

Circulating Lyso-Phosphatidylcholines are Markers of a metabolically benign Nonalcoholic Fatty Liver

Supplementary Table 1 Serum lipid levels of the 40 subjects with nonalcoholic fatty liver at baseline and after 9 months of lifestyle intervention

Characteristics	Insulin sensitive subjects		Insulin resistant subjects		p-value at baseline	p-value at follow-up	p-MANOVA group*time
	Baseline (N=20)	Follow-up	Baseline (N=20)	Follow-up			
Free fatty acids ($\mu\text{mol/l}$)	650 \pm 45	555 \pm 43*	645 \pm 54	591 \pm 43	0.77	0.51	0.60
Triglycerodes (mg/dl)	190 \pm 42	190 \pm 50	140 \pm 16	138 \pm 13	0.41	0.67	0.98
Total cholesterol (mg/dl)	202 \pm 2	202 \pm 11	201 \pm 7	195 \pm 6	0.85	0.72	0.72
HDL cholesterol (mg/dl)	49 \pm 3	50 \pm 3	50 \pm 3	48 \pm 3	0.76	0.80	0.23
LDL cholesterol (mg/dl)	130 \pm 6	120 \pm 8*	131 \pm 6	4127 \pm 6	0.94	0.38	0.65

Values are means \pm SEM. *p < 0.05 for change within each group; MANOVA: multivariate analysis of variance.

Supplementary Table 2 List of all measured metabolites and their biochemical classification.

	Metabolite	Metabolite class
1	Ala	amino acids
2	Arg	
3	Asn	
4	Asp	
5	Cit	
6	Gln	
7	Glu	
8	Gly	
9	His	
10	Ile	
11	Leu	
12	Lys	
13	Met	
14	Orn	
15	Phe	
16	Pro	
17	Ser	
18	Thr	
19	Trp	
20	Tyr	
21	Val	
22	C0	acylcarnitines
23	C2	
24	C3	
25	C3-DC (C4-OH)	
26	C3-OH	
27	C3:1	
28	C4	
29	C4:1	
30	C5-M-DC	
31	C5-OH (C3-DC-M)	
32	C5:1	
33	C5:1-DC	
34	C5	
35	C5-DC (C6-OH)	
36	C6 (C4:1-DC)	
37	C6:1	
38	C7-DC	
39	C8	
40	C8:1	
41	C9	
42	C10	
43	C10:2	
44	C10:1	

45	C12-DC	
46	C12:1	
47	C12	
48	C14:1	
49	C14:2	
50	C14	
51	C14:1-OH	
52	C14:2-OH	
53	C16	
54	C16:2	
55	C16-OH	
56	C16:1	
57	C16:1-OH	
58	C16:2-OH	
59	C18:1-OH	
60	C18	
61	C18:1	
62	C18:2	
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63	Dopamin	biogenic amines
64	Histamine	
65	Kynurenine	
66	Methionine-Sulfoxide	
67	Nitro-Tyrosine	
68	Hydroxykynurenine	
69	Hydroxyproline	
70	Phenylethylamine	
71	Putrescine	
72	Symmetric dimethylarginine	
73	Asymmetric dimethylarginine	
74	Sarcosine	
75	Serotonin	
76	Spermidine	
77	Spermine	
78	Taurine	
79	total dimethylarginine	
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80	lysoPC C14:0	lyso-PCs
81	lysoPC C16:0	
82	lysoPC C16:1	
83	lysoPC C17:0	
84	lysoPC C18:0	
85	lysoPC C18:1	
86	lysoPC C18:2	
87	lysoPC C20:3	
88	lysoPC C20:4	
89	lysoPC C24:0	
90	lysoPC C26:0	
91	lysoPC C26:1	

92 lysoPC C28:0
93 lysoPC C28:1

Phosphatidylcholines (diacyl)

94 PC aa C24:0
95 PC aa C26:0
96 PC aa C28:1
97 PC aa C30:0
98 PC aa C30:2
99 PC aa C32:0
100 PC aa C32:1
101 PC aa C32:2
102 PC aa C32:3
103 PC aa C34:1
104 PC aa C34:2
105 PC aa C34:3
106 PC aa C34:4
107 PC aa C36:0
108 PC aa C36:1
109 PC aa C36:2
110 PC aa C36:3
111 PC aa C36:4
112 PC aa C36:5
113 PC aa C36:6
114 PC aa C38:0
115 PC aa C38:1
116 PC aa C38:3
117 PC aa C38:4
118 PC aa C38:5
119 PC aa C38:6
120 PC aa C40:1
121 PC aa C40:2
122 PC aa C40:3
123 PC aa C40:4
124 PC aa C40:5
125 PC aa C40:6
126 PC aa C42:0
127 PC aa C42:1
128 PC aa C42:2
129 PC aa C42:4
130 PC aa C42:5
131 PC aa C42:6

