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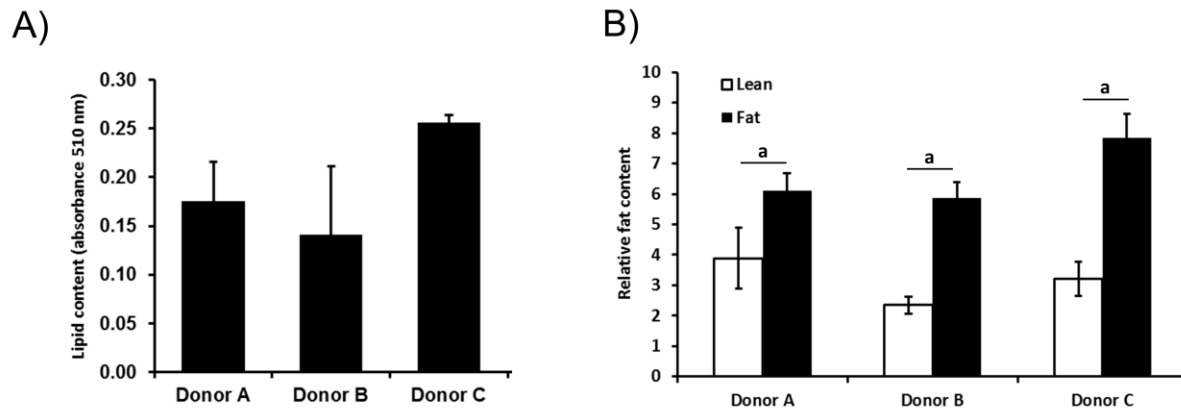
Three-dimensional perfused human *in vitro* model of non-alcoholic fatty liver disease

Kostrzewski T *et al.* Human *in vitro* model of NAFLD

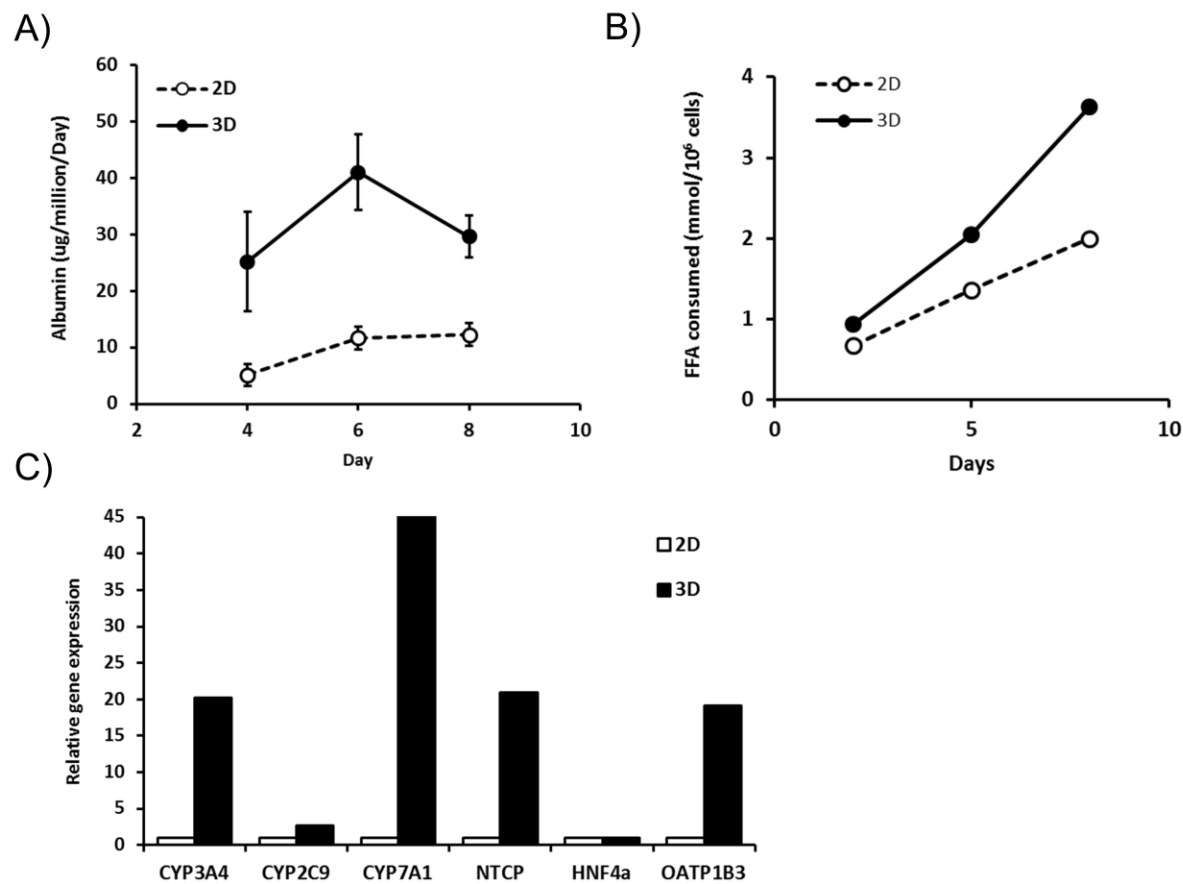
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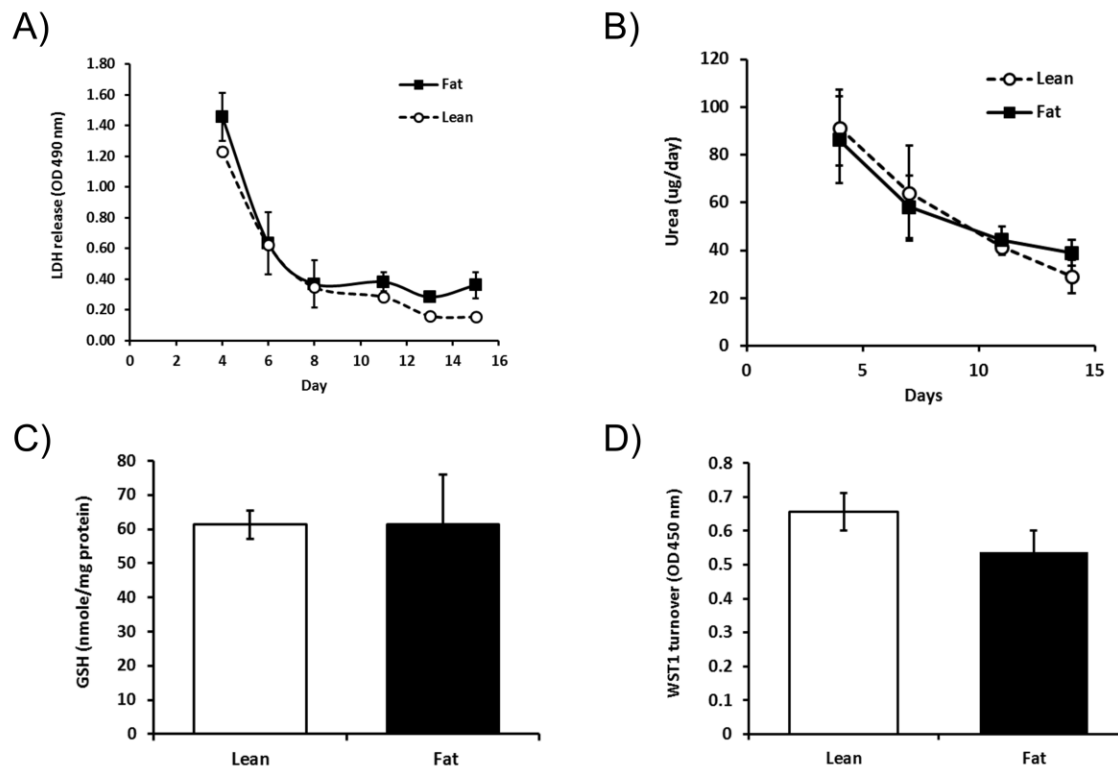
