

Serum, liver and bile sitosterol and sitostanol in obese patients with and without NAFLD

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Supplementary Table 1. Sequences of qPCR primers.

Gene	Forward primer (5' – 3')	Reverse primer (5' – 3')
<i>NPC1L1</i>	TACTGTGCCAATGCCCCGCT	GGGAAGACAGGGGCCCCGTA
<i>ABCG5</i>	TTCATCTTTGACCCCCGGA	GATGTGATGTCCCACCAGGG
<i>ABCG8</i>	GGAACCTGGAGGGAACAATAAC	GGCATCTTGCTGGTACATCTT
<i>RPLP0</i>	GGCGACCTGGAAGTCCAAC	CCATCAGCACACAGCCTTC

Supplementary Table 2. Clinical characteristics (mean±SD) of study subjects in groups that had plant sterol and plant stanol measurement available from serum, liver and bile and had either histologically normal liver or NAFLD. *P<0.05 compared to those with serum measurements. DPI (dietary phytosterol intake, serum campesterol to cholestanol ratio).

	Serum	Liver	Bile	P serum vs. liver	P serum vs. bile
	138	38	39		
Gender (male/female)	38/100	14/24	9/30	0.133	0.463
Age (years)	46.3±8.9	45.8±9.1	48.2±9.0	0.746	0.180
Body mass index (kg/m ²)	43.3±6.9	43.8±5.2	41.6±4.9	0.662	0.021
ALT (U/L)	49.6±33.4	49.9±42.1	48.4±30.0	0.629	0.903
Fasting glucose (mmol/L)	6.5±1.9	6.3±1.1	6.4±1.7	0.604	0.932
Fasting insulin (mU/L)	18.6±10.1	18.5±10.5	18.8±9.5	0.694	0.673
Total cholesterol (mmol/L)	4.5±0.9	4.3±1.0	4.5±0.7	0.031	0.301
HDL cholesterol (mmol/L)	1.1±0.3	1.1±0.3	1.2±0.3	0.683	0.004
LDL cholesterol (mmol/L)	2.7±0.8	2.5±0.8	2.6±0.7	0.085	0.849
Triglycerides (mmol/L)	1.3±0.5	1.3±0.5	1.7±0.9	0.166	0.898
DPI (dietary phytosterol intake)	1.0±0.4	0.9±0.4	1.0±0.5	0.577	0.526

Supplementary Table 3. Spearman correlations of serum, liver and bile plant sterol and sitostanol.
 **P<0.01 and *P<0.05. Bile avenasterol and campesterol were undetected.

	Serum			
	Campesterol	Sitosterol	Avenasterol	Sitostanol
Liver (n=38)	0.544**	0.488**	0.517*	0.214
Bile (n=41)		0.795**		0.236

Supplementary Table 4. Spearman correlations of liver mRNA gene expression (qPCR assay) with plant sterols and sitostanol ratios to cholesterol in serum, liver and bile. *P<0.05.

	<i>NPC1L1</i>	<i>ABCG5</i>	<i>ABCG8</i>
SERUM (n=102)			
Campesterol	-0.112	-0.042	-0.090
Sitosterol	-0.210*	-0.069	-0.172
Avenasterol	-0.046	0.117	-0.110
Sitostanol	0.248*	0.243*	-0.016
LIVER (n=38)			
Campesterol	-0.059	0.027	-0.194
Sitosterol	-0.168	-0.073	-0.310
Avenasterol	0.133	-0.025	-0.383*
Sitostanol	0.215	0.190	0.012
BILE (n=41)			
Sitosterol	-0.295	-0.138	-0.209
Sitostanol	0.227	0.247	0.376

Supplementary Table 5. Correlations of the inflammation, lipid and cholesterol metabolism associated genes (liver mRNA expression, qPCR assay) with sitosterol and sitostanol ratios to cholesterol in serum (n=102), liver (n=38) and bile (n=41). *P<0.05, **P<0.009 in bold. Spearman`s correlation analysis. N/A: data not available.

