Metabolic consequences of mouse hepatocyte culture and impact of TGFβ signaling

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Background. Culture of primary hepatocytes is highly important for medical applications [1,2] and liver research [3,4]. However, the metabolism of cultured hepatocytes undergoes substantial changes [5]. Autocrine and paracrine acting factors as TGFβ further enhance/modify this spontanously occurring process [6].

Metabolic genes are more expressed.

The average expression value of the mRNA associated with reactions of HepatoNet1 compared to the average expression value of all spots by time point and treatment series.

	1h (%)	**6h** (%)	**24h** (%)	**all** (%)
control	11.5	11.1	9.5	10.7
TGFβ	11.3	10.7	7.7	9.9
average	11.4	10.9	8.6	10.3

Data. Full genome RNA transcript profiles of primary mouse hepatocytes cultured on collagen 6-well plates / monolayer (3 time points, 1h, 6h, and 24h, control versus TGFβ stimulation, 3 repeats) [6–8].

Method. Flux distributions computed in the stoichiometric network of the hepatocyte's metabolism [9] for a plethora of metabolic functions are matched to transcript changes with a novel method combining the scoring approach [10] to complex functional flux distributions [11], implemented in [12].

$$\mathsf{Score}(M_k,V)=\frac{\sum_{i\in I_k} w_i \mathsf{score}_i(m_i,v_i)}{\sum_{i\in I_k} w_i} \qquad \text{total score}$$

$$I_k=\{i\,|\,m_i\neq 0\} \qquad \text{indices of nonzero values}$$

$$w_i=\sqrt{|m_i|\omega_i} \qquad \text{weights}$$

$$\omega_i \qquad \text{weight adjustment}$$

$$\mathsf{score}_i(m_i,v_i)=e^{-\frac{1}{2}\left(2\frac{\lambda v_i-m_i}{|m_i|}\right)^2} \qquad \text{score component}$$

$$M_k=(m_i)_i \qquad k\text{-th reference mode}$$

$$V=(v_i)_i \qquad \text{relative expression profile}$$

$$\lambda \text{ chosen such that} \qquad \mathsf{Score}(M_k,V) \quad \text{is maximal}$$

$$\mathsf{Impact}_i=w_i(\mathsf{score}_i(m_i,v_i)-\mathsf{Score}(M_k,V)) \qquad \text{influence measure}$$

Function-specific regulation in the time course.

Relative expression scores of the third time point (24h) with respect to the first (1h) for the control and TGFβ series sorted by the sum of amplitudes (inverse of scaling factor λ). Only the 40 most down-regulated shown.

	control			TGFβ		
Simulation	**rank**	**ampl**	**score**	**rank**	**ampl**	**score**
Anaerobic rephosph of ATP	2	-8.74	0.31	1	-12.6	0.29
Gluconeogen. from Glycerol	4	-6.38	0.42	3	-9.16	0.35
Aerobic ATP rephosph (FA)	3	-6.75	0.28	4	-8.68	0.31
Galactose degr	5	-5.49	0.26	5	-8.13	0.24
Complete oxidation of oleate	7	-5.31	0.28	6	-7.2	0.33
Ethanol(s) degr min	68	-0.2	0.24	2	-11.5	0.25
Aerobic reduction of NADP+	8	-5.25	0.24	7	-6.34	0.26
Triacylglycerol(r) synt phys	1	-10.6	0.23	64	-0.97	0.29
Gly-CD-cholate(b) phys	6	-5.41	0.27	10	-4.59	0.28
Lysine degr	10	-3.91	0.22	14	-4.22	0.28
Urea from alanine	12	-3.04	0.25	11	-4.52	0.28
Glycocholate(b) synt phys	20	-2.04	0.26	12	-4.42	0.27
LDL(s) degr phys	9	-4.12	0.2	37	-1.89	0.24
Alanine(c) synt min	49	-0.83	0.31	9	-4.61	0.31
Taurocholate(b) synt phys	17	-2.25	0.27	16	-3.16	0.28
ATP salvage from Adenosine	72	-0.08	0.21	8	-5.08	0.23
CTP(c) synt min	15	-2.41	0.21	23	-2.62	0.24
Acetoacetate	19	-2.19	0.35	19	-2.79	0.36
(R)-3-Hydroxybutanoate	21	-2.02	0.4	21	-2.66	0.43
THF(c) synt min	74	-0.03	0.33	13	-4.34	0.25
Aspartate degr	27	-1.59	0.24	22	-2.66	0.28
Gluconeogen. from Lactate	42	-1.09	0.26	18	-2.93	0.33
Methionine degr	13	-2.86	0.25	57	-1.13	0.32
Stearate(c) synt min	38	-1.23	0.35	20	-2.69	0.37
ATP salvage from Hypoxanthine	86	0.36	0.21	15	-4.21	0.23
Bilirubin conjugation	11	-3.45	0.23	88	-0.15	0.26
UTP(c) synt min	30	-1.51	0.2	33	-2.08	0.27
udpglcur(c) synt min	24	-1.74	0.22	39	-1.82	0.26
Gluconeogen. from Alanine	45	-0.96	0.31	24	-2.59	0.35
cmpneuNAc(n) synt min	26	-1.62	0.25	36	-1.9	0.25
udpgal(c) synt min	25	-1.7	0.23	40	-1.75	0.27
NH3 degr	40	-1.17	0.25	35	-2.01	0.32
Pyridoxal-P(c) synt min	73	-0.03	0.21	17	-3.13	0.23
Asparagine(c) synt min	16	-2.27	0.25	75	-0.76	0.32
UDP-glucose(c) synt min	37	-1.27	0.22	41	-1.73	0.27
Creatine(c) synt min	32	-1.38	0.25	44	-1.56	0.34
Histidine degr	18	-2.21	0.25	77	-0.72	0.29
Arginine(c) synt min	34	-1.35	0.35	43	-1.57	0.4
Glutamate degr	14	-2.75	0.21	87	-0.17	0.3
udpgalnac(c) synt min	31	-1.43	0.23	45	-1.47	0.25

