low High		P
LIPOPROTEIN PARTICLES																
VLDL																
Total VLDL particle concentration	1.52	1.30	1.78	2E-7	1.54	1.32	1.81	9E-8	1.38	1.16	1.64	3E-4	1.45	1.22	1.72	2E-5
Extremely large VLDL particles	1.83	1.50	2.22	1E-9	1.82	1.50	2.22	2E-9	1.42	1.19	1.69	8E-5	1.46	1.23	1.74	2E-5
Very large VLDL particles	1.67	1.35	2.08	4E-6	1.68	1.35	2.09	3E-6	1.51	1.20	1.89	3E-4	1.60	1.28	2.01	4E-5
Large VLDL particles	1.93	1.51	2.46	2E-7	1.98	1.54	2.54	8E-8	1.41	1.13	1.75	0.002	1.51	1.21	1.89	3E-4
Medium VLDL particles	1.70	1.45	1.99	4E-11	1.74	1.48	2.03	8E-12	1.45	1.21	1.72	3E-5	1.53	1.29	1.82	1E-6
Small VLDL particles	1.53	1.29	1.81	7E-7	1.55	1.31	1.84	3E-7	1.39	1.16	1.67	4E-4	1.48	1.23	1.77	2E-5
Very small VLDL particles	1.05	0.92	1.20	0.47	1.06	0.93	1.21	0.41	1.08	0.93	1.25	0.29	1.10	0.95	1.28	0.19
IDL and LDL																
Total IDL+LDL particle concentration	1.13	0.99	1.28	0.07	1.14	1.00	1.30	0.05	1.13	0.97	1.31	0.12	1.16	1.00	1.35	0.05
IDL particles	1.01	0.89	1.14	0.92	1.01	0.89	1.15	0.82	1.04	0.89	1.20	0.65	1.05	0.91	1.22	0.51
Large LDL particles	1.08	0.95	1.22	0.27	1.09	0.96	1.24	0.19	1.08	0.93	1.25	0.34	1.10	0.95	1.28	0.20
Medium LDL particles	1.15	1.01	1.31	0.03	1.17	1.02	1.33	0.02	1.14	0.98	1.32	0.10	1.17	1.01	1.36	0.04
Small LDL particles	1.22	1.07	1.40	0.002	1.24	1.09	1.41	0.001	1.23	1.06	1.43	0.007	1.28	1.10	1.48	0.001
HDL																
Total HDL particle concentration	1.05	0.91	1.21	0.51	1.10	0.95	1.27	0.18	1.05	0.89	1.24	0.54	1.04	0.88	1.22	0.64
Very large HDL particles	0.75	0.66	0.85	6E-6	0.74	0.65	0.84	3E-6	0.92	0.80	1.06	0.26	0.91	0.80	1.05	0.19
Large HDL particles	0.65	0.54	0.79	8E-6	0.67	0.55	0.80	3E-5	0.67	0.54	0.83	3E-4	0.64	0.51	0.79	3E-5
Medium HDL particles	1.18	1.03	1.35	0.02	1.23	1.07	1.41	0.003	1.13	0.97	1.33	0.13	1.12	0.95	1.31	0.17
Small HDL particles	1.50	1.30	1.73	2E-8	1.57	1.37	1.81	3E-10	1.34	1.15	1.56	1E-04	1.37	1.18	1.58	3E-5
PARTICLE SIZE																
VLDL particle size	1.74	1.51	2.00	4E-15	1.77	1.54	2.03	5E-16	1.58	1.35	1.84	1E-08	1.65	1.42	1.92	1E-10
LDL particle size	0.72	0.63	0.82	1E-6	0.71	0.62	0.81	3E-7	0.71	0.60	0.83	2E-05	0.68	0.58	0.79	1E-6

