SUPPORTING INFORMATION

Metabolomic assessment of the effect of dietary cholesterol in the progressive development of fatty liver disease

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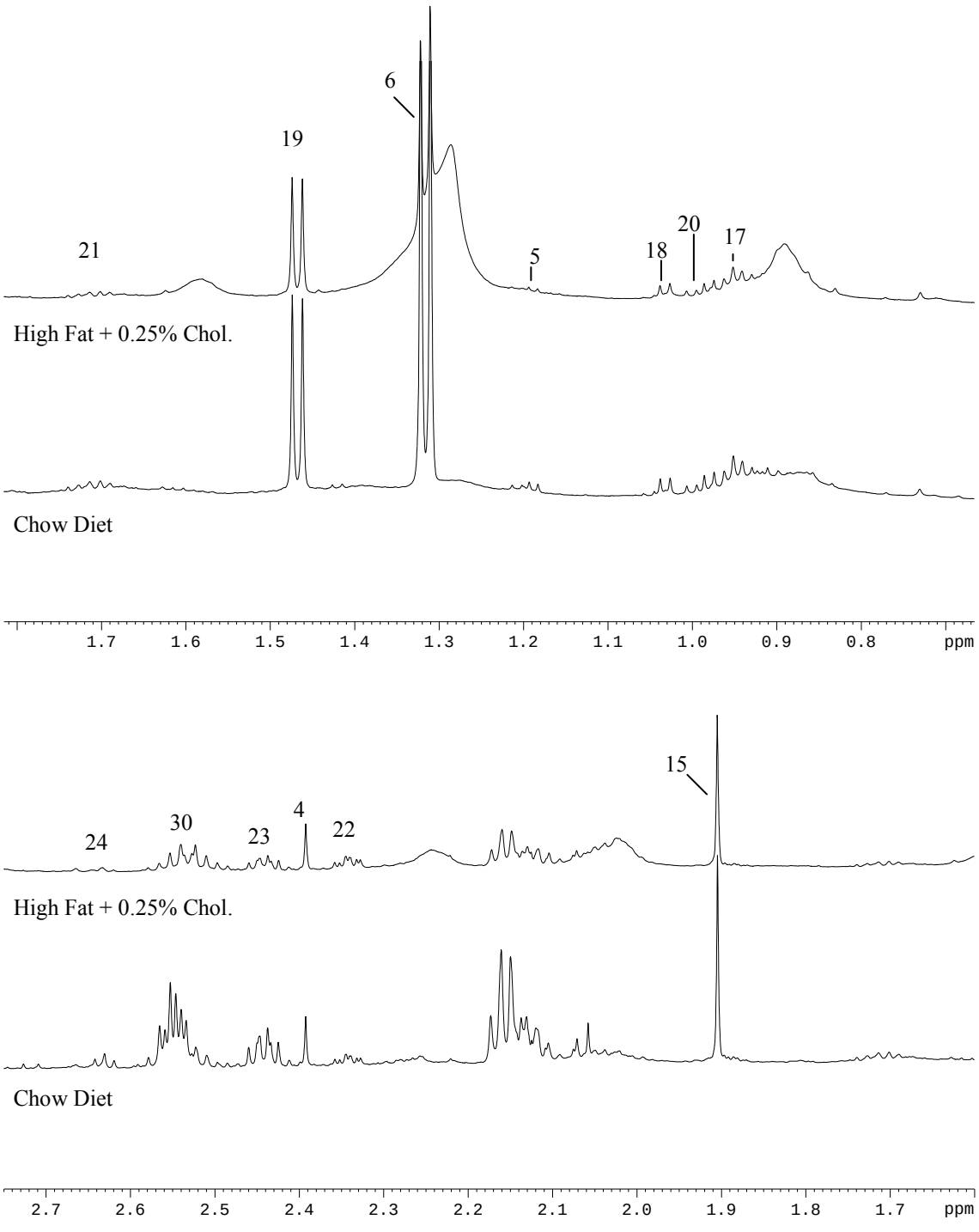