Supplementary material



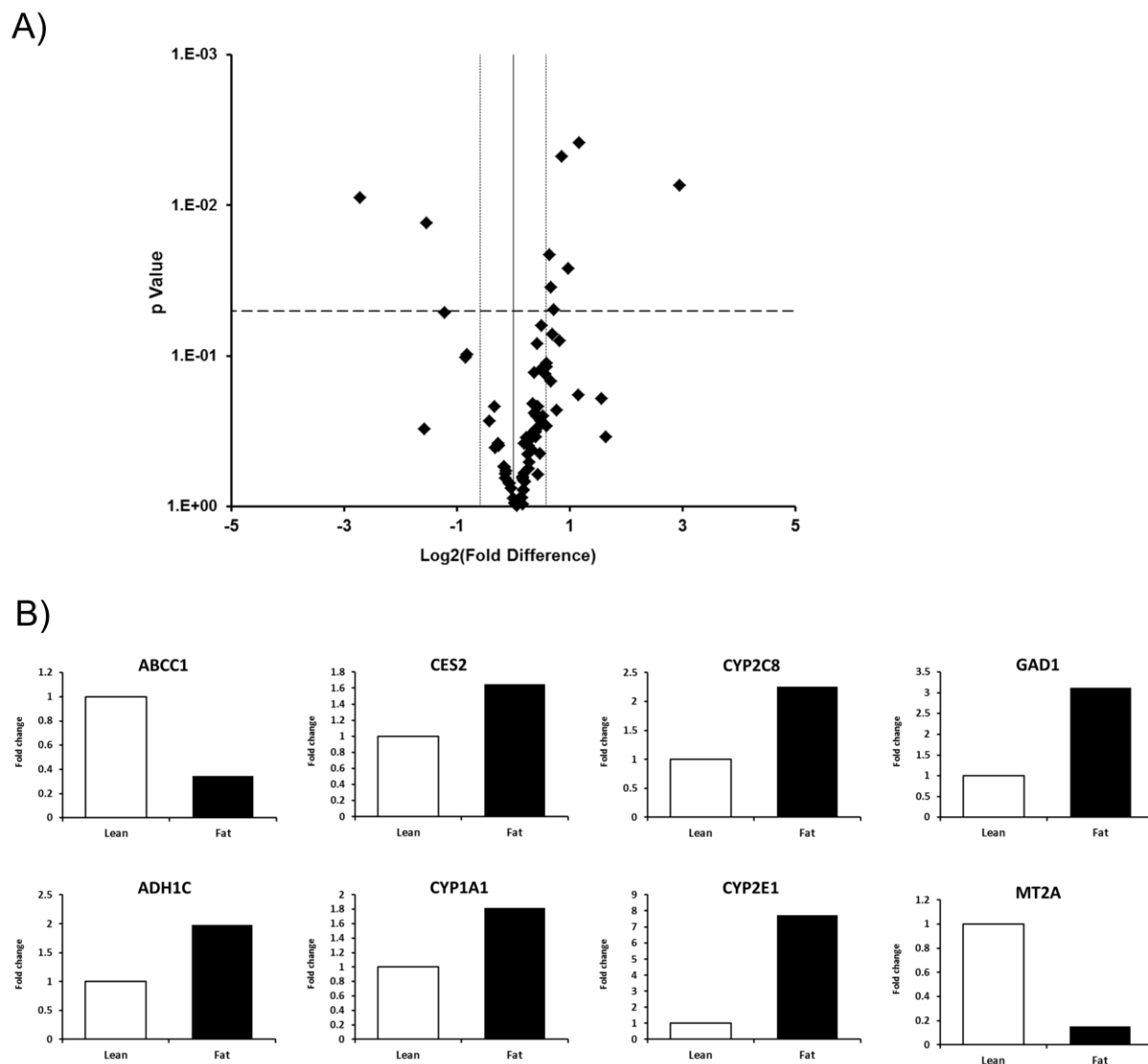
Supplemental Figure 1 –Cryopreserved human hepatocyte donors have varying lipid content but all load with fat in NAFLD model. A) Equivalent numbers of cells of cryopreserved human hepatocyte donors were thawed and stained with Oil Red O to measure fat content. Oil Red O staining was quantified by absorbance at 510 nm. B) Cells from three donors were cultured for 7 days in lean or fat conditions and fat loading was determined by measuring Oil Red O staining and normalising to total cellular protein content. Data is a mean \pm SD (n =3), a = P < 0.05.