Inflammation-related genes		Serum n=102		Liver n=38		Bile=41	
		Sitosterol	Sitostanol	Sitosterol	Sitostanol	Sitosterol	Sitostanol
Gene name	Gene symbol	10**2 mmol/mol of chol		ug/100 mg chol		ug/100mg chol	
ADAM Metallopeptidase Domain 8	<i>ADAM8</i>	-0,036	-0,069	-0,281	0,309	0,088	-0,211
Apoptotic Peptidase Activating Factor 1	<i>APAF1</i>	,199*	0,054	0,174	-,394*	0,227	-0,008
BCL2-Associated Agonist of Cell Death	<i>BAD</i>	-0,187	-,205*	-0,088	0,065	-,368*	0,050
BCL2-Associated Athanogene 6	<i>BAG6</i>	0,008	-,386**	-0,262	0,064	-0,146	-0,200
B-Cell CLL/Lymphoma 3	<i>BCL2</i>	0,034	0,070	-0,185	0,243	0,067	0,178
Complement Component 3	<i>C3</i>	-0,105	-,348**	-0,299	-0,013	-0,069	-0,107
Caspase 1, Apoptosis-Related Cysteine Peptidase	<i>CASP1</i>	-0,008	0,036	-0,255	0,203	-0,020	-0,077
Caspase 3, Apoptosis-Related Cysteine Peptidase	<i>CASP3</i>	-0,116	-0,001	0,043	0,043	-0,096	0,020
Caspase 8, Apoptosis-Related Cysteine Peptidase	<i>CASP8</i>	0,080	-0,165	-0,114	-0,091	0,013	-0,139
Caspase 9, Apoptosis-Related Cysteine Peptidase	<i>CASP9</i>	-0,013	-,306**	-0,323	0,044	0,277	-0,102
Chemokine (C-C Motif) Ligand 2	<i>CCL2</i>	0,130	0,142	0,202	-0,133	0,079	-0,173
CD68 Molecule	<i>CD68</i>	-0,040	-0,039	-0,275	0,120	-0,147	0,273
C-Reactive Protein, Pentraxin-Related	<i>CRP</i>	-0,092	-0,020	-0,188	0,206	-0,022	-0,014
Egf-Like Module Containing, Mucin-Like, Hormone Receptor-Like 1	<i>EMR1=ADGRE1</i>	0,041	-0,024	0,085	0,105	-0,054	0,060
Fas (TNFRSF6)-Associated Via Death Domain	<i>FADD</i>	0,153	-0,017	0,042	-0,001	0,130	-0,170
Fas Cell Surface Death Receptor	<i>FAS</i>	,238*	-0,030	0,064	-0,011	-0,036	-0,249
Guanylate Binding Protein 5	<i>GBP5</i>	-0,151	-0,009	-0,163	-0,117	-0,276	0,049
Heme Oxygenase 1	<i>HMOX1</i>	0,025	0,008	-0,024	0,282	-0,139	,359*
Interleukin 10	<i>IL10</i>	-0,032	0,167	-0,119	0,266	0,310	0,294
Interleukin 18	<i>IL18</i>	0,041	-0,146	-0,144	0,116	-0,065	0,264
Interleukin 1, Beta	<i>IL1B</i>	-0,106	-0,068	-0,217	,389*	-0,027	-0,162
Interleukin 1 Receptor, Type I	<i>IL1R1</i>	0,034	-0,125	-0,147	-0,172	0,151	0,016
Interleukin 1, Receptor, Type II	<i>IL1RN</i>	-0,175	0,066	-0,332	0,153	0,028	0,029
Interleukin 2	<i>IL2</i>	-0,040	0,067	-0,043	-0,216	0,025	0,041
Interleukin 32	<i>IL32</i>	-0,116	0,077	-0,281	,528**	-0,062	0,003
Interleukin 6	<i>IL6</i>	-0,015	0,007	N/A	N/A	0,047	0,219
Integrin, Alpha M (Complement Component 3 Receptor 3 Subunit)	<i>ITGAM</i>	-0,018	-0,125	-,437**	,507**	0,120	-0,040
MHC Class I Polypeptide-Related Sequence A	<i>MICA</i>	0,069	-,240*	-0,158	-0,013	-0,120	-,321*
Matrix Metallopeptidase 9	<i>MMP9</i>	-0,020	-0,010	-0,088	0,163	0,116	0,191
Nuclear Factor of Kappa Light Polypeptidase Gene Enhancer in B-Cells 1	<i>NFKB1</i>	-0,019	0,104	-0,016	0,239	-0,168	-0,235
nlr Family, Pyrin Domain Containing 3	<i>NLRP3</i>	-0,144	0,077	0,111	-0,129	-0,180	-0,055
Platelet Derived Growth Factor Beta Polypeptidase	<i>PDGFB</i>	-0,052	-,194*	-0,289	0,099	-,321*	0,180
PYD and Card Domain Containing	<i>PYCARD</i>	-0,023	0,023	0,104	-0,031	-0,116	0,236
Receptor (TNFRSF)-Interacting Serine-Threonine Kinase 1	<i>RIPK1</i>	,193*	0,190	0,228	-0,232	0,096	-0,059
Superoxide Dismutase 2, Mitochondrial	<i>SOD2</i>	-0,023	,210*	,361*	-0,119	-0,148	0,056
Signal Transducer and Activator of Transcription 5B	<i>STAT5B</i>	-0,007	0,042	-0,109	0,208	0,131	0,113
Toll-Like Receptor	<i>TLR4</i>	0,007	-0,143	-,405*	,391*	-0,035	-0,023
Tumor Necrosis Factor Alpha	<i>TNFα</i>	-0,060	0,038	-0,158	0,191	-0,172	0,051
Tumor Necrosis Factor Receptor Superfamily, Member 1A	<i>TNFRSF1A</i>	0,069	-,213*	-0,123	-0,333	-0,037	-0,162
Tumor Necrosis Factor Receptor Superfamily, Member 1B	<i>TNFRSF1B</i>	-0,098	0,063	-0,310	0,112	-0,101	0,185
Tumor Necrosis Factor Receptor Superfamily, Member 10	<i>TNFRSF10</i>	-0,008	-,372**	-0,159	-0,290	0,112	-0,076
Tumor Protein 53	<i>TP53</i>	-0,032	-0,032	-0,215	0,160	0,164	-0,050
TNFRSF1A-Associated Via Death Domain	<i>TRADD</i>	-0,093	-,222*	0,025	0,055	-0,086	-0,002
TNF Receptor-Associated Factor 2	<i>TRAF2</i>	-0,120	-0,088	-0,278	0,012	-0,305	0,027
Thioredoxin Interacting Protein	<i>TXNIP</i>	0,017	,285**	0,193	0,126	0,070	0,245