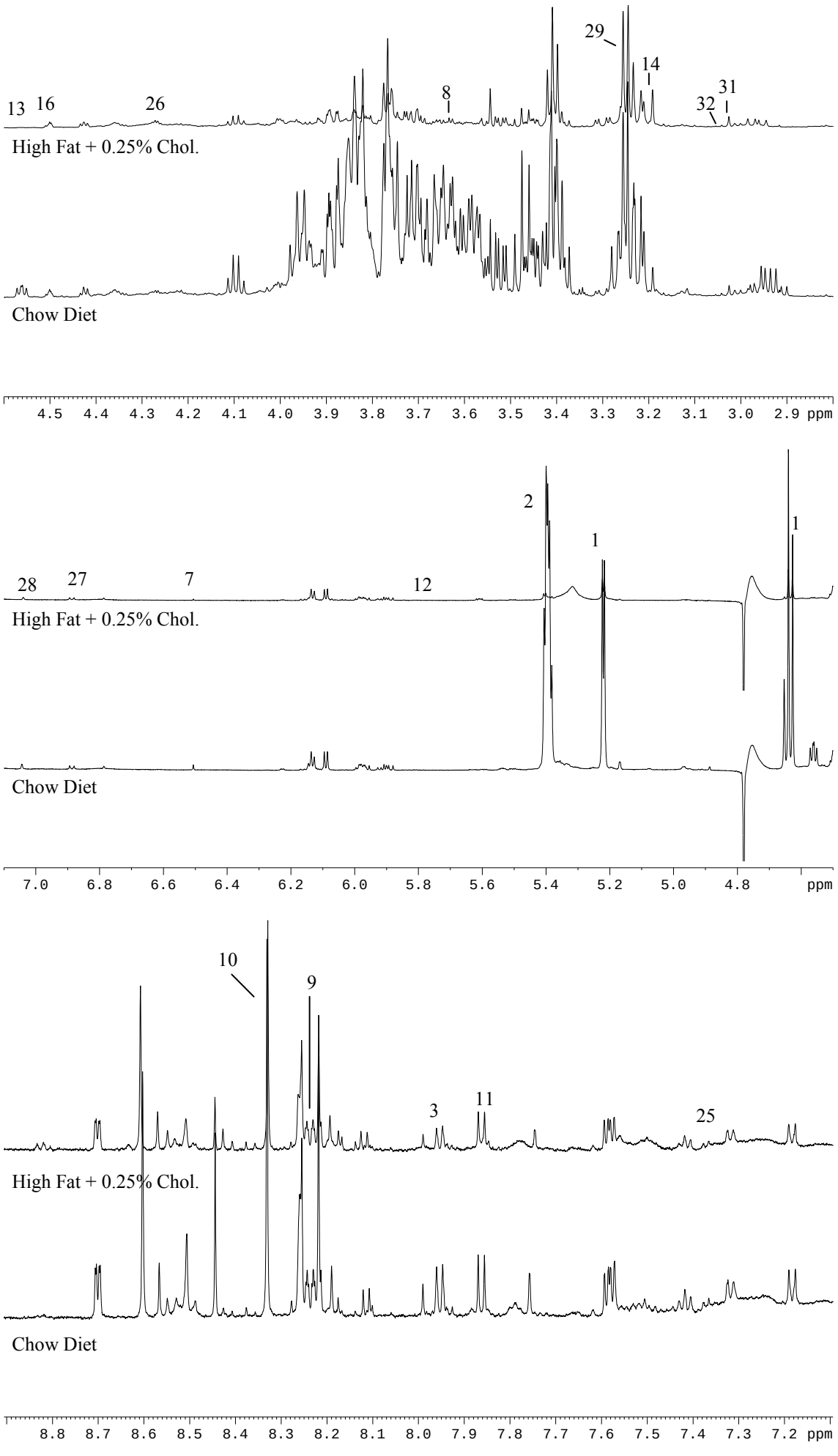
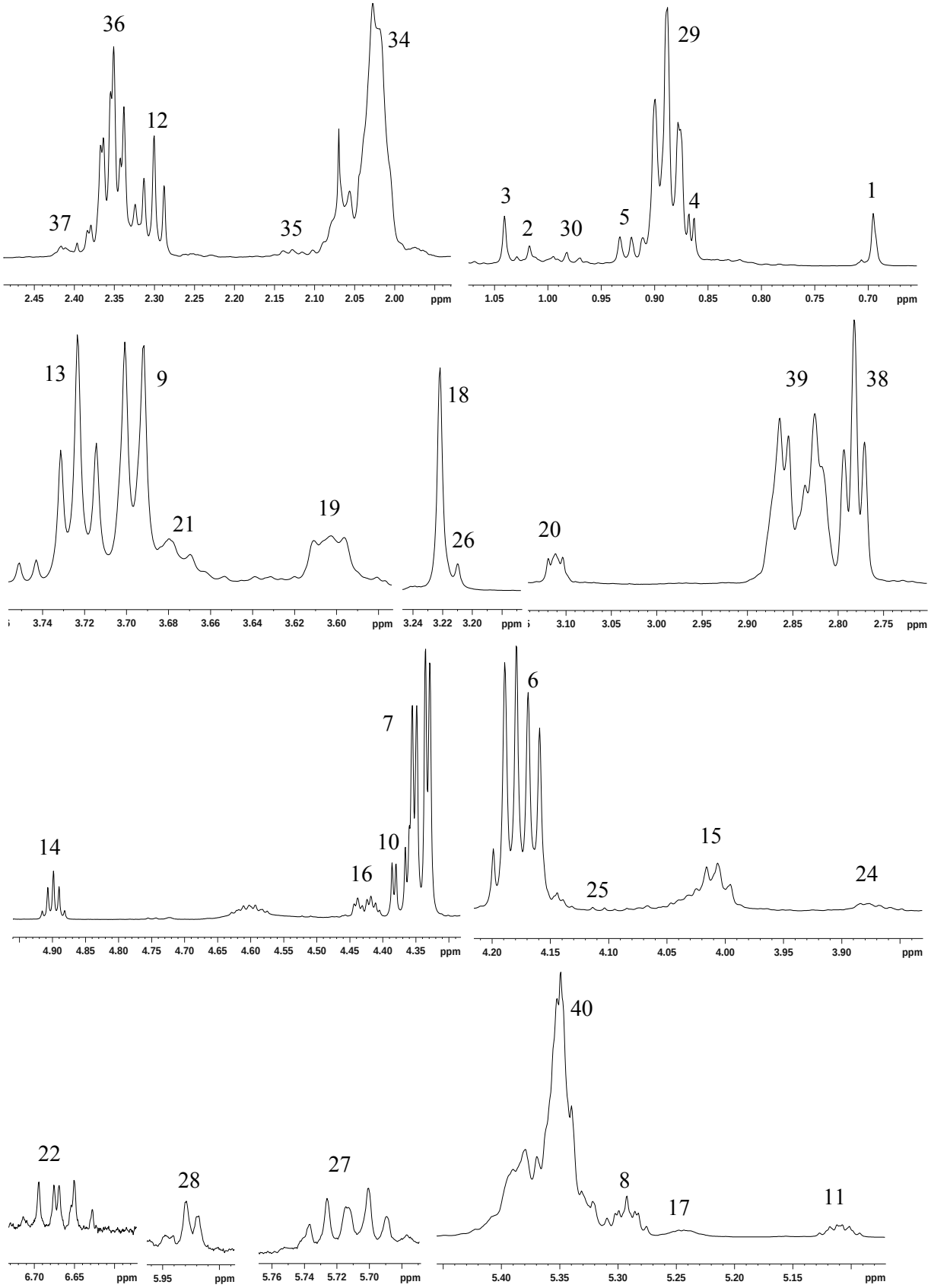
Supplementary Figure S1: ^{1}H NMR spectra (600 MHz) of liver aqueous extracts of Chow and High Fat + 0.25% Chol. diets at 16 weeks of age. For identification of the peak numbers, refer to codes in Table 1.

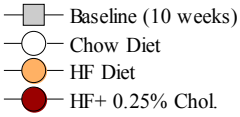



13
16
26
8
29
14
31
32
High Fat + 0.25% Chol.
