SUPPLEMENTARY INFORMATION

Small Metabolites, Possible Big Changes—a Microbiota-centered View of Non-alcoholic Fatty Liver Disease

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Supplementary Table 1 Change of metabolites associated with NAFLD in feces

Disease	comparision	Change of metabolites		Method	Ref
		Increased	Decreased		
NAFLD(n=11) Healthy(n=22)	NAFLD vs Healthy	ethanol; lysine	SCFAs(specifcally formate, acetate, and valerate); alanine	^{1}H NMR	Michail S[1]
NAFLD(n=53) Healthy(n=54)	NAFLD vs Healthy	4-methyl-2-pentanone; 1-butanol; 2-butanone	aromatic hydrocarbons; hydrazines	GC-MS	Del Chierico[2]
NAFLD(n=30) Healthy(n=30)	NAFLD vs Healthy	butanoic acid, propyl ester; propanoic acid, propyl ester; acetic acid, ethyl ester; acetic acid, pentyl ester; butanoic acid, 3-methyl-, butyl ester; n-Propyl acetate; butanoic acid, butyl ester; propanoic acid, ethyl ester; pentanoic acid, methyl ester; acetic acid, methyl ester; 2-propynoic acid methyl ester; butanoic acid, 3-methyl-, ethyl ester; propanoic acid, 2-methyl-, propyl ester; cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3Rtrans)-; phellandrene; 1,6-octadien-3-ol, 3,7-dimethyl; myrcene; 1-propanol	2-butanone; furan, 2-methyl; heptanal 2(3H)-furanone, dihydro-5- methyl; 2-heptanone, 6-methyl; 2,3-pentanedione; 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate; cyclohexanol, 5-methyl-2-(1-methylethyl)-; 2-octene, 3,7-dimethyl-, (Z)-; 3-hexanone, 2-methyl-; acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester; cyclohexane, hexyl-	GC-MS	Raman M[3]
NAFLD(n=86)	NAFLD enrichment	ethanol; l-lactate; formate; acetate; D-lactate; succinate; butyrate/acetate; butyrate; propionate		UHLC/MS/MS^{2} and GC/MS	Loomba R[4]

Comparison of condition A vs condition B: Increased signifies an increase in condition A relative to condition B. Decreased signifies a decrease in condition A relative to condition B.

NAFLD: non-alcoholic fatty liver disease; SCFAs: short-chain fatty acids; ^{1}H NMR: proton NMR; GC-MS: gas chromatography-mass spectrometry; UHLC/MS/MS^{2}：ultrahigh performance liquid chromatography/tandem mass spectrometry; GC/MS: gas chromatography/mass spectrometry.

Supplementary Table 2 Change of metabolites associated with NAFLD in serum/plasma