Phosphatidylcholines (acyl-alkyl)

132 PC ae C30:0
133 PC ae C30:1
134 PC ae C30:2
135 PC ae C32:1
136 PC ae C32:2
137 PC ae C34:0
138 PC ae C34:1

139 PC ae C34:2
140 PC ae C34:3
141 PC ae C36:0
142 PC ae C36:1
143 PC ae C36:2
144 PC ae C36:3
145 PC ae C36:4
146 PC ae C36:5
147 PC ae C38:0
148 PC ae C38:1
149 PC ae C38:2
150 PC ae C38:3
151 PC ae C38:4
152 PC ae C38:5
153 PC ae C38:6
154 PC ae C40:0
155 PC ae C40:1
156 PC ae C40:2
157 PC ae C40:3
158 PC ae C40:4
159 PC ae C40:5
160 PC ae C40:6
161 PC ae C42:0
162 PC ae C42:1
163 PC ae C42:2
164 PC ae C42:3
165 PC ae C42:4
166 PC ae C42:5
167 PC ae C44:3
168 PC ae C44:4
169 PC ae C44:5
170 PC ae C44:6

sphingomyelins

171 SM (OH) C14:1
172 SM (OH) C16:1
173 SM (OH) C22:1
174 SM (OH) C22:2
175 SM (OH) C24:1
176 SM C16:0
177 SM C16:1
178 SM C18:0
179 SM C18:1
180 SM C20:2
181 SM C22:3
182 SM C24:0
183 SM C24:1
184 SM C26:0
185 SM C26:1

186	Cholic Acid	bile acids
187	Chenodeoxycholic Acid	
188	Deoxycholic Acid	
189	Glycocholic Acid	
190	Glycochenodeoxycholic Acid	
191	Glycodeoxycholic Acid	
192	Glycolithocholic Acid	
193	Glycolithocholic Acid sulfate	
194	Glycoursodeoxycholic Acid	
195	Lithocholic Acid	
196	Taurocholic Acid	
197	Taurochenodeoxycholic Acid	
198	Taurodeoxycholic Acid	
199	Taurolithocholic Acid	
200	Taurolithocholic Acid sulfate	
201	Tauroursodeoxycholic Acid	
202	Ursodeoxycholic Acid	
203	Hexose	

204	C6:0 (caproic)	free fatty acids
205	C7:0 (heptanoic)	
206	C8:0 (caprylic)	
207	C10:0 (capric)	
208	C11:0 (undecanoic)	
209	C12:0 (lauric)	
210	C13:0 (tridecanoic)	
211	12Me-C13:0 (iso-myristic)	
212	C14:0 (myristic)	
213	c-C14:1w5 (myristoleic)	
214	13Me-C14:0 (iso)	
215	13Me-C15:0 (anteiso)	
216	14Me-C15:0 (iso)	
217	4Me-C6:0	
218	C12:2	
219	C21:0 (heneicosanoic)	
220	C22:0 (behenic)	
221	C23:0 (tricosanoic)	
222	C24:0 (lignoceric)	
223	c-C15:1w5 (c-10-pentadecenoic)	
224	c-C16:1w5	
225	c-C17:1w7 (c-10-heptadecenoic)	
226	c-C18:1w6	
227	c-C18:4w3 (stearidonic)	
228	c-C19:5w3	
229	c-C20:2w6 (c-11,14-eicosadinoic)	
230	c-C20:3w3 (c-11,14,17-C20:3)	

- 231 c-C20:3w6 (c-8,11,14-C20:3)
- 232 c-C20:3w9 (mead)
- 233 c-C20:4w3
- 234 c-C20:5w6
- 235 c-C22:1w9 (erucic)
- 236 c-C22:2w6 (c-13,16-C22:2)
- 237 c-C22:5w6
- 238 c-C24:1w9 (nervonic)
- 239 t-C18:2w6 (linolelaidic)
- 240 12Me-C14:0 (anteiso)
- 241 C15:0 (pentadecanoic)
- 242 C16:0 (palmitic)
- 243 c-C16:1w10 (sapienic)
- 244 c-C16:1w7 (palmitoleic)
- 245 15Me-C16:0 (iso)
- 246 14Me-C16:0 (anteiso)
- 247 C17:0 (heptadecanoic)
- 248 c-C17:1w8
- 249 16Me-C17:0 (iso)
- 250 C18:0 (stearic)
- 251 t-C18:1w9 (elaidic)
- 252 c-C18:1w9 (oleic)
- 253 c-C18:1w7 (vaccenic)
- 254 c-C18:2w6 (linoleic)
- 255 C19:0 (nonadecanoic)
- 256 c-C18:3w6 (gamma-linolenic)
- 257 c-C18:3w3 (linolenic)
- 258 C19:2
- 259 C20:0 (eicosanoic)
- 260 c-C20:1w9 (c-11-eicosenoic)
- 261 c-C20:4w6 (arachidonic)
- 262 c-C20:5w3 (EPA)
- 263 c-C22:4w6 (adrenic)
- 264 c-C22:5w3 (DPA)
- 265 cis-C22:6w3 (DHA; cervonic)

Supplementary Table 3 List of plasma metabolites that were excluded from data analysis. (Metabolites with >30% of the mass spectrometric signals below the limit of detection (LOD) were excluded from the data evaluation (LOD was defined as a signal-to-noise ratio of 3)).

