

**Supplementary Table 1.** Physical and biochemical characteristics of patients in the transcriptomics study.

	Low Liver Fat	High Liver Fat	P-value
n (women)	8 (4)	8 (5)	ns
Age (years)	46 ± 3	41 ± 4	ns
PNPLA3 genotype* (CC/CG/GG)	5/1/0	3/3/1	ns
<b>Body composition</b>			
Weight (kg)	153 ± 10	146 ± 7	ns
BMI (kg/m <sup>2</sup> )	51.8 ± 2.4	49.0 ± 1.1	ns
<b>Laboratory parameters</b>			
fP-Glucose (mmol/l)	5.4 ± 0.4	6.9 ± 0.8	ns
fS-Insulin (mU/l)	13 ± 3	18 ± 4	ns
HbA <sub>1c</sub> (%)	5.4 ± 1.0	5.9 ± 0.3	ns
S-ALT (U/l)	25 ± 4	53 ± 7	< 0.01
S-AST (U/l)	25 ± 2	39 ± 4	< 0.01
S-γGT (U/l)	28 ± 6	40 ± 11	ns
P-Albumin (g/l)	37 ± 2	39 ± 3	ns
fS-Triglycerides (mmol/l)	1.8 ± 0.3	2.1 ± 0.3	ns
fS-HDL cholesterol (mmol/l)	1.3 ± 0.1	1.0 ± 0.1	< 0.05
fS-LDL cholesterol (mmol/l)	2.3 ± 0.3	2.4 ± 0.3	ns
<b>Liver histology</b>			
Macrovesicular fat (%)	2 ± 1 (range 0-10)	41 ± 7 (range 20-80)	< 0.001
NASH (%)	0%	14%	ns
Stage (n, 0/1/2/3/4)	7/0/1/0/0	6/2/0/0/0	ns

Data shown as mean ± s.e.m.

\*at rs738409. Data are missing from two subjects in the low and one subject in the high liver fat group.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; fP, fasting plasma; fS, fasting serum; NASH, non-alcoholic steatohepatitis, P, plasma; S, serum.

**Supplementary Table 2.** Clinical characteristics of the study subjects from the metabolomics study.

All subjects	
n (women)	9 (1)
Age (years)	54 ± 5
<b>Body composition</b>	
Weight (kg)	93 ± 4
BMI (kg/m <sup>2</sup> )	29.6 ± 1.1
<b>Laboratory parameters</b>	
fP-Glucose (mmol/l)	7.1 ± 1.0
fS-Insulin (mU/l)	20 ± 4
HbA <sub>1c</sub> (%)	5.7 ± 0.7
S-ALT (U/l)	92 ± 14
S-AST (U/l)	56 ± 8
S-γGT (U/l)	140 ± 40
P-Albumin (g/l)	40 ± 1
fS-Triglycerides (mmol/l)	2.7 ± 1.0
fS-HDL cholesterol (mmol/l)	1.2 ± 0.1
fS-LDL cholesterol (mmol/l)	3.4 ± 0.3
<b>Liver histology</b>	
Macrovesicular fat (%)	52 ± 26 (range 5-80)
NASH (%)	67%
Stage (n, 0/1/2/3/4)	1/3/2/3/0

Data are mean ± s.e.m.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; fP, fasting plasma; fS, fasting serum; NASH, non-alcoholic steatohepatitis; P, plasma; S, serum.

**Supplementary Table 3.** Significantly changing metabolite ratios (hepatic vein vs. artery; two-sided *t*-test) in the basal state or in low-dose insulin infusion based on the measurements, as obtained by metabolomics. Data for all measured bile acids is shown.