HDL particle size	0.57	0.47	0.69	8E-9	0.57	0.47	0.69	8E-9	0.67	0.55	0.82	1E-04	0.64	0.52	0.78	1E-5
TRIGLYCERIDES																
Serum triglycerides	1.69	1.46	1.97	7E-12	1.72	1.48	2.00	2E-12	1.51	1.28	1.79	1E-6	1.60	1.35	1.89	4E-8
Extremely large VLDL triglycerides	2.28	1.72	3.02	1E-8	2.23	1.68	2.96	3E-8	1.68	1.36	2.07	2E-6	1.76	1.42	2.18	2E-7
VLDL triglycerides	1.78	1.51	2.10	5E-12	1.81	1.54	2.14	1E-12	1.55	1.29	1.85	2E-6	1.64	1.37	1.96	5E-8
IDL triglycerides	1.15	1.01	1.31	0.03	1.16	1.02	1.32	0.03	1.09	0.95	1.27	0.23	1.11	0.96	1.28	0.17
LDL triglycerides	1.24	1.08	1.43	0.003	1.23	1.06	1.41	0.005	1.31	1.11	1.55	0.002	1.36	1.15	1.61	4E-4
HDL triglycerides	1.22	1.07	1.38	0.002	1.24	1.10	1.41	0.0007	1.04	0.90	1.20	0.61	1.04	0.90	1.20	0.58
CHOLESTEROL AND APOLIPOPROTEINS																
Serum cholesterol	1.06	0.93	1.20	0.41	1.08	0.95	1.23	0.24	1.05	0.90	1.22	0.55	1.07	0.92	1.24	0.37
VLDL cholesterol	1.33	1.14	1.56	3E-4	1.35	1.16	1.58	2E-4	1.25	1.06	1.49	0.009	1.31	1.11	1.56	0.002
Non-HDL cholesterol	1.10	0.95	1.27	0.19	1.12	0.97	1.29	0.13	1.07	0.91	1.25	0.43	1.11	0.94	1.30	0.21
IDL cholesterol	0.97	0.86	1.10	0.68	0.98	0.87	1.12	0.80	1.00	0.86	1.16	0.98	1.02	0.88	1.18	0.82
LDL cholesterol	1.08	0.95	1.23	0.23	1.10	0.96	1.25	0.17	1.07	0.92	1.24	0.39	1.10	0.95	1.28	0.21
HDL cholesterol	0.74	0.64	0.87	2E-4	0.76	0.65	0.89	0.0007	0.82	0.69	0.98	0.03	0.80	0.68	0.96	0.01
HDL ₂ cholesterol	0.70	0.60	0.82	9E-6	0.72	0.61	0.84	4E-5	0.76	0.64	0.92	0.004	0.74	0.62	0.89	0.001
HDL ₃ cholesterol	1.05	0.92	1.19	0.49	1.07	0.94	1.22	0.28	1.22	1.06	1.41	0.006	1.27	1.10	1.46	0.001
Apolipoprotein B	1.38	1.19	1.61	2E-5	1.40	1.20	1.62	2E-5	1.29	1.09	1.52	0.003	1.35	1.15	1.60	3E-4
Apolipoprotein A-I	0.90	0.78	1.04	0.15	0.93	0.81	1.07	0.33	0.99	0.84	1.16	0.88	0.99	0.85	1.16	0.93
Apo B/Apo A-I	1.56	1.32	1.85	2E-7	1.54	1.30	1.83	4E-7	1.32	1.10	1.59	0.003	1.39	1.16	1.67	4E-4
FATTY ACIDS																
Total FA concentration	1.56	1.36	1.79	3E-10	1.58	1.38	1.82	7E-11	1.38	1.19	1.60	3E-05	1.43	1.24	1.66	2E-6
PUFA (%), relative to total fatty acids	0.45	0.39	0.53	1E-24	0.45	0.39	0.52	8E-25	0.55	0.47	0.64	2E-14	0.53	0.46	0.62	4E-16
n-3 (%), relative to total fatty acids	0.82	0.71	0.95	0.007	0.83	0.72	0.96	0.01	0.85	0.73	0.99	0.04	0.85	0.73	0.99	0.04
DHA (%), relative to total fatty acids	0.78	0.68	0.90	6E-4	0.78	0.68	0.90	0.0007	0.82	0.71	0.96	0.01	0.81	0.70	0.95	0.008
n-6 (%), relative to total fatty acids	0.47	0.40	0.54	2E-23	0.46	0.40	0.54	7E-24	0.56	0.48	0.66	3E-13	0.55	0.47	0.64	7E-15
Linoleic acid (%), relative to total fatty acids	0.46	0.39	0.54	2E-22	0.45	0.39	0.53	1E-22	0.56	0.48	0.66	7E-13	0.54	0.46	0.63	2E-14
MUFA (%), relative to total fatty acids	1.70	1.47	1.97	8E-13	1.72	1.49	1.99	2E-13	1.59	1.36	1.86	4E-9	1.63	1.40	1.90	4E-10
SFA (%), relative to total fatty acids	1.73	1.50	1.99	2E-14	1.71	1.49	1.97	5E-14	1.45	1.25	1.68	1E-6	1.48	1.28	1.72	2E-7
n-6 /n-3	0.80	0.69	0.92	0.002	0.79	0.69	0.91	0.001	0.91	0.79	1.06	0.23	0.90	0.77	1.04	0.16
PUFA/SFA	0.43	0.37	0.51	5E-25	0.43	0.37	0.51	6E-25	0.56	0.48	0.65	6E-13	0.54	0.46	0.63	2E-14
Double bonds per fatty acid	0.51	0.44	0.59	6E-19	0.51	0.44	0.59	1E-18	0.59	0.50	0.69	3E-11	0.57	0.49	0.67	3E-12

