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# The acute effect of metabolic cofactor supplementation, a potential therapeutic strategy against non-alcoholic fatty liver disease

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**Appendix Figure S5** 

**Appendix Figure S6** 

#### Appendix Table S1. Observations and measurements

SIG-41				
Group 1 females, Dos level 304 mg	/kg			
Observation	Number of animals	Number of obs./day	From study day	To study day
Nothing abnormally detected (NAD)	3	21	1	7
PILO	2	2	1	1
Excessive chewing (EXCH)	2	2	1	1

SIG-41						
Group 2 females, Dos level 3040 mg	/kg					
Observation	Number of animals	Number of obs./day	From study day	To study day		
Nothing abnormally detected (NAD)	3	21	1	7		
Plouing in the bedding (PLOU)	2	11	2	7		
Pilo erecction (PILO	3	3	1	1		
Excessive chewing (EXCH)	3	3	1	1		
Motor activity decreased (MADE)	2	2	1	1		
Eyes half shut (EYHS)	3	3	1	1		

SIG-41				
Group 3 females, Dos level 11820/7880	mg/kg			
Observation	Number of animals	Number of obs./day	From study day	To study day
Nothing abnormally detected (NAD)	3	21	1	7
Ploughing in the bedding (PLOU)	3	18	1	7
Pilo erection (PILO)	3	3	1	1
Excessive chewing (EXCH)	3	3	1	1
Vocalisation during handling (VODH)	1	2	6	7
Difficulty dosing (DIDO)	1	1	3	3
Ataxia (ATAX)	2	2	2	2
Cyanosis (CYAN)	1	1	2	2
Respiration irregular (REIR)	1	2	1	2
Respiration noisy (RENO)	1	1	1	1
Motor activity decreased (MADE)	3	5	1	2
Eyes half shut (EYHS)	3	4	1	2

Observations: Observations were made daily after dosing until "No

Adverse Finding" is observed and recorded. If adverse findings were observed, they were recorded during 0-30 MAD, 1-6 HAD, and 6-24 HAD, or more frequently if found

necessary.

Body weight Body weights were recorded prior to first dosing Day 1 and

anytime if decline in general condition was observed.

#### Appendix Table S2. Haematology data

SIG-41				WBC	LYM	MONO	GRAN	LYM	MONO	GRAN	HGB	HCT	RBC	MCV	МСН	MCHC	RDW%	RDWa	PLT	MPV
ID/Animal no	Gender	Group	/Analysis date	(10 <sup>9</sup> /L)	(10 <sup>9</sup> /L)	(10 <sup>9</sup> /L)	(10 <sup>9</sup> /L)	%	%	%	g/L	%	(10 <sup>12</sup> /L)	fL	pg	g/dL	%	fl	(10 <sup>9</sup> /L)	fL
1	F	1	20-Dec-17	8.1	7.2	0.2	0.7	88.9	1.4	9.7	152	42.5	8.35	50.9	18.2	357	17.9	32.9	574	5.8
2	F	1	20-Dec-17	5.4	4.7	0.1	0.6	86.5	2.1	11.4	145	39.4	7.93	49.7	18.3	369	18.6	32.8	632	5.8
3	F	1	20-Dec-17	6.4	4.9	0.2	1.3	77.2	2.5	20.3	151	39.8	8.02	49.6	18.8	380	18.1	32.3	592	6.0
4	F	2	20-Dec-17	7.7	6.0	0.4	1.3	78.3	4.9	16.8	152	40.5	7.79	52.0	19.5	375	18.6	34.7	607	5.9
5	F	2	20-Dec-17	9.6	8.7	0.3	0.6	91.4	1.6	7.0	152	40.7	7.88	51.6	19.4	375	18.6	34.2	701	5.9
6	F	2	20-Dec-17	6.9	5.7	0.2	1.0	83.4	2.1	14.5	143	37.7	7.72	48.9	18.6	380	18.0	31.0	656	5.4
7	F	3	20-Dec-17	6.9	5.5	0.2	1.2	79.2	2.4	18.4	154	40.9	8.30	49.2	18.6	378	19.2	32.7	737	5.7
8	F	3	20-Dec-17	7.1	5.5	0.3	1.3	76.9	4.8	18.3	141	36.9	7.28	50.7	19.4	383	18.5	33.6	543	5.7
9	F	3	20-Dec-17	7.2	5.8	0.3	1.1	81.3	2.5	16.2	145	38.8	7.60	51.1	19.1	374	18.7	33.8	697	5.7