Basal state		Low-dose insulin infusion		Metabolite name
<i>P</i> -value	fold	<i>P</i> -value	fold	
<b>0.021</b>	-1.12	0.233	-1.05	Glycine
<b>0.040</b>	1.19	0.325	1.30	2-Ketovaline
<b>0.052</b>	-5.53	<b>0.004</b>	-1.98	Glutamine
0.054	-1.90	<b>0.017</b>	-1.21	Methionine
0.055	-2.03	<b>0.001</b>	-3.25	Alanine
0.059	1.93	<b>&lt;0.001</b>	2.34	Glutamic acid
0.065	-1.74	<b>0.019</b>	-1.43	Threonine
0.065	-1.21	<b>0.014</b>	-1.29	L-Proline, hydroxy
0.080	-1.38	<b>0.003</b>	-1.21	Tyrosine
0.303	1.05	<b>0.006</b>	1.03	Pyroglutamic acid
0.446	-2.16	<b>0.012</b>	-1.55	l-Threonine
<b>0.728</b>	-1.08	<b>0.005</b>	1.41	Isoleucine
<b>0.034</b>	-1.03	0.957	1.05	Butane, 2,3-hydroxy
0.465	1.10	<b>0.032</b>	1.53	3-Oxobutanoic acid
<b>0.056</b>	1.03	<b>0.038</b>	1.19	$\alpha$ -Hydroxybutyric acid
<b>0.059</b>	1.35	<b>0.041</b>	1.23	$\beta$ -Hydroxybutyric acid
0.200	2.34	<b>0.028</b>	-2.08	Butanoic acid, 2-methyl-3-hydroxy
0.317	1.11	<b>0.035</b>	1.14	(R*,S*)-3,4-Dihydroxybutanoic acid
0.933	1.09	<b>0.001</b>	1.46	Alpha-ketoglutaric acid
0.301	-1.01	<b>0.005</b>	1.14	b-Sitosterol
0.554	1.04	<b>0.049</b>	1.10	2-O-Glycerol-à-d-galactopyranoside
0.836	1.09	<b>0.015</b>	2.09	1H-Indole-3-acetamide
<b>0.005</b>	-1.05	<b>0.001</b>	-1.51	Cyclopentanecarboxylic acid, 1-amino-
<b>&lt;0.001</b>	-1.08	0.193	-1.04	Linoleic acid
<b>0.014</b>	-1.73	<b>0.014</b>	-1.72	Lauric acid

<b>0.023</b>	-1.64	0.152	-1.20	2-Monopalmitin
<b>0.025</b>	-2.39	<b>0.012</b>	-1.91	9-Tetradecenoic acid
0.422	-1.18	<b>0.017</b>	-1.54	Octanoic acid
0.436	-1.11	<b>0.002</b>	1.12	Propanoic acid, 2,3-hydroxy
0.620	-1.98	<b>0.019</b>	-1.89	9-Octadecenoic acid, 2-ethyl hydroxyl ester
<b>0.048</b>	-1.69	<b>&lt;0.001</b>	-1.51	Glycerol
<b>0.030</b>	-2.34	<b>0.027</b>	-1.45	1-Monooleoylglycerol
<b>0.032</b>	1.04	<b>0.011</b>	1.08	Myo-Inositol
<b>0.033</b>	-1.03	0.410	-1.02	Arabinofuranose
0.115	-2.00	<b>0.028</b>	-3.31	Arabitol
<b>0.030</b>	-1.06	<b>0.082</b>	-1.15	4-hydroxy-4-phenylbut-1-ene
<b>0.033</b>	-1.29	0.332	-1.15	GDCA
0.306	-1.10	0.337	-1.16	GCDCA
0.212	1.67	0.534	-1.60	GCA
<b>0.048</b>	-1.71	0.948	-1.50	GUDGA
0.723	-1.16	0.325	-1.14	CDCA
0.107	-1.21	0.553	-1.46	TCA
0.259	-1.15	0.482	-1.30	TDCA
1.000	-1.14	0.308	1.00	GLCA
0.067	-2.07	0.359	-1.14	UDCA
0.142	-1.52	0.179	-1.20	DCA

**Supplementary Table 4.** Enriched pathways in the basal state and in low-dose insulin infusion state. 1 and -1 denote metabolic pathways that are enriched with active or inactive reactions, respectively (hyper-geometric  $P$ -value  $<0.01$ ). 0 denotes pathways that are not enriched neither with active nor with inactive reactions.