AMINO ACIDS

Isoleucine	1.86	1.58	2.19	2E-13	1.88	1.59	2.21	8E-14	1.67	1.40	1.99	8E-9	1.75	1.47	2.08	2E-10
Leucine	2.09	1.76	2.48	3E-17	2.09	1.76	2.49	3E-17	1.75	1.47	2.10	6E-10	1.84	1.54	2.19	9E-12
Valine	1.44	1.25	1.67	7E-7	1.44	1.25	1.66	7E-7	1.34	1.15	1.57	2E4	1.37	1.17	1.60	7E-5
Phenylalanine	1.69	1.46	1.97	7E-12	1.71	1.47	1.99	3E-12	1.17	1.00	1.37	0.06	1.23	1.05	1.44	0.01
Tyrosine	1.58	1.37	1.81	3E-10	1.60	1.39	1.84	6E-11	1.26	1.08	1.47	0.003	1.29	1.11	1.51	0.001
Alanine	1.61	1.41	1.84	2E-12	1.66	1.45	1.90	1E-13	1.31	1.14	1.52	2E-4	1.36	1.18	1.56	2E-5
Glutamine	0.78	0.68	0.90	0.0007	0.78	0.68	0.90	0.0008	0.90	0.77	1.07	0.23	0.90	0.76	1.06	0.19
Histidine	1.09	0.96	1.25	0.18	1.12	0.98	1.28	0.09	0.94	0.81	1.10	0.47	0.95	0.82	1.11	0.53
Glycine	0.86	0.74	1.01	0.06	0.86	0.74	1.01	0.06	0.94	0.80	1.10	0.45	0.94	0.80	1.10	0.43

GLYCOLYSIS-RELATED METABOLITES

Glycerol	1.44	1.23	1.68	4E-6	1.47	1.26	1.71	1E-6	1.24	1.05	1.47	0.01	1.25	1.06	1.48	0.007
Glucose	1.29	1.13	1.47	2E-4	1.33	1.17	1.52	2E-5	1.10	0.96	1.27	0.18	1.11	0.97	1.29	0.13
Lactate	1.46	1.27	1.67	7E-8	1.46	1.27	1.67	5E-8	1.06	0.92	1.22	0.44	1.06	0.92	1.23	0.41
Pyruvate	1.45	1.26	1.66	1E-7	1.52	1.32	1.74	2E-9	1.23	1.07	1.43	0.004	1.28	1.11	1.48	0.0008