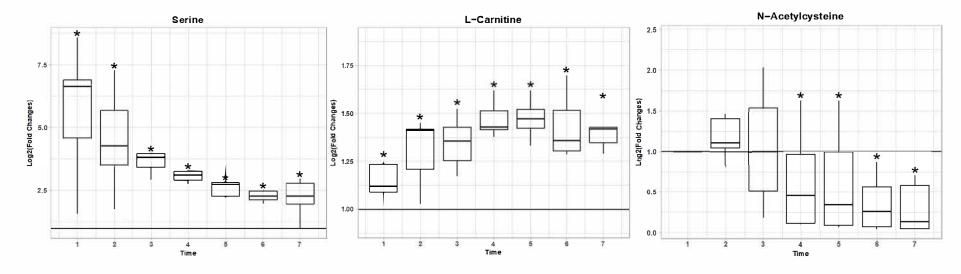
The below parameters were analyzed and evaluated. Red cell distribution width (absolute) and Mean platelet volume were also analyzed as part of the test kit used for the analyses, but are reported in the Pathology evaluation.

Red blood cells	RBC
Red cell distribution width (%)	RDW%
Red cell distribution width (absolute)	RDWa
Mean red cell hemoglobin	MCH
Hematocrit	HCT
Mean red cell hemoglobin concentration	MCHC
Mean red cell volume	MCV
Hemoglobin	HGB
Platelets	PLT
Mean platelet volume	MPV
White blood cells	WBC
Lymphocytes	LYM
Granulocytes	GRAN
Monocytes	MONO

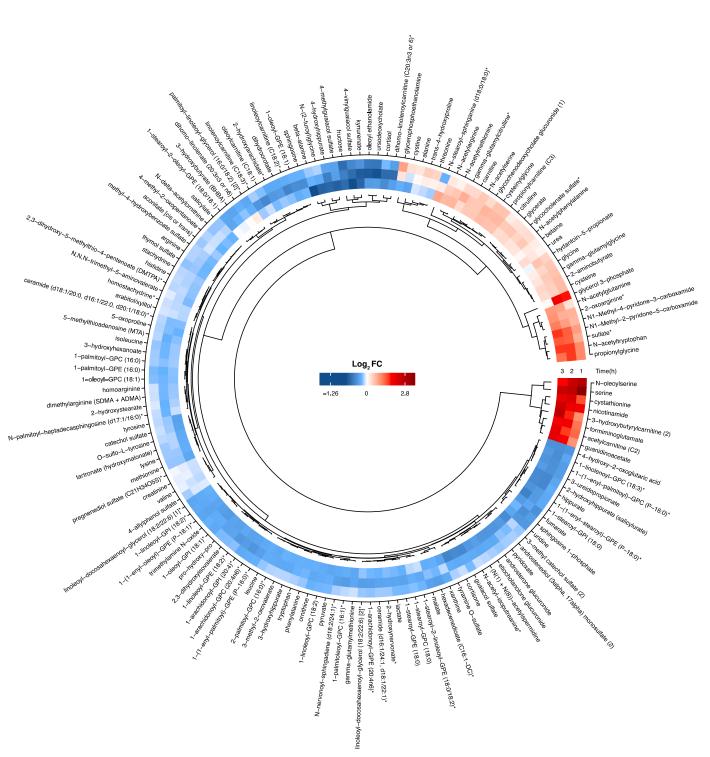
Appendix Table S3. Plasma chemistry data