Pathway	Basal state	Low-dose insulin infusion	Pathway	Basal state	Low-dose insulin infusion
Exchange	1	-1	Limonene and pinene degradation	0	-1
Alanine and Aspartate Metabolism	0	1	Lysine Metabolism	1	-1
Aminosugar Metabolism	-1	1	Methionine Metabolism	1	0
Arginine and Proline Metabolism	0	1	Miscellaneous	1	1
Bile Acid Biosynthesis	-1	-1	N-Glycan Biosynthesis	-1	1
Biotin Metabolism	-1	1	N-Glycan Degradation	-1	-1
beta-Alanine metabolism	0	1	Nucleotides	1	1
C5-Branched dibasic acid metabolism	1	0	Oxidative Phosphorylation	1	1
CYP Metabolism	-1	-1	Pentose Phosphate Pathway	0	1
Carnitine shuttle	-1	1	Phenylalanine metabolism	0	-1
Cholesterol Metabolism	-1	1	Purine Catabolism	-1	1
Citric Acid Cycle	1	1	Pyrimidine Biosynthesis	-1	1
CoA Biosynthesis	0	1	Pyrimidine Catabolism	-1	0
Cysteine Metabolism	0	-1	Pyruvate Metabolism	1	1
Eicosanoid Metabolism	-1	-1	R Group Synthesis	0	1
Exchange	-1	-1	Sphingolipid Metabolism	-1	1
Fatty acid activation	0	-1	Starch and Sucrose Metabolism	-1	0
Fatty acid elongation	0	1	Taurine and hypotaurine metabolism	0	-1
Fatty acid oxidation	-1	1	Tetrahydrobiopterin	1	0
Fatty acid oxidation, peroxisome	-1	0	Thiamine Metabolism	0	1
Folate Metabolism	-1	1	Transport, Endoplasmic Reticular	0	-1

Fructose and Mannose Metabolism	-1	1	Transport, Extracellular	1	0
Galactose metabolism	0	-1	Transport, Lysosomal	-1	1
Glutamate metabolism	0	1	Transport, Mitochondrial	1	1
Glutathione Metabolism	0	1	Transport, Nuclear	1	1
Glycerophospholipid Metabolism	-1	1	Transport, Peroxisomal	-1	0
Glycine, Serine, and Threonine Metabolism	0	1	Triacylglycerol Synthesis	-1	0
Glycolysis/Gluconeogenesis	1	1	Tryptophan metabolism	0	1
Glyoxylate and Dicarboxylate Metabolism	-1	0	Tyrosine metabolism	0	-1
Heme Biosynthesis	-1	-1	Urea cycle/amino group metabolism	0	1
Hyaluronan Metabolism	0	1	Vitamin A Metabolism	1	0
IMP Biosynthesis	-1	1	Vitamin B6 Metabolism	-1	0
Inositol Phosphate Metabolism	-1	1	Vitamin D	0	-1

**Supplementary Table 5.** Significant metabolite concentration changes in the low-dose insulin infusion vs. the basal state (two-sided *t*-test) based on the measurements, as obtained by metabolomics. Data for all measured bile acids is shown.

Artery		Vein		Name
<i>P</i> -value	fold	<i>P</i> -value	fold	
<b>0.001</b>	-1.72	0.003	-1.72	4-Methyl-2-oxovaleric acid
<b>0.001</b>	0.00	0.139	0.00	Methyl cis-2-trimethylsilyl-cyclopropane-1-carboxylate
<b>0.006</b>	-1.23	0.289	-1.11	Stearic acid
<b>0.006</b>	-1.31	0.549	1.12	9-Tetradecenoic acid
<b>0.006</b>	-1.39	<b>0.003</b>	-1.16	Methionine
<b>0.007</b>	3.12	<b>0.020</b>	1.38	Butanoic acid, 2-methyl-3-hydroxy
<b>0.008</b>	2.86	0.297	-1.62	Acetic acid, hydroxyl
<b>0.008</b>	-1.14	0.061	-1.04	Isoleucine
<b>0.009</b>	-1.31	0.361	-1.07	4-hydroxy-4-phenylbut-1-ene
<b>0.010</b>	-2.62	0.684	-1.34	1H-Indole, 2,6-dimethyl-
<b>0.011</b>	-1.14	0.346	-1.11	Valine
<b>0.019</b>	-1.18	0.702	-1.04	Linoleic acid
<b>0.021</b>	1.15	0.304	1.05	1-Dimethyl(isopropyl)silyloxypropane
<b>0.021</b>	-1.21	0.522	-1.13	Hydroxyproline
<b>0.022</b>	-1.28	0.737	1.08	Butanoic acid, 2-methyl-3-hydroxy
<b>0.022</b>	-1.11	0.281	-1.11	Palmitic acid
<b>0.022</b>	-1.17	0.176	-1.19	Oleic acid
<b>0.023</b>	-1.06	0.244	-1.13	Arachidonic acid
<b>0.023</b>	-1.13	0.074	-1.07	Leucine
<b>0.024</b>	-2.05	0.823	1.62	1-Methyl-1H-1,2,4-triazole
<b>0.025</b>	-2.17	0.069	-2.48	$\beta$ -Hydroxybutyric acid
<b>0.027</b>	-1.32	<b>0.011</b>	-1.16	$\alpha$ -Hydroxybutyric acid
<b>0.028</b>	-1.18	0.211	1.05	1,3-Dioxolane
<b>0.028</b>	-1.08	0.202	-1.07	Xylitol
<b>0.029</b>	1.43	0.855	1.02	Azelaic acid
<b>0.031</b>	-1.12	0.377	-1.19	Proline, 4-hydroxy-, trans-