Chow Diet
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 ppm
28
27
7
12
2
1
1
High Fat + 0.25% Chol.
Chow Diet
7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 ppm
10
9
3
11
25
High Fat + 0.25% Chol.
Chow Diet
8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 ppm

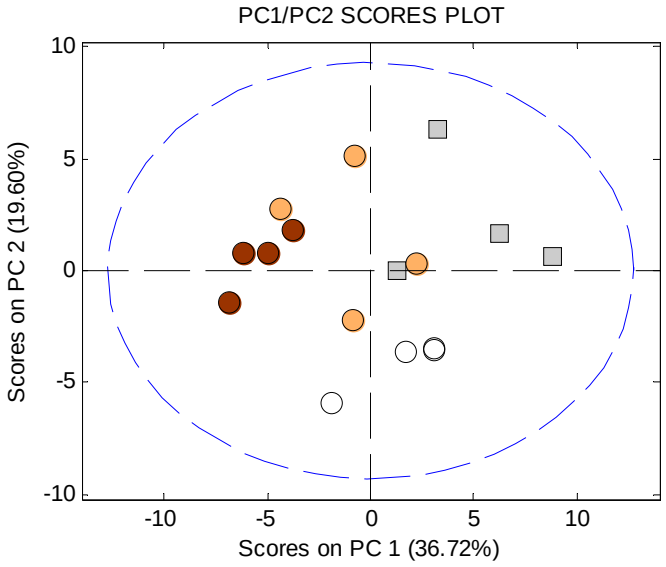
Supplementary Figure S2: ^{1}H NMR spectra (600 MHz) of liver lipidic extract. For identification of the peak numbers, refer to codes in Table 1.

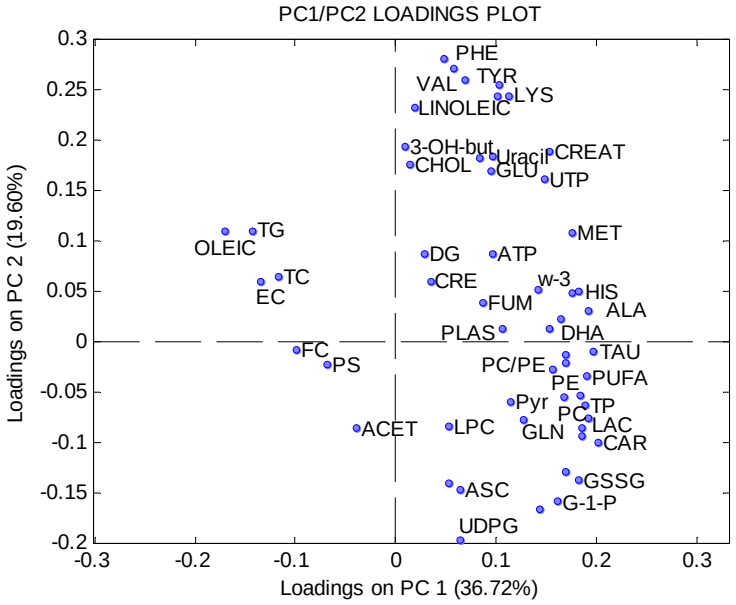


Supplementary Figure S3: Results of the two PCA models fit either at 16-weeks time point (A) and 32-weeks time point (B) using data derived from concentrations of metabolites identified in both lipophilic and hydrophilic liver extracts.