Supplemental Figure 2 – Hepatocytes cultured in 3D outperform those cultured in 2D and accumulate greater quantities of fat. Hepatocytes were cultured in LiverChip (3D) or in static 2D plates for seven days under fat conditions. Equivalent samples were compared for A) Albumin production. B) Fat consumed by cells over 7 days of culture was calculated by analysing culture medium for the presence of free fatty acids using enzyme-based colorimetric assay. C) Relative expression of hepatic genes, as measured by QPCR; expression of each gene was normalised to GAPDH and is shown as relative to 2D sample. Data shown are a mean \pm SD, n =3 [C] n=2].



Supplemental Figure 3 - Hepatocytes are viable under fat conditions and have functional mitochondria. Hepatocytes were cultured for 14 days (A, B) or 7 days (C, D) in fat and lean conditions and compared for (A) LDH release; (B) urea production; (C) cellular glutathione (GSH) concentrations; and (D) WST-1 turnover. Each data point is a mean of 3 independent cultures, \pm SD.



Supplemental Figure 4 – Hepatocytes cultured in fat media have altered gene expression profiles. Hepatocytes were cultured in fat or lean conditions for 7 days before total RNA was extracted and gene expression was compared using Drug Metabolism RT² Profiler PCR Arrays. A) Gene expression changes were defined by a fold change >1.95 and $P = < 0.05$. B) Fold change in expression in fat vs lean condition of key genes, filled bars = fat, white bars = lean. Data are means \pm SEM from three independent cultures.

Supplemental Table 1 - Gene expression changes in NAFLD model analysed by Human Fatty Liver RT² Profiler™ PCR Arrays

Gene	Lean		Fat		Fold regulation
	Ct average	2 ⁻ ΔCt	Ct average	2 ⁻ ΔCt	
ABCA1	25.44	1.28E-02	25.8	1.32E-02	1.04
ACACA	23.88	3.78E-02	24.2	4.01E-02	1.06
ACADL	23.03	6.82E-02	23.26	7.73E-02	1.13
ACLY	21.87	1.52E-01	22.1	1.72E-01	1.13
ACOX1	21.92	1.47E-01	21.97	1.89E-01	1.29
ACSL5	22.35	1.09E-01	22.86	1.02E-01	-1.07
ACSM3	24.98	1.76E-02	24.61	3.04E-02	1.73
ADIPOR1	22.8	7.97E-02	23.05	8.94E-02	1.12
ADIPOR2	22.93	7.32E-02	22.89	9.97E-02	1.36
AKT1	23.87	3.82E-02	23.96	4.75E-02	1.24