Disease	Comparison	Change of metabolites		Method and tissue	Ref
		Increased	Decreased		
NAFLD(n=15) Non-NAFLD(n=30) at baseline	NAFLD vs Non NAFLD	monoetherglycerophosphocholineO_plasmenyles; monoetherglycerophosphocholineP_plasmenyles; 1-monoetherglycerophosphocholine; L-cystine to L-glutamate ratio	1-ether, 2-acylglycerophosphocholine P_plasmenyles;ceramides; N-acyl ceramides; N-acyl sphingosines; total sphingolipids;sum of triacylglycerols & cholesteryl esters; activity index of sphingomyelinase (Cer/SM)	LC-MS/MS, serum	Papandreou C[5]
NAFLD(n=75) Lean normal control(n=50)	NAFLD vs Lean normal control	total plasma fatty acids; total saturated fatty acids; monounsaturated fatty acids; palmitoleic(16:1 n7) acid; oleic(18:1 n9) acid; vaccenic (18:1 n7) acid; DAG; TAG; DAG and TAG: palmitoleic (16:1 n7), oleic (18:1 n9); PC, PE, and LyPC: palmitoleic acid (16:1 n7), stearic acid (18:0), stearic (18:0) acid to palmitic (16:0) acid ratio; monounsaturated fatty acids: palmitoleic acid (16:1 n7) to palmitic acid (16:0) ratio, oleic acid (18:1 n9); polyunsaturated fatty acids: gamma-linolenic (18:3n6) acids, dihomo γ-linolenic (20:3n6) acids, linoleic acid (18:2n6), dihomo γ-linolenic acid (20:3n6), docosapentaenoic acid (DPA, 22:5n3); 5-HETE; 8-HETE; 15-HETE; 11-HETE	DAG and TAG: stearic acid (18:0), lignoceric acid (24:0), behenic acid (22:0); monounsaturated fatty acids: stearic acid (18:0); polyunsaturated fatty acids: linoleic acid (18: 2n6), α-linolenic acid (18:3n3), DHA:DPA ratio; plasmalogen (dm) and dm16:0	GC and LC-MS, plasma	Puri P[6]
NAFLD(n=914) Healthy(n=714)	NAFLD vs Healthy	TMAO	betaine	HPLC-MS/MS, serum	Chen YM[7]
NASH(n=16) Healthy(n=11)	NASH vs Healthy	total bile acid; CA; CDCA; DCA; UDCA; secondary bile acids to primary bile acids ratio; DCA to CDCA ratio		LC-MS/MS, serum	Jiao N[8]

Supplementary Table 2 continued

Disease	Comparison	Change of metabolites		Method and tissue	Ref
		Increased	Decreased		
NASH(n=7) Healthy(n=15)	NASH vs Healthy	total bile acid; taurine-conjugated bile acids; glycine-conjugated bile acids; primary bile acids; secondary bile acids; CA; LCA; CDCA; DCA; UDCA		UPLC-MS/MS, serum	Ferslew BC[9]
NAFLD(n=64) Healthy(n=64)	NAFLD vs Healthy NASH vs Not-NASH	homocysteine; cysteine; cysteinylglycine	glutathione homocysteine; cysteine	HPLC, plasma	Pastore A[10]
Obese patients with NAFLD(n=154) Obese patients without NAFLD(n=76)	Obese patients with NAFLD vs Obese patients without NAFLD	leucine; isoleucine; valine; 3-methyl-2-oxobutyrate; 3-methyl-2-oxovalerate; 4-methyl-2-oxopentanoate; α-ketoglutarate; succinylcarnitine; bradykinin; des-Arg9-bradykinin; dicarboxylic acid 2-hydroxybutyrate(α–hydroxybutyrate); glycocholate; taurocholate	3-hydroxybutyrate; 1,5-anhydroglucitol ; 4-androsten-3β; 17-β-dioldisulphate 1; 17-β-diol disulphate 2; 5α-androstan-3β; 5α-pregnan-3β; 20α-diol disulphate; pregnen-diol disulphate; pregnen steroid monosulphate; andro steroid monosulphate 2; 21-hydroxypregnenolone disulphate	LC/GC-QTOF-MS, plasma	Rodr ǵuez-Gallego E[11]
NAFLD(n=32) Healthy(n=28)	NAFLD vs Healthy	lactate; glutamate; glucose; taurine		^{1}H NMR, serum	Li H[12]
NAFLD(n=105) Healthy(n = 48)	NAFLD vs Healthy	16-OH-DHEA-S	etiocholanolone-S; DHEA-S	CE-TOF-MS andLC-TOF-MS, serum	Tokushige K[13]
Obese patients with NAFLD(n=24) NASH(n=9)	NAFLD vs Control	LPC; DCA; sphingomyelin: (SM 36:3), (d18:2/16:0), (d18:2/14:0), (d18:1/18:0), (d18:1/16:0), (d18:1/12:0), (d18:0/16:0); arachidonic acid	creatine; linoleic(18:2n-6) ; α-linolenic(18:3n-3)	UPLC-MS, serum	Barr J[14]
Obese patients without NAFLD(n=9)	NASH vs NAFLD	PC(14:0/20:4); LPC(18:1)	LPC(24:0); arachidonic acid(20:4n-6); PC(P-24:0/0:0); PC(P-22:0/0:0)		