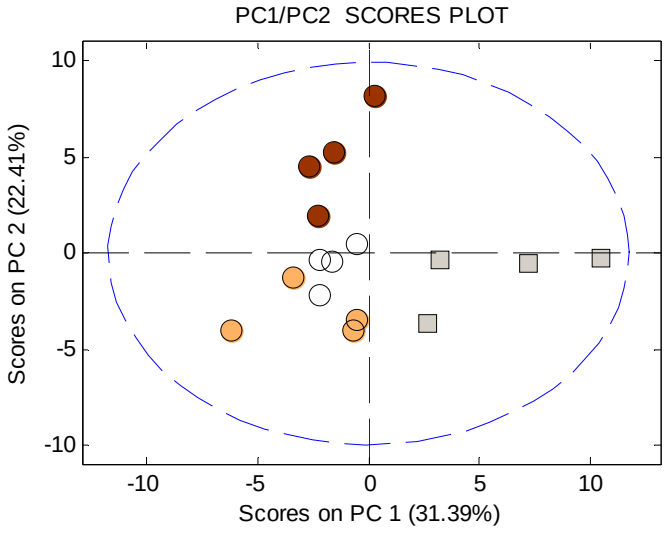


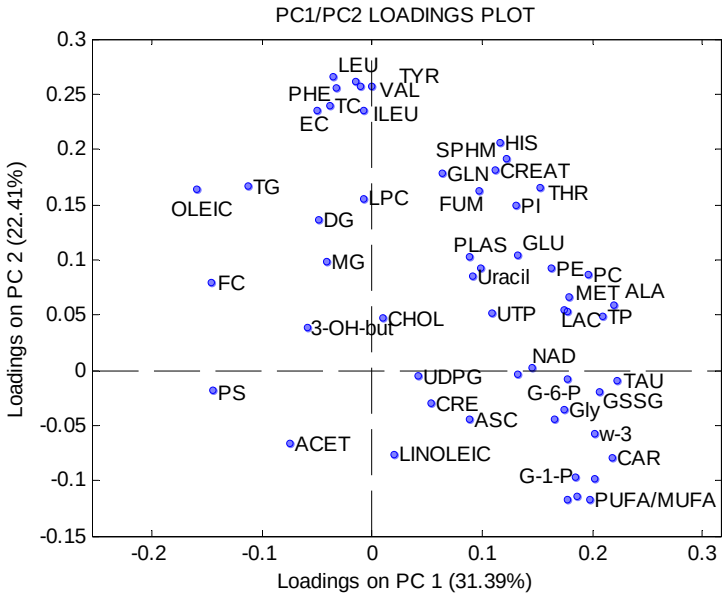
A) PCA ANALYSIS 16 WEEKS OF AGE





B) PCA ANALYSIS 32 WEEKS OF AGE





Total Cholesterol: TC; Free Cholesterol: FC; Esterified Cholesterol: EC; Triglycerides : TG; Diglycerides: DG; Monoglycerides : MG; Total Phospholipids : TP; Phosphatidylethanolamine: PE; Phosphatidylcholine: PC; Phosphatidylinositol : PI; Phosphatidylserine : PS; Lysophosphatidylcholine : LPC; Sphyngomyelin : SPHM; Plasmalogen : PLAS; omega-3 : w-3; Arachidonic acid + Eicosapentaenoic acid: ARA+EPA; Oleic acid : OLEIC; Docohexanoic Acid: DHA; Linoleic acid: LINOLEIC; Polyunsaturated Fatty Acyls: PUFA; Monounsaturated Fatty Acyls: MUFA; PUFA/MUFA ratio: PUFA/MUFA; Phosphatidylcholine/Phosphatidyletanolamine ratio: PC/PE; Glucose-6-Phophate: G-6-P; Glucose-1-Phosphate (Glycogen): G-1-P; UDPG: UDPG; Pyruvate: Pyr; 3-hydroxybutyrate: 3-OH-but; Lactate: LAC; Fumarate: FUM; Free Glycerol: Free Gly; NADH/NADP/NADPH: NADH/NADP/NADPH; ATP/ADP/AMP: ATP/ADP/AMP; UTP/UDP/UMP: UTP/UDP/UMP; Uracil: Uracil; Carnitine: CAR; Cholines: CHOL; Acetates: ACET; Ascorbic acid: ASC; Leucine: LEU; Valine: VAL; Alanine: ALA; Isoleucine: ILEU; Lysine: LYS; Glutamine: GLN; Glutamate: GLU; Methionine: MET; Phenylalanine: PHE; Threonine: THR; Tyrosine: TYR; Histidine: HIS; Taurine: TAU; Glutathione (oxidized): GSSG;Creatine: CRE; Creatinine: CREAT.