<b>0.031</b>	-1.02	0.768	-1.00	2,2-Dimethyl-1-(2,4,6-trimethylphenyl)propan-1-one
<b>0.031</b>	1.27	0.285	1.13	Succinic acid
<b>0.035</b>	-3.36	0.103	-2.62	3-Oxobutanoic acid
<b>0.038</b>	-1.43	0.207	-1.52	Gluconic acid, $\gamma$ -lactone, 5-methoximine
<b>0.039</b>	-1.08	0.981	-1.02	Threonine
<b>0.041</b>	-1.47	0.619	1.03	Galacturonic acid
<b>0.042</b>	2.82	<b>0.009</b>	2.66	Propanedioic acid, hydroxyl
<b>0.047</b>	-1.14	0.287	-1.06	Proline
<b>0.048</b>	-1.16	0.518	1.04	Phenylalanine
<b>0.048</b>	-1.04	0.615	1.01	Hexestrol
<b>0.049</b>	1.04	0.904	-1.00	2-Butenedioic acid (Z)-
0.459	-1.13	<b>0.005</b>	2.48	1-Iodo-2-methylundecane
0.506	1.01	<b>0.003</b>	1.21	b-Sitosterol
0.577	1.05	<b>0.031</b>	-1.07	1,11-dihydroxyundecane
0.762	-1.02	<b>0.003</b>	-1.05	2,6-Bis(1,1-dimethylethyl)-4-methyl-4-methoxycyclohexa-2,5-dien-1-one
0.852	1.01	<b>0.028</b>	2.76	Urea
0.862	-1.10	<b>0.023</b>	1.47	Indole-3-lactic acid
0.330	1.08	0.762	1.21	TCA
0.488	1.16	0.242	1.10	TDCA
0.967	2.67	0.634	1.00	UDCA
0.157	-1.33	0.269	-1.17	CDCA
0.763	1.29	0.932	1.32	DCA
0.361	2.40	0.533	2.00	GCA
0.700	1.62	0.907	1.44	GUDGA
0.374	-1.14	0.304	1.00	GLCA
0.826	1.29	0.525	2.34	GDCA
0.827	1.11	0.778	1.40	GCDCA



**Supplementary Table 6.** Metabolite fluxes (or fold changes\*) across the splanchnic bed which significantly correlate with the amount of liver fat based on the measurements (without modelling).