KETONE BODIES

Acetoacetate	0.88	0.76	1.01	0.07	0.87	0.76	1.01	0.07	1.02	0.88	1.19	0.75	1.02	0.88	1.19	0.77
3-hydroxybuturate	1.00	0.88	1.15	0.96	0.97	0.85	1.11	0.65	0.93	0.81	1.08	0.37	0.97	0.83	1.12	0.64

MISCELLANEOUS

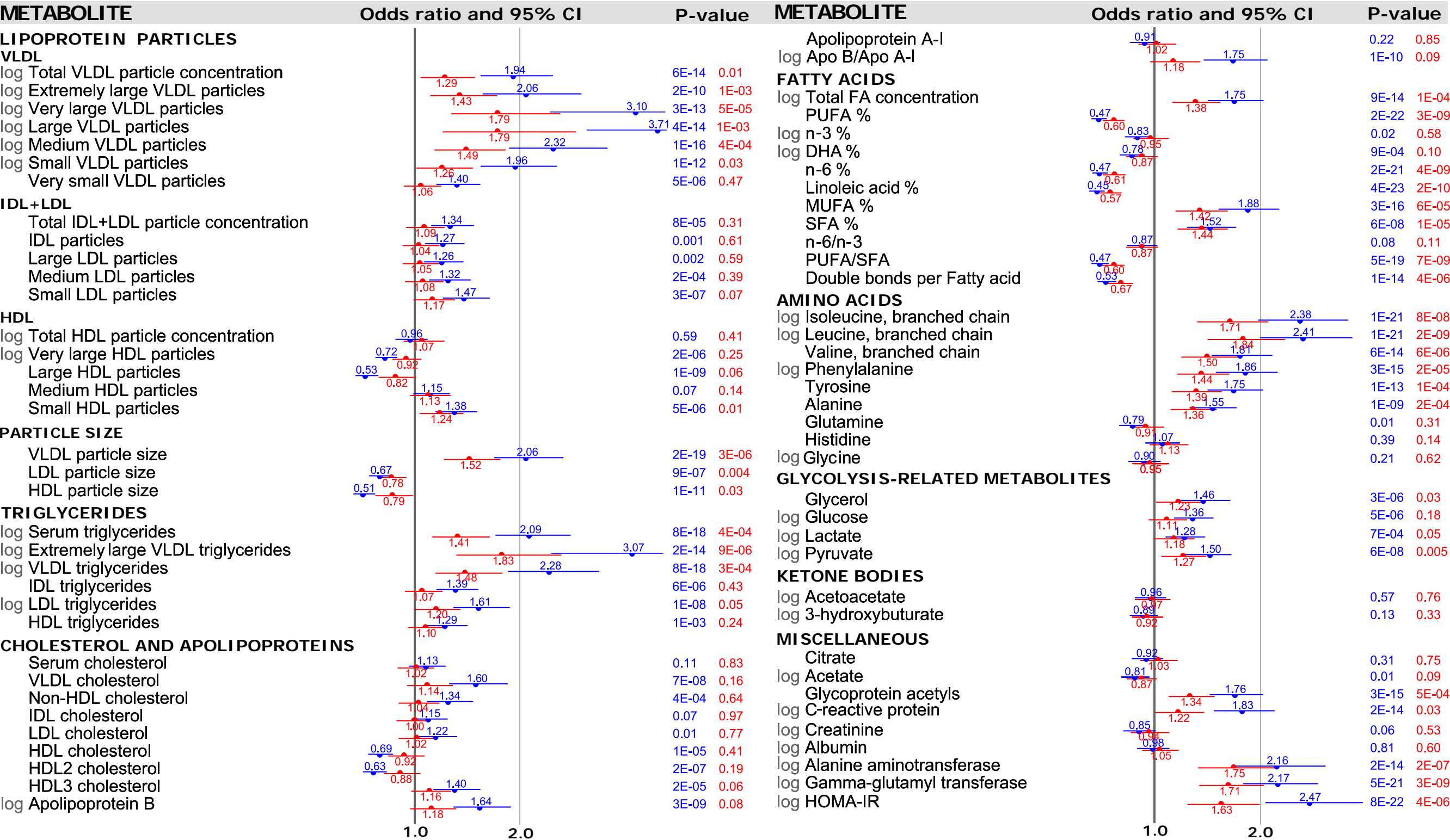
Citrate	1.12	0.97	1.28	0.13	1.09	0.95	1.26	0.22	0.97	0.83	1.13	0.67	0.99	0.85	1.15	0.89
Acetate	0.68	0.58	0.80	2E-6	0.70	0.60	0.82	1E-5	0.99	0.86	1.13	0.85	0.96	0.84	1.10	0.59
Glycoprotein acetyls	1.53	1.33	1.75	9E-10	1.56	1.36	1.79	1E-10	1.34	1.16	1.55	6E-5	1.42	1.23	1.64	1E-6
C-reactive protein	1.38	1.18	1.62	6E-5	1.37	1.17	1.60	1E-4	1.01	0.86	1.19	0.91	1.04	0.89	1.23	0.62
Creatinine	0.91	0.78	1.06	0.23	0.89	0.77	1.04	0.15	1.07	0.90	1.27	0.42	1.04	0.88	1.23	0.65
Albumin (signal area)	1.01	0.88	1.15	0.93	1.06	0.93	1.22	0.37	0.99	0.85	1.16	0.90	1.00	0.86	1.17	0.97
Alanine aminotransferase	2.47	2.09	2.93	5E-26	2.49	2.11	2.95	1E-26	1.43	1.23	1.67	6E-6	1.48	1.27	1.73	4E-7
Gamma-glutamyl transferase	2.07	1.78	2.42	2E-20	2.08	1.79	2.43	5E-21	1.59	1.35	1.88	2E-8	1.67	1.42	1.96	7E-10
HOMA-insulin resistance index	2.25	1.86	2.72	5E-17	2.26	1.87	2.72	2E-17	1.53	1.28	1.82	2E-6	1.63	1.37	1.94	4E-8