APOA1	17.56	3.01E+00	17	5.92E+00	1.95
APOB	17.62	2.90E+00	17.64	3.80E+00	1.31
APOC3	15.32	1.43E+01	15.19	2.08E+01	1.46
APOE	19.86	6.14E-01	19.75	8.82E-01	1.44
ATP5C1	20.69	3.46E-01	20.81	4.21E-01	1.22
CASP3	24.39	2.66E-02	24.67	2.91E-02	1.09
CD36	30.21	4.70E-04	30.23	6.13E-04	1.31
CEBPB	23.37	5.38E-02	23.17	8.21E-02	1.53
CNBP	21.63	1.80E-01	21.8	2.12E-01	1.18
CPT1A	25.3	1.41E-02	25.29	1.89E-02	1.34
CPT2	24.11	3.22E-02	23.68	5.76E-02	1.79
CYP2E1	23.57	4.68E-02	21.59	2.46E-01	5.26
CYP7A1	23.41	5.24E-02	21.36	2.88E-01	5.49
DGAT2	21.91	1.49E-01	21.97	1.89E-01	1.27
FABP1	18.68	1.39E+00	18.1	2.76E+00	1.98
FABP3	27.05	4.21E-03	26.62	7.51E-03	1.78
FABP5	26.39	6.64E-03	26.24	9.77E-03	1.47
FAS	22.9	7.44E-02	23.31	7.45E-02	1.00
FASN	19.36	8.68E-01	19.21	1.28E+00	1.47
FOXA2	24.74	2.08E-02	24.86	2.54E-02	1.22
FOXO1	27.66	2.76E-03	27.71	3.54E-03	1.28
G6PC	24.96	1.79E-02	24.56	3.14E-02	1.75
G6PD	29.73	6.56E-04	29.61	9.49E-04	1.45
GCK	26.19	7.60E-03	25.99	1.16E-02	1.53
GK	26.18	7.68E-03	25.72	1.40E-02	1.82
GSK3B	23.75	4.14E-02	24.02	4.55E-02	1.10
HMGCR	21.91	1.48E-01	21.79	2.14E-01	1.44
HNF4A	23.25	5.87E-02	23.25	7.74E-02	1.32
IFNG	35	1.70E-05	35	2.30E-05	1.33
IGF1	24.61	2.27E-02	24.3	3.75E-02	1.65
IGFBP1	25.06	1.67E-02	23.99	4.64E-02	2.78
IL10	35	1.70E-05	35	2.30E-05	1.33
IL1B	32.83	7.70E-05	34.38	3.50E-05	-2.21
IL6	32.94	7.10E-05	33.82	5.10E-05	-1.39
INSR	23.8	4.00E-02	23.76	5.45E-02	1.36

IRS1	26.1	8.09E-03	25.97	1.18E-02	1.46
LDLR	22.48	9.99E-02	22.41	1.39E-01	1.39
LEPR	24.54	2.40E-02	24.3	3.75E-02	1.57
LPL	32.01	1.35E-04	32.31	1.46E-04	1.08
MAPK1	22.88	7.55E-02	22.99	9.30E-02	1.23
MAPK8	25.11	1.61E-02	25.33	1.84E-02	1.14
MLXIPL	22.82	7.91E-02	23.02	9.12E-02	1.15
MTOR	22.74	8.33E-02	22.76	1.10E-01	1.32
NDUFB6	26.33	6.93E-03	26.55	7.90E-03	1.14
NFKB1	25.59	1.15E-02	25.64	1.49E-02	1.29
NR1H2	25.53	1.20E-02	25.64	1.48E-02	1.23
NR1H3	24.54	2.39E-02	24.66	2.92E-02	1.22
NR1H4	22.63	8.98E-02	23.19	8.12E-02	-1.11
PCK2	22.69	8.64E-02	22.79	1.07E-01	1.23
PDK4	25.22	1.49E-02	23.45	6.77E-02	4.54
PIK3CA	24.55	2.37E-02	24.62	3.00E-02	1.27
PIK3R1	23.55	4.77E-02	23.52	6.45E-02	1.35
PKLR	21.9	1.49E-01	21.8	2.13E-01	1.43
PNPLA3	23.92	3.69E-02	23.98	4.69E-02	1.27