SIG-41			Sample/	ALB	ALP	ALT	AMY	TBIL	BUN	CA	PHOS	CRE	GLU	NA+	K+	TP	GLOB
ID/Animal no	Gender	Group	Analysis dat	g/L	U/L	U/L	U/L	μmol/L	mmol/L	mmol/L	mmol/L	μmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L
1	F	1	20-Dec-17	61	162	33	550	5	4,8	2,84	2,08	62	9,4	139	3,7	68	7
2	F	1	20-Dec-17	72	139	26	603	5	6,3	2,92	1,72	49	8,7	138	4,1	77	0
3	F	1	20-Dec-17	65	158	44	606	5	5,4	3,01	2,52	43	7,8	139	4,5	76	11
4	F	2	20-Dec-17	72	198	49	689	5	7,0	2,93	2,04	28	7,6	137	4,1	78	0
5	F	2	20-Dec-17	64	163	35	725	5	6,1	3,02	2,24	40	8,6	140	4,5	71	7
6	F	2	20-Dec-17	62	111	27	647	5	7,1	2,87	2,20	18	9,2	136	4,6	67	5
7	F	3	20-Dec-17	65	128	32	644	5	6,0	2,90	2,25	26	8,9	138	4,6	72	8
8	F	3	20-Dec-17	62	123	26	566	5	5,3	2,91	2,24	43	8,8	139	5,1	68	6
9	F	3	20-Dec-17	61	102	28	650	5	5,6	2,89	2,06	39	7,5	138	4,0	69	8

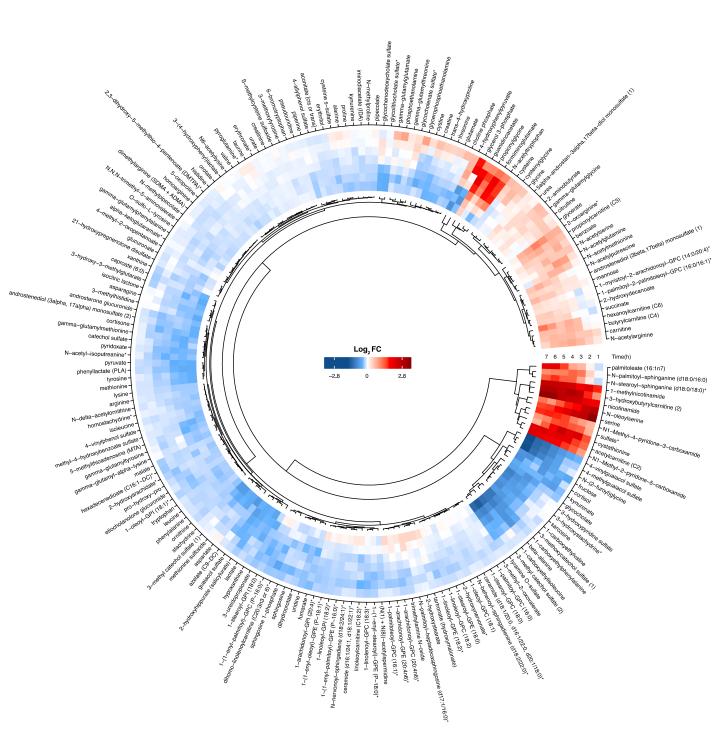
ALB Albumin Alanine aminotransferase ALT Alkaline phosphatase ALP Amylase AMYL Bilirubin (total) **TBIL** Blood urea nitrogen BUN Calcium  $Ca^{++}$ Creatinine CREA Globulin GLOB Glucose GLU Phosphate PHOS Potassium  $K^{+}$  $Na^{+}$ Sodium Total protein TP



**Appendix Figure S1**. Changes in plasma level of each metabolic cofactor (except for NR) in supplementation study compared to baseline based on targeted metabolomics measurement.