Supporting Table S1: Absolute individual concentrations of metabolites identified in both aqueous and lipophilic extracts. Data comparison between high-fat and high-fat high-cholesterol diets with respect to chow diet used the Mann-Whitney U test ($p<0.05$). Data is expressed as mean ± SEM.

	10 weeks age	16 weeks age			32 weeks age		
	Chow Diet	Chow Diet	HF Diet	HF+0.25 Chol Diet	Chow Diet	HF Diet	HF+0.25 Chol Diet
Lipid-soluble (µmol/ g liver)							
Total Cholesterol	17.80 ± 3.11	14.09 ± 1.56	17.44 ± 1.60	74.40 ± 15.96*	17.02 ± 0.65	14.15 ± 2.24	120.18 ± 8.13*
Free Cholesterol	1.72 ± 0.22	5.42 ± 0.71	5.45 ± 0.70	6.58 ± 1.30	9.05 ± 1.14	9.05 ± 1.24	10.17 ± 1.59
Esterified Cholesterol	4.58 ± 0.98	3.90 ± 0.51	7.52 ± 1.51	61.03 ± 13.46*	7.66 ± 0.70	5.88 ± 0.99	91.78 ± 9.19*
Triglycerides	13.60 ± 1.37	20.14 ± 3.71	78.15 ± 28.14*	133.02 ± 38.23*	116.42 ± 23.89	128.41 ± 19.09	332.46 ± 75.74*
Diglycerides	5.42 ± 0.56	5.09 ± 0.71	5.71 ± 0.92	5.39 ± 0.93	8.84 ± 0.67	4.88 ± 0.48*	26.21 ± 14.11*
Monoglycerides	0.35 ± 0.11	0.68 ± 0.22	0.43 ± 0.04	0.37 ± 0.09	0.44 ± 0.10	0.35 ± 0.06	9.25 ± 8.86
Total Phospholipids	50.05 ± 7.20	48.36 ± 5.33	39.24 ± 4.99	28.34 ± 4.78	36.94 ± 2.20	31.54 ± 6.14	38.51 ± 2.49
Phosphatidylethanolamine	19.54 ± 2.73	19.25 ± 1.96	17.45 ± 1.68	13.56 ± 2.13	17.49 ± 0.85	14.38 ± 2.67	18.46 ± 1.04
Phosphatidylcholine	37.62 ± 5.69	36.17 ± 4.55	29.17 ± 4.13	21.44 ± 4.11	26.67 ± 1.73	24.58 ± 4.43	32.43 ± 1.85
Phophatidylinositol	4.65 ± 0.77	3.83 ± 0.39	3.92 ± 0.49	2.91 ± 0.54	3.18 ± 0.30	3.37 ± 0.55	5.06 ± 0.63
Phosphatidylserine	0.16 ± 0.03	0.85 ± 0.11	0.92 ± 0.15	0.69 ± 0.12	1.66 ± 0.17	1.56 ± 0.23	1.10 ± 0.25
Lysophophsatidylcholine	0.81 ± 0.12	2.01 ± 1.26	0.76 ± 0.06	0.79 ± 0.07	1.32 ± 0.46	0.80 ± 0.19	1.41 ± 0.22
Sphyngomyeline	2.11 ± 0.34	1.97 ± 0.28	1.68 ± 0.30	1.49 ± 0.31	1.68 ± 0.13	1.48 ± 0.29	2.53 ± 0.20*
Plasmalogen	1.13 ± 0.22	0.75 ± 0.09	0.65 ± 0.08	0.65 ± 0.12	0.86 ± 0.06	0.64 ± 0.12	1.11 ± 0.04*
PUFA/MUFA	0.38 ± 0.01	0.34 ± 0.02	0.26 ± 0.04	0.15 ± 0.01*	0.45 ± 0.06	0.30 ± 0.05	0.21 ± 0.01*