Lyso-PCs

lysoPC C14:0
lysoPC C24:0
lysoPC C26:0
lysoPC C26:1
lysoPC C28:0

Acylcarnitines

C3:1
C3-DC (C4-OH)
C3-OH
C4:1
C5-M-DC
C5-OH (C3-DC-M)
C5:1
C5:1-DC
C6:1
C10:1
C12-DC
C12:1
C14
C14:1-OH
C14:2-OH
C16-OH
C16:1
C16:1-OH
C16:2-OH
C18:1-OH

Phosphatidylcholines (diacyl)

PC aa C24:0
PC aa C26:0
PC aa C30:2
PC aa C38:1
PC aa C40:1

Phosphatidylcholines (acyl-alkyl)

PC ae C40:0
PC ae C42:0

Free fatty acids

4Me-C6:0
C12:2
13Me-C15:0 (anteiso)
14Me-C15:0 (iso)
c-C15:1w5 (c-10-pentadecenoic)
c-C16:1w5

c-C17:1w7 (c-10-heptadecenoic)
c-C18:1w6
t-C18:2w6 (linolelaidic)
c-C18:4w3 (stearidonic)
c-C19:5w3
c-C20:2w6 (c-11,14-eicosadinoic)
c-C20:3w3 (c-11,14,17-C20:3)
c-C20:3w6 (c-8,11,14-C20:3)
c-C20:3w9 (mead)
c-C20:4w3
c-C20:5w6
C21:0 (heneicosanoic)
C22:0 (behenic)
c-C22:1w9 (erucic)
c-C22:2w6 (c-13,16-C22:2)
c-C22:5w6
C23:0 (tricosanoic)
C24:0 (lignoceric)
c-C24:1w9 (nervonic)

Biogenic amines

Dopamin
Histamine
Kynurenine
Methionine-Sulfoxide
Nitro-Tyrosine
Hydroxykynurenine
Hydroxyproline
Phenylethylamine
Putrescine
Symmetric dimethylarginine
total dimethylarginine
Sarcosine
Serotonin
Spermidine
Spermine
Taurine

Bile acids

Chenodeoxycholic Acid
Deoxycholic Acid
Glycodeoxycholic Acid
Glycolithocholic Acid
Glycoursodeoxycholic Acid
Lithocholic Acid
Taurocholic Acid
Taurochenodeoxycholic Acid
Taurodeoxycholic Acid
Taurolithocholic Acid

Tauroursodeoxycholic Acid
 Ursodeoxycholic Acid

Supplementary Table 4 Significant correlations of metabolites with insulin sensitivity (ISI) in all 40 subjects with nonalcoholic fatty liver (NAFL) and the insulin sensitive (IS) and insulin resistant (IR) NAFL subgroups at baseline.

Metabolite class	all	IS	IR
Lysophosphatidylcholines			
Total lyso-PC	+		+
Total lysoPC/PC	+		+
lysoPC C16:0	+		+
lysoPC C17:0	+	-	+
lysoPC C18:0	+		+
lysoPC C18:1	+		+
lysoPC C18:2	+		+
lysoPC C20:4	+		
lysoPC C28:1			+
Amino acids			
Glu	-		-
Ile	+		
Leu	+		
Sphingomyelins			
Total SM	+		
Total SM(non-OH)	+		
Total SM/(SM+PC)	+	+	+
SM (OH) C16:1	+		
SM (OH) C22:2	+		+
SM (OH) C24:1		+	
SM C18:0	+	+	+
SM C18:1	+	+	+
SM C26:1		+	
Acylcarnitines			
C2 (acetyl)		+	
C7-dicarboxylic (pimelyl)	+	+	
C8 (octanoyl)		+	
C10 (decanoyl)		+	
C12 (dodecanoyl)	+		
Phosphatidylcholines (diacyl)			
Total PC	-		
PC aa C32:2		-	
PC aa C32:3		-	+
PC aa C34:3		-	
PC aa C34:4		-	
PC aa C36:0		-	
PC aa C36:2		-	
PC aa C36:3		-	
Metabolite class			
Phosphatidylcholines (acyl-alkyl)			
PC ae C30:1			+
PC ae C30:2			+
PC ae C34:0		-	
PC ae C34:2		-	
PC ae C36:2		-	
PC ae C36:3		-	
PC ae C38:2		-	
PC ae C38:3		-	
PC ae C40:1		-	
PC ae C44:3		-	
Fatty acids			
Total FFA			-
FFA (even)			-
FFA (ω9)			-
FFA (ω10)			-
total SFA			-
total MUFA			-
PUFA/SFA			+
C16:0 (palmitic)			-
C16:1ω10 (sapienic)			-
C18:1ω9 (oleic)			-
C18:1ω7 (vaccenic)			-
C18:3ω6 (γ-linolenic)		-	
C20:1ω9 (c-11-eicosenoic)			-
C22:4ω6 (adrenic)			-