<b>Metabolite</b>	<b>Pearson correlation (liver fat vs. metabolite flux or fold change*)</b>
<i>Basal state</i>	
Glucose	-0.966
Lactic acid	-0.962
L-valine	-0.954
Leucine	-0.946
2-Ethyl-3-hydroxybutyric acid	-0.946
Pyruvic acid	-0.939
Alanine	-0.876
Succinic acid	-0.872
<i>Cholesterol</i>	-0.841
Azelaic acid	-0.774
Arabifuranose	-0.747
$\beta$ -Sitosterol	-0.708
<i>Tricarballic acid</i>	0.712
Methione	0.727
1H-Indelo-3-acetic acid	0.729
3-Hydroxybenzoic acid	0.730
<i>Aminomaloic acid</i>	0.735
l-Tryphtophan	0.765
<i>Urea</i>	0.778
Citric acid	0.788
Phenylalanine	0.791
Glutamine	0.824
Creatinine	0.846
<i>l-Proline, 4-hydroxy</i>	0.848
<i>Altronic acid, lactone</i>	0.849

Butanedioic acid, methylene	0.876
Decanoic acid	0.848
Hexadecanoic acid	0.880
9-Tetradecenoic acid	0.883
2,3,4-Trihydroxybutyric acid	0.892
<i>Ethanolamine</i>	0.921
<i>Glycerol-3-phosphate</i>	0.924
Butanal, 2,3,4-hydroxy	0.926
Butane, 2,3-hydroxy	0.929
<i>Threonic acid</i>	0.940
Aspartic acid	0.950
Eicosanoic acid	0.954
Oleic acid	0.962
<i>Glyceric acid-3-phosphate</i>	0.971
Docosahexenoic acid	0.990
Monopalmitin	0.995
<b><i>Low-dose insulin</i></b>	
$\alpha$ -Hydroxybutyric acid	-0.945
Stearic acid	-0.859
Ornithine	-0.842
4-Methyl-2-oxovaleric acid	-0.818
Adipic acid	-0.789
<i>Cinnamic acid</i>	0.753
Citric acid	0.819

\*For metabolites (marked with *cursive*) which were not quantified (*i.e.*, were determined semi-quantitatively), fold changes were used instead of fluxes. For quantified metabolites we found that there is practically no difference in correlation values if fluxes or fold changes are used.

**Supplementary Table 7.** Reactions whose maximal flux rate in the basal state is correlated with liver fat levels, according to the flux-based modelling.

Reaction	Subsystem	Spearman (empirical) <i>P</i> -value*	Correlation	Maximal flux rate at different fat levels				
				5	30	65	70	70
pyruvate carboxylase	Pyruvate Metabolism	0.033 (8.26E-03)	0.975	0	86.4	524.98	814.97	1000
dimethylglycine dehydrogenase, mitochondrial	Glycine, Serine, and Threonine Metabolism	0.033 (8.26E-03)	0.975	25	50	63.76	84.28	191.83
Sarcosine dehydrogenase (m)	Urea cycle/amino group metabolism	0.033 (8.26E-03)	0.975	25	50	100.43	168.56	507.72
guanylate kinase (GMP:ATP)	Nucleotides	0.033 (8.26E-03)	0.975	0	57.35	97.62	143.72	1000
5'-nucleotidase (GMP)	Nucleotides	0.033 (8.26E-03)	0.975	0	7.35	97.62	156.69	1000
pyruvate mitochondrial transport via proton symport	Transport, Mitochondrial	0.033 (8.26E-03)	0.975	-201.61	-79.61	517.9	648.04	1000
1-acylglycerol-3-phosphate O-acyltransferase 1	Triacylglycerol Synthesis	0.033 (8.26E-03)	0.975	21.77	83.99	342.06	613.06	443.46
Deoxyadenosine deaminase	Nucleotides	0.033 (8.26E-03)	0.975	101.92	126.79	150	252.64	1000
glucose 6-phosphate endoplasmic reticular transport via diffusion	Transport, Endoplasmic Reticular	0.033 (8.26E-03)	0.975	0	49.45	50.99	210.4	1000
L-proline reversible transport via proton symport	Transport, Extracellular	0.033 (8.26E-03)	1.000	863.6	982.77	998.46	1000	1000