Supplementary Table 2 continued

Disease	Comparison	Change of metabolites		Method and tissue	Ref
		Increased	Decreased		
NAFL(n=25) NASH(n=37) Non-NAFLD(n=24)	NAFLD vs Non-NAFLD	proportion of total plasma primary BAs; GCA; TCA; GCDCA; TCDCA; glycoursodeoxycholate; tauroursodeoxycholate; DCA; ratio of conjugated to unconjugated CA; ratio of conjugated to unconjugated CDCA; ratios of the total CA to total CDCA; hydrophilic trihydroxy bile acids	proportion of total secondary BAs; hydrophobic bile acids to hydrophilic bile acids ratio	LC/MS, plasma	Puri P[15]
	NASH vs NAFL	proportion of total plasma primary BAs; GCA; TCA; GCDCA; TCDCA; glycoursodeoxycholate; tauroursodeoxycholate; ratio of conjugated to unconjugated CA; ratio of conjugated to unconjugated CDCA;	proportion of total secondary BAs		
Hepatic steatosis (n=11) NASH(n=24) Healthy(n=25)	Hepatic steatosis vs Healthy	homocysteine; cysteine; taurocholate; glutamyl dipeptides; glutamyl valine; glutamyl leucine; glutamyl phenylalanine; glutamyl tyrosine; free carnitine; butyrylcarnitine; gamma-glutamylphenylalanine; gamma-glutamyltyrosine; mannose; lactate; erythronate; glutamate; lysine; tyrosine; isoleucine; glycocholate; glycochenodeoxycholate; propionylcarnitine; 2-methylbutyroylcarnitine; glucose;pyruvate; phenylalanine; branched chain amino acids; leucine; valine; aspartate; others(e.g.,x-11546 and x-11529)	glutathione; cysteine-glutathione disulfide; caprate(C10:0); 10-undecenoate(C11:1n1); eicosopentaenoate(C20:5n3); docosohexaenoate(C22:6n3); arachidonate(C20:4n6); glycerophosphocholine; 1-oleoylglycerophosphocholine; 1-linoleoylglycerophosphocholine; 1-arachidonoylglycerophosphocholine	UHLC/MS/MS and GC/MS, plasma	Kalhan SC[16]
	NASH vs. Steatosis	glutamate; creatine; pyruvate; unknown X-01911_200	undecenoate(C11:0); linolenate(alpha or gamma); linolenate(C18:3n3 or 6)		