PPA1	21.01	2.75E-01	21.28	3.04E-01	1.10
PPARA	23.88	3.78E-02	23.74	5.52E-02	1.46
PPARG	25.85	9.66E-03	25.88	1.26E-02	1.30
PPARGC1A	25.69	1.08E-02	26.03	1.13E-02	1.05
PRKAA1	23.33	5.53E-02	23.63	5.95E-02	1.08
PTPN1	26.28	7.19E-03	26.45	8.47E-03	1.18
RBP4	15.63	1.15E+01	15.98	1.20E+01	1.05
RXRA	23.48	4.99E-02	23.28	7.61E-02	1.52
SCD	18.02	2.19E+00	18.27	2.45E+00	1.12
SERPINE1	20.71	3.41E-01	21.39	2.82E-01	-1.21
SLC27A5	24.63	2.25E-02	24.87	2.53E-02	1.12
SLC2A1	30.91	2.90E-04	31.55	2.46E-04	-1.18
SLC2A2	21.22	2.39E-01	21.35	2.90E-01	1.21
SLC2A4	28.52	1.51E-03	27.72	3.52E-03	2.32
SOCS3	33.56	4.60E-05	33.3	7.30E-05	1.59
SREBF1	25.05	1.68E-02	25.56	1.57E-02	-1.07
SREBF2	23.47	5.04E-02	23.53	6.38E-02	1.27
STAT3	22.36	1.08E-01	22.74	1.11E-01	1.02
TNF	34.57	2.30E-05	34.38	3.50E-05	1.51
XBP1	20.71	3.39E-01	20.91	3.93E-01	1.16

Supplemental Table 2 – Gene expression changes in NAFLD model analysed by Human Drug Metabolism RT² Profiler™ PCR Arrays

Gene	<u>Lean</u>		<u>Fat</u>		Fold regulation
	Ct average	2 ^{^-ΔCt}	Ct average	2 ^{^-ΔCt}	
ABCB1	22.97	6.8E-02	22.96	8.6E-02	1.27
ABCC1	30.36	4.0E-04	32.24	1.4E-04	-2.91
AOC1	31.83	1.5E-04	31.68	2.1E-04	1.41
ADH1B	18.46	1.5E+00	17.99	2.7E+00	1.76
ADH1C	18.15	1.9E+00	17.51	3.8E+00	1.98
ADH4	23.05	6.4E-02	24.21	3.6E-02	-1.76
ADH5	22.37	1.0E-01	22.60	1.1E-01	1.08
ADH6	22.61	8.7E-02	22.90	9.0E-02	1.04
AHR	22.96	6.8E-02	23.11	7.8E-02	1.15
ALAD	22.79	7.7E-02	22.73	1.0E-01	1.32
ALDH1A1	18.67	1.3E+00	19.15	1.2E+00	-1.10
ALOX12	31.78	1.5E-04	31.64	2.1E-04	1.40
ALOX15	30.70	3.2E-04	31.36	2.5E-04	-1.25
ALOX5	32.28	1.1E-04	32.45	1.2E-04	1.13
APOE	19.24	9.0E-01	19.62	8.7E-01	-1.03
ARNT	25.57	1.1E-02	25.89	1.1E-02	1.02
ASNA1	24.28	2.7E-02	24.26	3.5E-02	1.29
BLVRA	26.99	4.2E-03	27.03	5.1E-03	1.23
BLVRB	21.12	2.4E-01	21.31	2.7E-01	1.11
CES1	17.57	2.8E+00	17.85	3.0E+00	1.05
CES2	20.54	3.6E-01	20.16	6.0E-01	1.65
CES3	25.92	8.8E-03	26.07	1.0E-02	1.14
CHST1	34.08	3.1E-05	33.27	6.8E-05	2.23
COMT	20.82	3.0E-01	21.23	2.9E-01	-1.05
CYB5R3	21.50	1.9E-01	21.60	2.2E-01	1.18
CYP11B2	34.38	2.5E-05	33.16	7.3E-05	2.95
CYP17A1	31.13	2.4E-04	31.21	2.8E-04	1.19
CYP19A1	34.80	2.0E-05	34.53	2.8E-05	1.39
CYP1A1	25.83	9.3E-03	25.30	1.7E-02	1.82