formaldehyde transport via diffusion (mitochondrial)	Transport, Mitochondrial	0.033 (8.26E-03)	0.975	25	50	63.76	84.28	191.83
5'-nucleotidase (dGMP)	Nucleotides	0.033 (8.26E-03)	0.975	50	61.44	150.02	158.99	1000
CO2 transport (diffusion), mitochondrial	Transport, Mitochondrial	0.033 (8.26E-03)	0.975	149.82	249.81	568.21	925.3	1000
Inosine transport (diffusion)	Transport, Extracellular	0.033 (8.26E-03)	0.975	51.92	76.79	100	202.64	1000
purine-nucleoside phosphorylase (Deoxyinosine)	Purine Catabolism	0.033 (8.26E-03)	0.975	51.92	76.79	100	202.64	1000
methenyltetrahydrofolate cyclohydrolase, mitochondrial	Folate Metabolism	0.033 (8.26E-03)	0.975	25	50	100.43	168.56	666.78
Proline transport (sodium symport) (2:1)	Transport, Extracellular	0.033 (8.26E-03)	1.000	863.6	982.77	998.46	1000	1000
betaine-homocysteine S-methyltransferase	Glycine, Serine, and Threonine Metabolism	0.033 (8.26E-03)	0.975	25	50	63.76	84.28	191.83
H2O endoplasmic reticulum transport	Transport, Endoplasmic Reticular	0.033 (8.26E-03)	0.975	535.8	559.34	600.5	846.6	1000
3-oxoacid CoA-transferase	Valine, Leucine, and Isoleucine Metabolism	0.033 (8.26E-03)	0.975	0	98.48	137.83	373.71	1000
Citrate exchange		0.033 (8.26E-03)	1.000	466.51	638.71	832.58	1000	1000
retinol acyltransferase	Vitamin A Metabolism	0.033 (8.26E-03)	0.975	0	50	301.15	1000	551.86
R total flux	R Group Synthesis	0.033 (8.26E-03)	0.975	0	50	50.41	106.25	384.81
dimethylglycine transport via diffusion (cytosol to mitochondria)	Transport, Mitochondrial	0.033 (8.26E-03)	0.975	25	50	63.76	84.28	191.83

phosphoglycerate mutase	Glycolysis/Gluconeogenesis	0.033 (8.26E-03)	0.975	-102.73	0	695.05	995.07	1000
monoacylglycerol acyltransferase	Triacylglycerol Synthesis	0.033 (8.26E-03)	0.975	0	52.71	299.16	1000	555.29
formaldehyde dehydrogenase	Tyrosine metabolism	0.033 (8.26E-03)	0.975	-2.32	100	113.76	134.28	288.96
glutamine-fructose-6-phosphate transaminase	Aminosugar Metabolism	0.033 (8.26E-03)	0.975	0	38.23	210.9	222.97	1000
glycerol kinase	Glycerophospholipid Metabolism	0.033 (8.26E-03)	0.975	0	29.87	198.25	607.39	295.91
deoxyinosine transport via diffusion	Transport, Extracellular	0.033 (8.26E-03)	0.975	51.92	76.79	100	202.64	1000
Deoxyinosine exchange		0.033 (8.26E-03)	0.975	338.05	527.74	728.4	891.31	1000
Deoxyadenosine deaminase, extracellular	Nucleotides	0.033 (8.26E-03)	0.975	101.92	126.79	150	252.64	1000
L-carnitine transport out of mitochondria via diffusion	Transport, Mitochondrial	0.033 (8.26E-03)	0.975	0	2.95	7.9	262.41	1000
formate-tetrahydrofolate ligase	Folate Metabolism	0.033 (8.26E-03)	1.000	-52.32	137.11	776.91	1000	1000
Adenosine deaminase, extracellular	Nucleotides	0.033 (8.26E-03)	0.975	101.92	126.79	150	252.64	1000
S-Formylglutathione hydralase	Tyrosine metabolism	0.033 (8.26E-03)	0.975	-2.32	100	113.76	134.28	288.96
Adenosine deaminase	Purine Catabolism	0.033 (8.26E-03)	0.975	101.92	126.79	150	252.64	1000
glycerol-3-phosphate acyltransferase	Triacylglycerol Synthesis	0.033 (8.26E-03)	0.975	21.77	83.99	342.06	613.06	443.46
glucose-6-phosphate phosphatase, edoplasmic	Glycolysis/Gluconeogenesis	0.033 (8.26E-03)	0.975	0	49.45	50.99	210.4	1000

reticular								
-----------	--	--	--	--	--	--	--	--

\*Corrected for multiple hypothesis *via* FDR, with false discovery rate limited to 0.1.