Supplementary Table 2 continued

Disease	Comparison	Change of metabolites		Method and tissue	Ref
		Increased	Decreased		
NAFLD(n=56)	NAFLD enrichment	succinate; malate; α-ketoglutarate; glutamine; serine; fumarate; α-ketobutyrate, glutamate; lactate; hypoxanthine; inosine		UHLC/MS/MS^2 and GC/MS, serum	Loomba R[4]
NAFLD (n = 36) Non-NAFLD (n = 120)	NAFLD vs Non NAFLD	2-hydroxy-3-methylvalerate; 3-(4-hydroxyphenyl)lactate; 3-methyl-2-oxobutyrate; 3-methyl-2-oxovalerate; 4-acetamidobutanoate; 4-methyl-2-oxopentanoate; C-glycosyltryptophan*; creatinine; indolelactate; isoleucine; kynurenate; leucine; N6-acetyllysine; N-acetylisoleucine; N-acetylphenylalanine; N-acetylvaline; N-formylmethionine; phenylalanine; phenyllactate(PLA); S-adenosylhomocysteine(SAH); valine; erythronate*; glucose; lactate; mannitol; sorbitol; malate; succinylcamitine; 3-hydroxy-3-methylglutarate; 4-androsten-3beta, 17beta-diol disulfate(1); maleate(cis-Butenedioate); TL 18:1n9(oleic acid); 7-methylguanine; allantoin; N1-methyladenosine; N1-methylguanosine; N2,N2-dimethylguanosine; N6-carbamoylthreonyladenosine; N6-succinyladenosine; orotidine; psuedouridine; urate; xanthosine; cyclo(leu-pro); cyclo(L-phe-L-pro); gamma-glutamylisolecine*; gamma-glutamyleucine; gamma-glutamyphenylalanine; gamma-glutamyvaline; prolylglycine; pyroglutamylvaline; 1,3,7-trimethylurate; N-(2-fuyoylglycine)	heptanoate(7:0); nervonoyl sphingomyelin*; cinnamoylglycine	UPLC-MS/MS and GC-MS, serum	Caussy C[17]
No advanced fibrosis(stage 0-2)(n=133) Advanced fibrosis (stage 3-4)(n=23)	Advanced fibrosis vs No advanced fibrosis	malate; sorbitol; 3-(4-hydroxyphenyl)lactate; 7-methylguanine; cyclo(leu-pro); cyclo(L-phe-L-pro)	leucine; creatinine; urate; valine; gamma-glutamylleucine; 4-androsten-3beta, 17beta-diol disulfate(1); gamma-glutamylvaline		

Supplementary Table 2 continued

Disease	Comparison	Change of metabolites		Method and tissue	Ref
		Increased	Decreased		
Simple steatosis (n =9) NASH (n = 11)	NASH vs Simple steatosis		γ-glutamyl dipeptides (γ-Glu-Val; γ-Glu-Thr; γ-Glu-Leu, γ-Glu-His; γ-Glu-Phe; γ-Glu-Arg)	LC–MS/MS, serum	Soga T[18]
NAFLD(n=102)	NAFLD activity score	glycoprotein (2.03 ppm); lactate (1.35 ppm); VLDL lipid (0.88 ppm); leucine (1.73 ppm); leucine (1.70 ppm); isoleucine (1.01 ppm); phenylacetate (7.39 ppm); leucine (0.95 ppm); alanine (1.46 ppm); valine (0.97 ppm); valine (1.02 ppm); citrate (2.51 ppm); citrate (2.67 ppm); alanine (3.79 ppm); alanine (1.48 ppm); lactate (4.12 ppm); glucose (3.90 ppm); glucose (3.88 ppm)	histidine (7.04 ppm); acetate (1.91 ppm); small albumin lysyl (2.98 ppm); choline (3.21 ppm); phosphocholine (3.22 ppm); scyllo-Inositol (3.35 ppm);	^{1}H NMR, plasma	Hoyles L[19]

Comparison of condition A vs condition B: Increased signifies an increase in condition A relative to condition B. Decreased signifies a decrease in condition A relative to condition B.

NAFLD: non-alcoholic fatty liver disease; LC-MS/MS: liquid chromatography tandem mass spectrometry; DAG: diacylglycerol; TAG: triacylglycerol; HETE: hydroxyeicosatetraenoic acid; GC: gas chromatography; LC-MS: liquid chromatography-mass spectrometry; DHA: docosahexanoic acid; TMAO: trimethylamine N-oxide; HPLC-MS/MS: high-performance liquid chromatography tandem mass spectrometry; NASH: non-alcoholic steatohepatitis; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; UDCA: ursodeoxycholic acid; LCA: lithocholate; UPLC-MS/MS: ultra-performance liquid chromatography tandem mass spectrometry; LC/GC-QTOF-MS: liquid and gas chromatography–quadruple time-of-flight–mass spectrometry; ^{1}H NMR: proton NMR; DHEA-S: dehydroepiandrosterone sulfate; CE-TOF-MS: capillary electrophoresis time-of-flight–mass spectrometry; LPC: lysophosphatidylcholine; PC: phosphatidylcholine; UPLC-MS: ultra-performance liquid chromatography-mass spectrometry; GCA: glycocholate; TCA: taurocholate; GCDCA: glycochenodeoxycholate; TCDCA: taurochenodeoxycholate; LC/MS: liquid chromatography tandem mass spectrometry; UHLC/MS/MS: ultrahigh performance liquid chromatography/tandem mass spectrometry;. GC/MS: gas chromatography tandem mass spectrometry; GC-MS: gas chromatography-mass spectrometry.