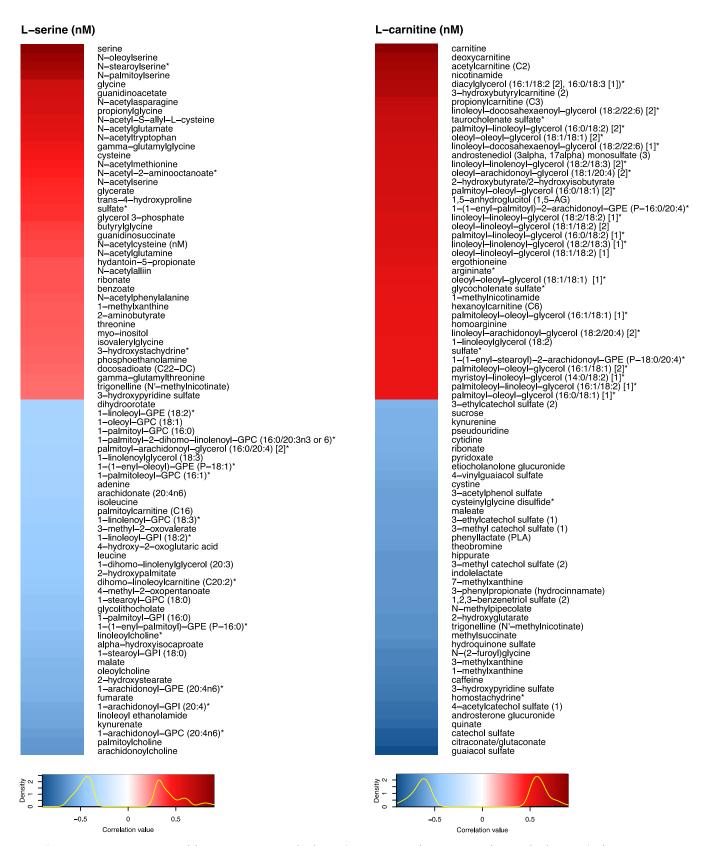


**Appendix Figure S2.** Changes in plasma level of metabolites in at least 2 out of 3 time points compared to baseline based on un-targeted metabolomics measurement. Identified metabolites were clustered based on hierarchical analysis using log2 FC as a distance metric. Significance level of P-value<0.05 after FDR adjustment.



**Appendix Figure S3.** Changes in plasma level of metabolites in at least 3 out of 7 time points compared to baseline based on un-targeted metabolomics measurement. Identified metabolites were clustered based on hierarchical analysis using log2 FC as a distance metric. Significance level of P-value<0.05 after FDR adjustment.

A



**Appendix Figure S4.** Top and bottom 40 metabolites (un-targeted & targeted metabolomics) that are significantly associated with supplemented L-Serine and L-Carnitine based on Spearman correlation analysis.