PC/PE	1.93 ± 0.08	1.87 ± 0.09	1.65 ± 0.07	1.56 ± 0.05	1.52 ± 0.04	1.73 ± 0.06	1.74 ± 0.06
Lipid-soluble (% mol)							
ω-3	7.83 ± 0.20	5.92 ± 0.35	5.87 ± 0.46	5.77 ± 0.19	4.99 ± 0.59	3.83 ± 0.58	3.99 ± 0.37
ARA+EPA	14.37 ± 0.36	17.72 ± 1.37	9.78 ± 1.97*	6.09 ± 0.98*	9.16 ± 2.07	7.06 ± 1.39	3.24 ± 0.37*
Oleic	24.84 ± 0.34	25.26 ± 1.87	32.48 ± 2.69	40.41 ± 0.89*	32.34 ± 2.38	36.87 ± 1.52	46.90 ± 0.33*
DHA	5.47 ± 0.40	3.78 ± 0.25	4.10 ± 0.36	2.25 ± 0.12*	2.86 ± 0.47	2.40 ± 0.45	1.64 ± 0.11*
Linoleic	18.55 ± 1.00	16.96 ± 0.82	19.23 ± 1.26	17.11 ± 0.47	25.87 ± 1.74	14.94 ± 0.71*	13.41 ± 0.65*
PUFA	64.32 ± 1.40	49.80 ± 3.84	39.23 ± 5.50	22.91 ± 1.44*	32.53 ± 4.31	22.84 ± 3.96	16.21 ± 1.10*
MUFA	84.78 ± 0.77	72.73 ± 3.38	75.43 ± 2.36	76.79 ± 0.72	72.73 ± 3.38	75.43 ± 2.36*	76.79 ± 0.72
Water-soluble (µmol/ g liver)							
Glucose-6-Phophate	19.34 ± 2.79	18.45 ± 3.44	8.87 ± 3.85	3.10 ± 0.47*	2.67 ± 0.98	12.29 ± 1.42*	9.18 ± 3.24
Glucose-1-Phosphate (Glycogen)	40.63 ± 10.31	50.56 ± 10.27	19.70 ± 11.49	2.39 ± 0.56*	0.57 ± 0.14	25.82 ± 3.72*	4.41 ± 1.65
UDPG	0.17 ± 0.05	0.41 ± 0.05	0.21 ± 0.05*	0.22 ± 0.04	0.20 ± 0.10	0.16 ± 0.08	0.22 ± 0.02
Pyruvate	0.53 ± 0.29	0.56 ± 0.08	0.31 ± 0.06*	0.42 ± 0.13	0.25 ± 0.08	0.38 ± 0.17	0.21 ± 0.05
3-hidroxybutyrate	0.21 ± 0.02	0.18 ± 0.03	0.24 ± 0.03	0.23 ± 0.04	0.39 ± 0.12	0.24 ± 0.05	0.30 ± 0.04

	10 weeks age	16 weeks age			32 weeks age		
	Chow Diet	Chow Diet	HF Diet	HF+0.25 Chol Diet	Chow Diet	HF Diet	HF+0.25 Chol Diet
Water-soluble (μmol/ g liver)							
Lactate	7.26 ± 0.63	6.98 ± 1.06	3.86 ± 0.90	3.00 ± 0.09*	3.51 ± 0.64	4.42 ± 0.89	5.54 ± 1.20
Fumarate	0.14 ± 0.03	0.09 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.06 ± 0.00*	0.14 ± 0.02
Free Glycerol	13.25 ± 2.60	14.27 ± 3.01	6.61 ± 3.12	1.53 ± 0.25*	2.36 ± 0.29	7.99 ± 0.84*	5.49 ± 2.40
NADH/NADP/NADPH	0.45 ± 0.08	0.44 ± 0.06	0.44 ± 0.05	0.32 ± 0.06	0.42 ± 0.04	0.33 ± 0.07	0.39 ± 0.03
ATP/ADP/AMP	1.22 ± 0.18	1.15 ± 0.17	1.48 ± 0.16	1.04 ± 0.15	1.28 ± 0.15	1.03 ± 0.15	1.28 ± 0.12