Lipid metabolism - related genes		Serum n=102		Liver n=38		Bile=41	
		Sitosterol	Sitostanol	Sitosterol	Sitostanol	Sitosterol	Sitostanol
Gene name	Gene symbol	10**2 mmol/mol of chol		ug/100 mg chol		ug/100mg chol	
Peroxisome Proliferator-Activated Receptor Alpha	<i>PPARA</i>	-0,045	0,192	0,006	-0,040	-0,062	-,365*
Peroxisome Proliferator-Activated Receptor Gamma (total)	<i>PPARG_tot</i>	-0,086	-,273**	-,370*	-0,036	-0,270	-0,057
Peroxisome Proliferator-Activated Receptor Gamma (variant 1A)	<i>PPARGC1A</i>	0,082	-,302**	-0,286	-0,155	-0,033	0,086
Peroxisome Proliferator-Activated Receptor Gamma (variant 1B)	<i>PPARGC1B</i>	-,204*	-,432**	-,428*	0,109	-0,181	-0,082
Sterol Regulatory Element Binding Transcription Factor 1	<i>SREBF1_tot</i>	-0,072	-0,070	-0,253	-0,121	-0,131	0,118
ATP Citrate Lyase	<i>ACLY</i>	-0,006	-0,033	-0,202	0,242	-0,054	0,265
Acetyl-CoA Carboxylase Alpha	<i>ACACA=ACC</i>	-0,093	-,271**	-0,300	-0,025	0,045	-0,108
Fatty Acid Synthetase	<i>FASN</i>	-0,052	0,004	-0,072	0,247	-0,101	0,269
Fatty Acid Elongase 1	<i>ELOVL1</i>	-0,069	0,001	-,414*	,496**	-0,125	0,122
Fatty Acid Elongase 2	<i>ELOVL2</i>	-0,047	,331**	0,118	0,321	0,049	0,300
Fatty Acid Elongase 3	<i>ELOVL3</i>	0,062	0,098	-0,004	0,128	0,175	0,070
Fatty Acid Elongase 4	<i>ELOVL4</i>	-0,125	0,083	-0,118	0,094	-0,066	0,094
Fatty Acid Elongase 5	<i>ELOVL5</i>	-0,060	,306**	0,314	0,074	-0,246	,352*
Fatty Acid Elongase 6	<i>ELOVL6</i>	0,110	0,134	0,185	-0,218	0,303	0,014
Fatty Acid Elongase 7	<i>ELOVL7</i>	0,070	-0,043	-0,244	0,152	-0,048	0,015
Stearoyl-CoA Desaturase (Delta-9 Desaturase)	<i>SCD</i>	0,040	-0,174	-0,202	0,095	0,007	0,054
Fatty Acid Desaturase 1	<i>FADS1</i>	-0,061	0,022	0,109	0,332	-0,291	-0,128
Fatty Acid Desaturase 2	<i>FADS2</i>	-0,092	-0,064	-0,018	0,306	-0,207	-0,083
Fatty Acid Desaturase 3	<i>FADS3</i>	-0,015	-,233*	0,011	0,095	-0,234	-0,152
Carnitine Palmitoyltransferase 1A (Liver)	<i>CPT1A</i>	-0,046	0,170	-0,161	,375*	0,146	-0,066
Carnitine Palmitoyltransferase 1B (Muscle)	<i>CPT1B</i>	0,170	0,164	-0,201	0,228	0,128	0,156