A

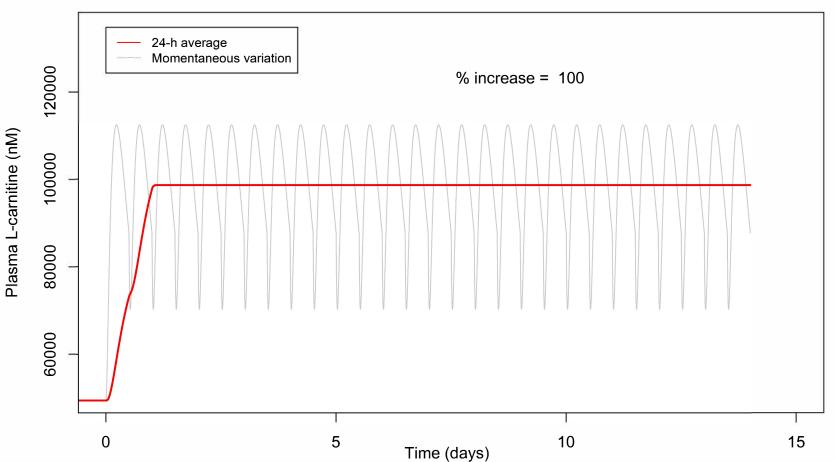
#### **Nicotinamide** N-acetylcysteine (nM) acetylcarnitine (C2) syringol sulfate o-cresol sulfate glycerol 3-phosphate N-acetyl-S-allyl-L-cysteine 4-methylguaiacol sulfate 3–hýdroxybutyrylcárnitine (2) carnitine L-carnitine (nM) 1-methylnicotinamide propionylcarnitine (C3) beta-alanine N1-Methyl-4-pyridone-3-carboxamide N1-Methyl-2-pyridone-5-carboxamide deoxycarnitine proline trans-4-hydroxyproline alanine tiglylcarnitine (C5:1-DC) 1-carboxyethylphenylalanine isocitric lactone threonine Detaile 2–hydroxybutyrate/2–hydroxyisobutyrate hexanoylcarnitine (C6) diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])\* choline phosphate phenyllactate (PLA) cystathionine linoleoyl–docosahexaenoyl–glycerol (18:2/22:6) [2]\* 1–palmitoyl–2–arachidonoyl–GPC (16:0/20:4n6) 1,5–anhydroglucitol (1,5–AG) oleoyl–oleoyl–glycerol (18:1/18:1) [2]\* 2–aminobutyrate N–palmitoyl–sphinganine (d18:0/16:0) linoleoyl–linoleoyl–glycerol (18:2/18:3) [1]\* linoleoyl–docosahexaenoyl–glycerol (18:2/22:6) [1]\* palmitoyl–linoleoyl–glycerol (16:0/18:2) [2]\* eroothioneine cystathionine pyruvate cysteine N-acetyltryptophan creatiné 2,5-dimethylphenol sulfate mannitol/sorbitol sarcosine 1-carboxyethylvaline 3-methoxycatechol sulfate (1) iminodiacetate (IDA) 1-carboxyethylisoleucine tyramine O-sulfate ergothioneine ergourioneine oleoyl-linoleoyl-glycerol (18:1/18:2) [2] linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]\* oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]\* 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)\* ásparagine hydantoin–5–propionate 1–carboxyethylleucine 2-piperidinone xylose 4-hydroxyglutamate oleoyl–arachidonoyl–glycerol (18:1/20:4) [1]\* taurocholenate sulfate\* N-acetylleucine 1–(1–enyl–palmitoyl)–2–arachidonoyl–GPE (P–16:0/20:4)\* alpha–ketobutyrate 3-hydróxystachydrine\* cysteine s-sulfate 3-methylhistidine 2'-deoxyuridine 2 – deoxyurlarie 1–palmitoyl–2–docosahexaenoyl–GPC (16:0/22:6) linoleoyl–linoleoyl–glycerol (18:2/18:2) [1]\* androstenediol (3alpha, 17alpha) monosulfate (3) formiminoglutamate trigonelline (N'-methylnicotinate) thioproline prolylglycine glycine 4–hydroxyphenylacetate dihomo–linoleate (20:2n6) dihydroorotate 1-stearoyl-2-linoleoyl-GPI (18:0/18:2) stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]\* kyńurenine imidazole lactate 3-hydroxyoctanoate myristoylcarnitine (C14) syringol sulfate asparagine 3-hydróxyhexanoàte linolenate [alpha or gamma; (18:3n3 or 6)] N1–Methyl–4–pyridone–3–carboxamide eicosapentaenoate (EPA; 20:5n3) palmitoleate (16:1n7) galactonate sucrose 4–vinylphenol sulfate indolelactate 1-methylxanthine (16 or 17)-methylstearate (a19:0 or i19:0) dihomo-linolenate (20:3n3 or n6) cytidine 1,7-dimethylurate 2-hydroxystearate docosapentaenoate (n3 DPA; 22:5n3) maleate ferulic acid 4-sulfate hexadecadienoate (16:2n6) etiocholanolone glucuronide homostachydrine\* 10-heptadecenoatè (17:1n7) arachidonate (20:4n6) oleate/vaccenate (18:1) N–palmitoyl–sphinganine (d18:0/16:0) 5–dodecenoate (12:1n7) theobromine 3-ethylcatechol sulfate (2) hydroquinone sulfate phenyllactate (PLA) 4–acetylcatechol sulfate (1) 2-hydroxypalmitate succinate 1-arachidonoyl-GPE (20:4n6)\* linoleate (18:2n6) cystine 7-methylxanthine 3-ethylcatechol sulfate (1) myristoleate (14:1n5) 3-methylxanthine 3-acetylphenol sulfate androsterone glucuronide palmitate (16:0) dodecadienoate (12:2)\* pentadecanoate (15:0) margarate (17:0) 3-methyl catechol sulfate (1) 10-nonadecenoate (19:1n9) stearate (18:0) nonadecanoate (19:0) pyridoxate quinate 2-hydroxyglutarate citraconate/glutaconate 3-hydroxybutyrate (BHBA) stearoyl ethanolamide laurate (12:0) 3–hydroxydecanoate N–acetylputrescine 3-methyl catechol sulfate (2) 3-hydroxypyridine sulfate 4-vinylguaiacol sulfate catechol sulfate myristate (14:0) guaiacol sulfate N-(2-furoyl)glycine 3-hydroxylaurate (14 or 15)-methylpalmitate (a17:0 or i17:0)

**Appendix Figure S5.** (A – B) Top and bottom 40 metabolites (un-targeted & targeted metabolomics) that are significantly associated with supplemented N-acetylcysteine and Nicotinamide based on Spearman correlation analysis.

Correlation value

Correlation value

### L-carnitine, dose = 8.3 g every 12 h



**Appendix Figure S6.** Predicted plasma L-carnitine during two weeks of simulated twice-daily supplementation: 24-hour moving average value indicated in red and continuous/momentaneous indicated in gray.