UTP/UDP/UMP	0.50 ± 0.07	0.34 ± 0.05	0.42 ± 0.04	0.32 ± 0.05	0.51 ± 0.05	0.40 ± 0.07	0.40 ± 0.06
Uracil	0.13 ± 0.04	0.07 ± 0.01	0.12 ± 0.02	0.05 ± 0.01	0.08 ± 0.03	0.07 ± 0.01	0.09 ± 0.03
Carnitine	2.37 ± 0.55	2.38 ± 0.32	0.90 ± 0.44	0.32 ± 0.12*	0.51 ± 0.05	1.06 ± 0.34	0.43 ± 0.02
Cholines	0.31 ± 0.04	0.24 ± 0.04	0.43 ± 0.06	0.32 ± 0.08	0.46 ± 0.09	0.27 ± 0.04	0.34 ± 0.04
Acetates	1.27 ± 0.10	1.88 ± 0.39	1.77 ± 0.39	1.77 ± 0.34	1.92 ± 0.29	1.79 ± 0.19	1.47 ± 0.07
Ascorbic acid	1.42 ± 0.33	2.54 ± 0.36	1.54 ± 0.21*	1.56 ± 0.38	1.49 ± 0.54	1.17 ± 0.38	1.15 ± 0.13
Leucine	0.59 ± 0.08	0.36 ± 0.05	0.56 ± 0.05	0.48 ± 0.03	0.70 ± 0.06	0.50 ± 0.06	1.04 ± 0.15
Valine	0.52 ± 0.06	0.35 ± 0.03	0.45 ± 0.05	0.43 ± 0.03	0.59 ± 0.07	0.41 ± 0.04	0.89 ± 0.15
Alanine	4.99 ± 0.89	3.38 ± 0.29	3.19 ± 0.69	2.44 ± 0.31	3.15 ± 0.20	2.98 ± 0.47	3.70 ± 0.26
Isoleucine	0.25 ± 0.03	0.16 ± 0.02	0.23 ± 0.02	0.16 ± 0.01	0.29 ± 0.03	0.20 ± 0.02	0.34 ± 0.06
Lysine	0.89 ± 0.08	0.59 ± 0.04	0.76 ± 0.09	0.61 ± 0.04	1.01 ± 0.11	0.67 ± 0.08	1.26 ± 0.14
Glutamine	1.64 ± 0.14	2.16 ± 0.35	1.62 ± 0.33	1.30 ± 0.18	1.37 ± 0.25	1.30 ± 0.24	2.11 ± 0.24
Glutamate	1.56 ± 0.40	0.75 ± 0.05	0.74 ± 0.09	0.98 ± 0.23	0.93 ± 0.05	0.68 ± 0.09	1.27 ± 0.04*
Methionine	0.93 ± 0.14	0.41 ± 0.08	0.45 ± 0.05	0.33 ± 0.05	0.75 ± 0.12	0.30 ± 0.03*	0.57 ± 0.05
Phenylalanine	0.23 ± 0.03	0.11 ± 0.02	0.21 ± 0.02*	0.15 ± 0.02	0.27 ± 0.04	0.19 ± 0.03	0.39 ± 0.06
Threonine	0.76 ± 0.10	0.69 ± 0.10	0.70 ± 0.07	0.52 ± 0.07	0.69 ± 0.01	0.56 ± 0.06	0.78 ± 0.06
Tyrosine	0.23 ± 0.03	0.12 ± 0.02	0.18 ± 0.02	0.14 ± 0.02	0.24 ± 0.02	0.16 ± 0.02	0.32 ± 0.04
Histidine	0.24 ± 0.03	0.22 ± 0.03	0.18 ± 0.03	0.14 ± 0.02	0.18 ± 0.02	0.14 ± 0.01	0.26 ± 0.04
Taurine	27.46 ± 3.17	24.98 ± 2.66	19.38 ± 2.48	13.46 ± 2.35*	14.54 ± 2.30	12.32 ± 2.15	15.00 ± 1.65
Glutathione (oxidized)	2.39 ± 0.42	3.03 ± 0.29	1.87 ± 0.43	1.20 ± 0.20*	1.24 ± 0.14	1.56 ± 0.39	1.56 ± 0.08
Creatine	0.87 ± 0.42	0.25 ± 0.03	0.31 ± 0.05	0.28 ± 0.04	0.39 ± 0.03	0.26 ± 0.03	0.46 ± 0.04
Creatinine	0.14 ± 0.02	0.05 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.14 ± 0.02