CYP2B6	23.99	3.3E-02	23.73	5.1E-02	1.51
CYP2C19	23.42	4.9E-02	23.20	7.3E-02	1.48
CYP2C8	20.95	2.7E-01	20.12	6.2E-01	2.26
CYP2C9	20.29	4.3E-01	20.21	5.8E-01	1.35
CYP2D6	20.59	3.5E-01	20.76	4.0E-01	1.13
CYP2E1	23.73	4.0E-02	21.13	3.1E-01	7.71
CYP2F1	34.61	2.1E-05	34.51	2.9E-05	1.36
CYP2J2	22.83	7.5E-02	22.58	1.1E-01	1.50
CYP3A4	20.17	4.7E-01	20.35	5.3E-01	1.12
CYP3A5	20.46	3.9E-01	20.27	5.6E-01	1.45
EPHX1	19.41	8.0E-01	19.06	1.3E+00	1.62
FAAH	27.41	3.1E-03	27.43	3.9E-03	1.25
FBP1	21.80	1.5E-01	21.70	2.1E-01	1.36
GAD1	33.21	5.6E-05	31.91	1.7E-04	3.12
GAD2	39.71	1.8E-05	34.60	2.8E-05	1.60
GCKR	23.77	3.9E-02	23.60	5.5E-02	1.43

GPI	21.63	1.7E-01	21.94	1.7E-01	1.02
GPX1	19.20	9.2E-01	19.71	8.2E-01	-1.12
GPX2	24.99	1.7E-02	25.67	1.3E-02	-1.26
GPX3	22.28	1.1E-01	21.95	1.7E-01	1.59
GPX4	19.09	9.9E-01	19.06	1.3E+00	1.30
GPX5	37.74	1.6E-05	34.93	2.2E-05	1.33
GSR	27.54	2.9E-03	28.02	2.6E-03	-1.11
GSTA3	27.61	2.7E-03	28.80	1.5E-03	-1.80
GSTA4	25.29	1.4E-02	25.53	1.5E-02	1.07
GSTM2	27.39	3.1E-03	27.56	3.6E-03	1.13
GSTM3	23.40	5.0E-02	23.47	6.0E-02	1.20
GSTM5	34.86	1.8E-05	34.72	2.5E-05	1.40
GSTP1	28.75	1.2E-03	29.16	1.2E-03	-1.05
GSTT1	20.91	2.8E-01	21.38	2.6E-01	-1.09
GSTZ1	23.96	3.4E-02	24.56	2.9E-02	-1.20
HK2	31.99	1.3E-04	33.90	4.4E-05	-2.98
HSD17B1	30.56	3.5E-04	30.57	4.4E-04	1.26
HSD17B2	21.42	2.0E-01	21.33	2.7E-01	1.35
HSD17B3	27.41	3.1E-03	27.57	3.5E-03	1.13
LPO	36.81	1.4E-05	38.21	2.1E-05	1.50
MGST1	16.55	5.8E+00	16.49	7.6E+00	1.32
MGST2	20.84	3.0E-01	20.91	3.6E-01	1.20
MGST3	21.24	2.2E-01	21.17	3.0E-01	1.33
MPO	34.97	1.7E-05	34.53	2.8E-05	1.71
MT2A	18.37	1.6E+00	21.43	2.5E-01	-6.58
MT3	36.58	1.6E-05	34.94	2.1E-05	1.32
MTHFR	27.84	2.3E-03	28.01	2.6E-03	1.12
NAT1	29.23	8.8E-04	29.37	1.0E-03	1.15
NAT2	23.00	6.6E-02	22.93	8.8E-02	1.33
NOS3	34.39	2.5E-05	34.14	3.7E-05	1.51
NQO1	27.91	2.2E-03	28.52	1.8E-03	-1.21
PKLR	22.07	1.3E-01	22.41	1.3E-01	-1.00
PKM	25.34	1.3E-02	25.01	2.1E-02	1.59
PON1	19.77	6.2E-01	20.27	5.6E-01	-1.11
PON2	21.42	2.0E-01	21.68	2.1E-01	1.06
PON3	21.73	1.6E-01	21.78	2.0E-01	1.22
SNN	27.22	3.6E-03	26.92	5.5E-03	1.56
SRD5A1	23.07	6.3E-02	23.83	4.7E-02	-1.34
SRD5A2	23.15	6.0E-02	24.71	2.6E-02	-2.32