Glycerol-3 Phosphate Acyltransferase, Mitochondrial	<i>GPAM=GPAT1</i>	-0,047	-0,063	-0,303	0,183	-0,031	0,164
Glycerol-3 Phosphate Acyltransferase 2, Mitochondrial	<i>GPAT2</i>	0,021	-0,147	-0,272	0,276	-0,082	-0,100
1-Acylglycerol-3-Phosphate O-Acyltransferase 1	<i>AGPAT1</i>	-,270**	0,065	-0,329	0,218	-0,010	0,084
1-Acylglycerol-3-Phosphate O-Acyltransferase 2	<i>AGPAT2</i>	-0,065	-,199*	-0,090	0,059	0,001	0,181
1-Acylglycerol-3-Phosphate O-Acyltransferase 3	<i>AGPAT3</i>	0,102	-0,006	0,054	-0,043	0,253	-0,205
1-Acylglycerol-3-Phosphate O-Acyltransferase 4	<i>AGPAT4</i>	0,025	-0,007	-0,025	0,141	-0,165	0,058
1-Acylglycerol-3-Phosphate O-Acyltransferase 5	<i>AGPAT5</i>	-0,014	-0,077	-0,004	-0,031	-0,210	-0,202
1-Acylglycerol-3-Phosphate O-Acyltransferase 9	<i>AGPAT9=GPAT3</i>	0,008	-0,095	-0,034	-0,275	0,153	-0,252
1-Acylglycerol-3-Phosphate O-Acyltransferase 6	<i>AGPAT6=GPAT4</i>	-0,014	-,294**	0,007	-0,321	-0,209	-0,222
Lipin 1	<i>LPIN1</i>	-0,082	-,406**	-0,334	-0,058	0,023	-,340*
Lipin 2	<i>LPIN2</i>	0,155	-,238*	0,133	-,444**	0,078	-0,251
Lipin 3	<i>LPIN3</i>	-0,001	-0,186	-,373*	-0,187	-0,138	0,045
Diacylglycerol O-Acyltransferase 1	<i>DGAT1</i>	-,204*	0,047	-0,278	0,174	-0,127	-0,110
Diacylglycerol O-Acyltransferase 2	<i>DGAT2</i>	-0,090	0,014	-0,167	0,222	-0,123	0,172
Lipoprotein Lipase	<i>LPL</i>	0,000	0,082	-0,019	,393*	0,179	-0,091
Patatin-Like Phospholipase Domain Containing 2	<i>PNPLA2=ATGL</i>	-0,088	-0,010	-0,270	0,048	0,038	-0,111
Lipase, Hormone Sensitive	<i>LIPE=HSL</i>	0,056	,197*	-0,071	-0,013	0,062	-0,169
Perlipin 1	<i>PLIN1</i>	-0,005	-0,006	-0,230	0,322	0,028	0,210
Patatin-Like Phospholipase Domain Containing 3	<i>PNPLA3</i>	0,026	-0,190	-0,239	0,081	-0,114	-0,020
Phospholipid Phosphatase 2A	<i>PPAP2A</i>	0,128	,318**	0,233	0,118	-0,008	0,104
Phospholipid Phosphatase 2B	<i>PPAP2B</i>	-0,135	0,078	-0,167	0,128	0,001	0,290
Phospholipid Phosphatase 2C	<i>PPAP2C</i>	0,120	0,139	0,024	0,267	0,135	0,052
Uncoupling Protein 1 (Mitochondrial, Proton Carrier)	<i>UCP1</i>	-0,028	0,033	N/A	N/A	-0,137	-0,094
Uncoupling Protein 2 (Mitochondrial, Proton Carrier)	<i>UCP2</i>	-,229*	-0,049	-0,179	,433*	-0,313	,343*