Supplementary Table 3 change of metabolites associated with NAFLD in urine

Disease	Comparison	Change of metabolites		Method	Ref
		Increased	Decreased		
NASH(n=7) Healthy(n=15)	NASH vs Healthy	DCA; CA; CDCA; UDCA; 6α-hydroxylated bile acid		UPLC-MS/MS	Ferslew BC[9]
NAFLD(n=12) Healthy(n=14)	NAFLD vs Healthy	glucose; 1-Methyl histidine(1-MHis); sebacic acid; pseudo uridine(PSI); glucono-1, 4-lactone; cresyl sulphate; cysteine	xilitol; 4-phenyl acetic acid; oleic acid; 4-Deoxyerythronate; oxalic acid; N-methyl nicotinate	GC-MS	Troisi J[20]
NAFLD(n=33) NASH(n=45) Healthy(n=30)	NAFLD vs Healthy	citrulline, arginine, valine; indole acetic acid; glucose; gluconic acid; 7-methylxanthine; 2-methylguanosine; indoxylsulfuric acid; lysine; threonine; tyrosine; leucine; hippuric acid; 3-indoleacetic acid; cAMP; acetyl-DL-leucine	hypoxanthine, xanthine, carnitine; 5-hydroxy indole acetic acid; indole-3-formic acid; cortisol	LC-MS	Dong S[21]
	NASH vs NAFLD	methyl xanthine; tryptophan; 3-indole acetic acid; gluconic acid; L-carnitine; pyroglutamic acid; indolelactic acid	proline		
NAFLD(n=56)	NAFLD activity score	citrate (2.66 ppm); valine (0.99 ppm); valine (1.00 ppm); glutamine (2.47 ppm); glutamine (2.46 ppm); lysine (1.71 ppm); lysine (1.73 ppm); α-Ketoisovalerate (1.12 ppm); paracetamol sulfate (7.33 ppm); creatinine (3.06 ppm); creatinine (4.07 ppm); alanine (3.18 ppm); 1-Methylnicotinamide (8.89 ppm); N-Acetylalanine (2.03 ppm); choline (3.20 ppm); inosine (8.34 ppm); p-Hydroxyphenylpyruvate (6.67 ppm); 1-Methylnicotinamide (9.29 ppm)； 1-Methylnicotinamide (8.91 ppm)； inosine (8.33 ppm)； pyruvate (2.37 ppm)； oxaloacetate (2.38 ppm)； 1-Methylnicotinamide (4.48 ppm)； isoleucine (0.94 ppm)； methylmalonate (1.24 ppm)；α-Hydroxybutyrate (0.90 ppm); lysine (3.02 ppm)； dimethylglycine (2.94 ppm)； valine (1.05 ppm)； α-Hydroxyisovalerate (0.97 ppm)； leucine (0.96 ppm)	hippurate (7.64 ppm); hippurate (7.56 ppm); hippurate (7.85 ppm); hippurate (7.82 ppm); trigonelline (8.83 ppm); hippurate (3.98 ppm); histamine (7.12 ppm); p-Hydroxyphenylpyruvate (7.01 ppm); trigonelline (9.12 ppm); trigonelline (8.85 ppm); paracetamol glucuronide (5.11 ppm); paracetamol glucuronide (7.35 ppm);	^{1}H NMR	Hoyles L[19]

Comparison of condition A vs condition B: Increased signifies an increase in condition A relative to condition B. Decreased signifies a decrease in condition A relative to condition B.