Cross-sectional associations included in 1,939–2,002 individuals of whom 339–372 had diagnosed fatty liver in 2011. Prospective associations included in 1,516–1,575 individuals with metabolite data at the 2001-baseline, of whom 263–275 had fatty liver diagnosed in 2011.

Prior to prospective analyses, individuals with suspected fatty liver in 2001 were excluded (alanine aminotransferase > 30 U/L).

P-values in the exponential format denote metabolite associations that were statistically significant associations when accounting for Bonferroni-correction ($P < 0.0007$).

DHA, docosahexaenoic acid; HDL, high-density lipoprotein; HOMA-IR, homeostatic model-based insulin resistance index; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; VLDL, very-low-density lipoprotein.



SUPPLEMENTARY FIGURE 1: Prospective associations of metabolic measures with 4-year risk for fatty liver (n=1,455–1,509 with metabolite data at the 2007-survey, of whom 207-212 had fatty liver diagnosed in 2011). Odds ratios (95% confidence intervals) are per 1-SD increment in the metabolic measures, and shown with adjustment for sex and age (blue), and additionally for baseline waist, alcohol intake, leisure-time physical activity and smoking (red). P-values listed in exponential format denote metabolite associations that were statistically significant associations when accounting for Bonferroni-correction ($P < 0.0007$). Individuals with suspected fatty liver in 2007 (alanine aminotransferase > 30 U/L) were excluded from analyses.