Cholesterol metabolism - related genes		Serum n=102		Liver n=38		Bile=41	
		Sitosterol	Sitostanol	Sitosterol	Sitostanol	Sitosterol	Sitostanol
Gene name	Gene symbol	10**2 mmol/mol of chol		ug/100 mg chol		ug/100mg chol	
Nuclear Receptor Subfamily 1, Group H, Member 2	<i>NR1H2=LXRB</i>	-0,077	-,201*	-0,125	0,122	-0,203	0,219
Sterol Regulatory Element Binding Transcription Factor 2	<i>SREBF2</i>	-0,105	-0,052	-0,029	,354*	-0,135	0,205
Acyl-CoA Synthetase Short-Chain Family Member 2	<i>ACSS2</i>	-0,013	-0,022	-0,060	0,186	-0,181	-0,122
Acetyl-CoA Acetyltransferase 1	<i>ACAT1</i>	-0,048	0,143	-0,050	0,263	0,090	0,228
3-Hydroxy-3-Methylglutaryl-CoA Synthetase 1 (Soluble)	<i>HMGCS1</i>	0,046	0,055	0,230	0,179	0,191	0,155
3-Hydroxy-3-Methylglutaryl-CoA Synthetase 3 (Mitochondrial)	<i>HMGCS2</i>	-0,084	,285**	-0,169	-0,016	-0,078	0,180
3-Hydroxy-3-Methylglutaryl-CoA Reductase	<i>HMGCR</i>	-0,135	-,213*	-0,117	0,091	0,037	0,219
Farnesyl-Diphosphate Farnelystransferase 1	<i>FDFT1</i>	-0,004	-0,112	-0,018	0,219	-0,018	-0,145
Squalene Epoxidase	<i>SQLE</i>	-0,074	-0,100	-0,042	0,308	0,002	0,063
Lanosterol Synthetase (2,3-Oxidosqualene-Lanosterol Cyclase)	<i>LSS</i>	-0,019	-0,112	-0,107	0,191	-0,200	-,324*
Transmembrane 7 Superfamily Member 2	<i>TM7SF2</i>	0,003	-0,011	0,068	0,159	-0,067	-0,199
Emopamil Binding Protein (Sterol Isomerase)	<i>EBP</i>	0,125	-,233*	-0,042	-0,134	0,104	-,331*
Methylsterol Mono-oxygenase 1	<i>MSMO1=SC4MO</i>	-0,101	-0,030	0,157	-0,127	-0,046	-0,035
Sterol C5-Desaturase	<i>SC5DL</i>	-0,023	,232*	0,151	0,050	-0,062	0,094
24-Dehydrocholesterol Reductase	<i>DHCR24</i>	-,237*	-0,127	-0,150	-0,110	0,068	0,057
7-Dehydrocholesterol Reductase	<i>DHCR7</i>	-0,124	,224*	-0,083	,569**	0,122	0,143
ATP-Binding Cassette, Sub-Family A (ABC1), Member 1	<i>ABCA1</i>	-0,076	-,287**	-,366*	-0,026	-0,031	-0,007
ATP-Binding Cassette, Sub-Family G (WHITE), Member 1	<i>ABCG1</i>	0,006	-0,073	-0,207	0,060	0,028	-0,034
ATP-Binding Cassette, Sub-Family G (WHITE), Member 5	<i>ABCG5</i>	-0,069	,243*	-0,073	0,190	-0,138	0,190
ATP-Binding Cassette, Sub-Family G (WHITE), Member 8	<i>ABCG8</i>	-0,172	-0,016	-0,310	0,012	-0,209	0,146
Niemann-Pick C1-Like 1	<i>NPC1L1</i>	-,210*	,248*	-0,168	0,215	-0,295	0,227
Low Density Lipoprotein Receptor	<i>LDLR</i>	-0,009	-0,030	0,045	0,005	0,057	-0,244
Proprotein Convertase Subtilisin/Kexin Type 9	<i>PCSK9</i>	-,200*	0,005	-0,186	0,047	-0,038	-0,026
3-Oxoacid CoA Transferase 1	<i>OXCT1</i>	-0,011	0,127	-0,249	0,201	0,062	0,301
3-Hydroxybutyrate Dehydrogenase, Type 1	<i>BDH1</i>	-0,102	,229*	-0,119	0,120	0,013	0,026
Scavenger Receptor Class B, Member 1	<i>SCARB1=SRB1</i>	-0,128	0,024	-0,139	0,116	-0,158	0,149
Fibroblast Growth Factor 21	<i>FGF21</i>	-0,047	-0,135	-0,222	0,111	0,205	,347*
Cytocrome P450, Family 51, Subfamily A, Polypeptide 1	<i>CYP51A1</i>	0,066	,196*	0,188	0,157	0,032	-,321*

Supplementary Figure. 1. Serum plant sterols and sitostanol ratios to cholesterol (mean \pm SD) in individuals with normal liver (n=44) and NAFLD (n=94). Serum campesterol, sitosterol, avenasterol or sitostanol were not different between normal liver and NAFLD.