NASH: non-alcoholic; DCA: deoxycholic acids; CA: cholic acids; CDCA: chenodeoxycholic acid; UDCA: ursodeoxycholic acid; UPLC-MS/MS: ultra-performance liquid chromatography tandem mass spectrometry; NAFLD: non-alcoholic fatty liver disease; GC-MS: gas chromatography-mass spectrometry; cAMP: cyclic adenosine monophosphate protein; LC-MS: liquid chromatography-mass spectrometry; ^{11}H NMR: proton NMR .

Supplementary Table 4 change of metabolites associated with NAFLD in liver

Disease	Comparison	Change of metabolites		Method	Ref
		Increased	Decreased		
NAFLD(n=41) Healthy(n=17)	NAFLD vs Healthy	GCDCA; TCA; TDCA; isoleucine; phenylalanine; tyrosine; valine; succinic acid; arachidonoyl-lyso-PC (20:4); butyryl carnitine (C4); lauryl carnitine (C12); stearoyl carnitine (C18:0); tetradecanoyl carnitine(C14); 4-hyddroxyproline; betaine; glucose; taurine	CA; DCA; histidine; methionine; tryptophan; creatine; arachidonic acid; choline; linoleic acid; stearoyl-lyso-PE (18:0)	LC-MS/MS	Han J[22]
Obese patients with NAFLD(n=154) Obese patients without NAFLD(n=76)	Obese patients with NAFLD vs Obese patients without NAFLD	leucine; isoleucine; valine,; 3-methyl-2-oxobutyrate; 3-methyl-2-oxovalerate; 4-methyl-2-oxopentanoate; α-ketoglutarate; succinylcarnitine; bradykinin; des-Arg9-bradykinin; dicarboxylic acid 2-hydroxybutyrate (α–hydroxybutyrate); glycocholate; taurocholate	3-hydroxybutyrate; 4-androsten-3β; 17-β-dioldisulphate 1; 17-β-diol disulphate 2; 5α-androstan-3β; 5α-pregnan-3β; 20α-diol disulphate; pregnen-diol disulphate; pregnen steroid monosulphate; andro steroid monosulphate 2; 21-hydroxypregnenolone disulphate	LC/GC-QTOF-MS	Rodríguez-Gallego E[11]
NASH(n=14) Healthy(n=16)	NASH vs Healthy	leucine; valine; isoleucine; betaine; succinate; lactate; propylene glycol		^{1}H-NMR	Schofield Z[23]
Steatosis(n=23) Non-steatosis(n=23)	Steatosis vs Non-steatosis	dextrin; glycochenodeoxycholate-3-sulfate; taurochenodeoxycholic acid; glycerophosphocholine; LysoPC(16:0); LysoPC(18:3); LysoPE(16:0/0:0); LysoPE(18:0/0:0); LysoPE(18:3); PC(36:2); PC(36:4)	L-glutamyl-L-lysine; L-glutamic acid; GSH; GSSG; GSH/GSSG ratio	UPLC-Q-ToF	García-Cañaveras JC[24]
NASH(n=15) Normal control(n=8)	NASH vs Normal control	CA; CDCA; DCA		gas–liquid chromatography	Aranha MM[25]

Comparison of condition A vs condition B: Increased signifies an increase in condition A relative to condition B. Decreased signifies a decrease in condition A relative to condition B.

NAFLD: non-alcoholic fatty liver disease; GCDCA: glycochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TCA: taurocholic acid; CA: cholic acid; DCA: deoxycholic acid; LC-MS/MS: liquid chromatography tandem mass spectrometry; LC/GC-QTOF-MS: liquid and gas chromatography–quadruple time-of-flight–mass spectrometry; ^{1}H NMR: proton NMR; GSH: glutathione; GSSG: glutathione disulfide; UPLC-Q-ToF: ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; CDCA: chenodeoxycholic acid.

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