No metabolite out of the classes of bile acids and biogenic amines did show a significant correlation with ISI. Dark grey fields indicate $p < 0.01$, light grey fields indicate $p < 0.05$. A positive correlation is indicated by plus and a negative correlation by minus.

Supplementary Table 5 Selected characteristics of the 17 subjects without nonalcoholic fatty liver at baseline and after 9 months of lifestyle intervention

Characteristics	Insulin sensitive subjects		Insulin resistant subjects				
	Baseline (N=8)	Follow-up	Baseline (N=9)	Follow-up	p-value at baseline	p-value at follow-up	p-MANOVA time*group
Insulin sensitivity (arb. u)	17.1 ± 1.80	19.3 ± 2.23	8.5 ± 0.66	10.36 ± 1.48	<0.0001	<0.0001	0.81
Liver fat _{MRS} (%)	2.0 ± 0.51	1.2 ± 0.30	3.0 ± 0.44	3.9 ± 0.92	0.16	0.005	0.22
Gender (males / females)	1 / 7		5 / 4		0.13		
Age (years)	51 ± 3		43 ± 4		0.13		
Body mass index (kg·m ⁻²)	24.2 ± 1.57	23.3 ± 1.28	28.7 ± 1.64	28.2 ± 1.38	0.06	0.02	0.46
Waist circumference (cm)	81.3 ± 2.84	77.5 ± 2.35	97.0 ± 4.92	94.4 ± 4.0	0.02	0.003	0.65
Metabolic syndrome (%) [#]	0	22	0	44	0.09	0.01	.
Total body fat _{MRT} (kg)	19.4 ± 3.70	17.7 ± 3.43	22.9 ± 4.13	23.4 ± 4.24	0.55	0.28	0.48
Visceral fat _{MRT} (kg)	1.4 ± 0.23	1.3 ± 0.26	3.2 ± 0.61	3.0 ± 0.60*	0.06	0.08	0.60
LBM _{MRT} (kg)	47.2 ± 2.4	46.9 ± 2.7	58.7 ± 4.3	58.6 ± 5.0	0.04	0.07	0.28
IMCL _{tibialis anterior} (arb.units)	3.4 ± 0.8	3.6 ± 0.4	3.5 ± 0.8	4.1 ± 0.5	0.80	0.51	0.92
AST (U/L)	22.8 ± 1.39	23.4 ± 1.81	21.9 ± 1.66	23.1 ± 3.45	0.70	0.94	0.85
ALT (U/L)	18.1 ± 21.47	18.9 ± 2.27	26.6 ± 2.66	26.7 ± 6.44	0.02	0.30	0.91
Hs-CRP (mg/dl)	0.14 ± 0.04	0.19 ± 0.11	0.14 ± 0.05	0.16 ± 0.07	0.56	0.70	0.95
Free fatty acids (μmol/l)	686 ± 59	614 ± 62	656 ± 79	568 ± 67	0.77	0.63	0.86
Fasting glucose (mM)	5.0 ± 0.15	5.1 ± 0.20	5.6 ± 0.21	5.5 ± 0.23	0.07	0.29	0.38
2 h glucose (mM)	7.8 ± 0.63	8.1 ± 0.67	8.0 ± 0.56	8.8 ± 0.67	0.77	0.46	0.79

PAI-1 (ng/ml)	2.8 ± 0.57	3.0 ± 0.48	5.0 ± 0.76	4.5 ± 0.86	0.04	0.18	0.76
Fetuin-A (µg/ml)	251 ± 19	.	259 ± 19	.	0.79	.	

Values are means ± SEM. # χ^2 test; *p < 0.05 for change within each group. MANOVA: multivariate analysis of variance; AT: adipose tissue; MRT: magnetic resonance tomography; MRS: magnetic resonance spectroscopy; LBM: lean body mass; IMCL: intramyocellular lipids; AST: aspartate transaminase; ALT: alanine transaminase; hs-CRP: high sensitivity C-reactive protein; PAI-1: plasminogen activator inhibitor 1