Overall down-regulation.

The average expression value of the mRNA of two samples (in the lower two sections) or averages of samples (upper section) is compared for all spots (second column) and for reactions associated with reactions of HepatoNet (last column).

	all (%)	**metabolic** (%)
24h vs. 1h	-0.12	-2.6
6h vs. 1h	-0.03	-0.49
24h vs. 6h	-0.09	-2.1
TGFβ vs. contr	0.002	-0.68
control series		
24h vs. 1h	-0.09	-1.8
6h vs. 1h	-0.02	-0.4
24h vs. 6h	-0.07	-1.5
TGFβ treated series		
24h vs. 1h	-0.15	-3.4
6h vs. 1h	-0.03	-0.6
24h vs. 6h	-0.11	-2.8

Detailed Flux chart

Synthesis of Glycochenodeoxycholate

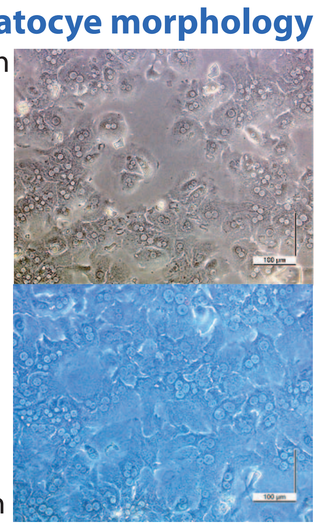
Rea ID	control Score	Impct	Expr diff	Expr 1h	Expr 24h	Expr *=0.18*	TGFβ Score	Impct	Expr diff	Expr 1h	Expr 24h	Expr *=0.22*	Ref value	Wght	Reaction
	0.27						*0.28*								
r0144	0.99	0.92	-9.66	10.2	0.58	1.78	0.96	0.88	-8.67	9.19	0.52	1.89	1.65	1.28	Arginine(c) + H2O(c) ⇌ Urea(c) + Ornithine(c)
r0192	0.75	0.66	-6.49	17	10.5	1.2	0.97	0.97	-7.85	15.2	7.32	1.71	1.94	1.39	Fructose-6P(c) ⇌ Glucose-6P(c)
r0322	0.96	0.6	-3.55	7.67	4.13	0.66	0.94	0.58	-4.12	7.32	3.2	0.9	0.76	0.87	HMG-CoA(m) ⇌ Acetyl-CoA(m) + Acetoacetate(m)
r0019	0.14	-0.54	-0.05	0.69	0.64	0.01	0.14	-0.55	-0.14	0.69	0.55	0.03	15.6	3.94	ADP(m) + Pi(m) + 3 H+(PG)(c) → ATP(m) + H2O(m) + 3 H+(PG)(m)
r2473	0.97	0.55	-3.77	4.72	0.95	0.7	0.91	0.5	-3.47	4.18	0.71	0.76	0.62	0.79	Glucose-6P(c) + Pi(r) → Glucose-6P(r) + Pi(c)
r0487	0.97	0.56	-3.06	4.99	1.93	0.56	0.89	0.49	-2.24	4.36	2.12	0.49	0.65	0.8	Fructose-1,6PP(c) + H2O(c) → Fructose-6P(c) + Pi(c)
r0080	0.29	0.02	-1.87	7.01	5.14	0.35	0.81	0.69	-5.11	6.98	1.86	1.11	1.65	1.28	Alanine(c) + AKG(c) ⇌ Pyruvate(c) + Glutamate(c)
r0081	0.29	0.02	-1.87	7.01	5.14	0.35	0.81	0.69	-5.11	6.98	1.86	1.11	1.65	1.28	Alanine(m) + AKG(m) ⇌ Pyruvate(m) + Glutamate(m)
r0017	0.13	-0.35	0.19	7.35	7.53	-0.03	0.13	-0.35	0.08	7.32	7.39	-0.02	2.94	2.43	O2(m) + 4 H+(PG)(m) + 4 Ferrocytochrome C(m) → 4 Ferricytochrome C(m) + 2 H2O(m) + 4 H+(PG)(c)
r0507	0.13	-0.34	0.13	7.93	8.06	-0.02	0.14	-0.34	-0.04	7.93	7.89	0.01	5.89	2.43	Ubiquinol(m) + 2 Ferricytochrome C(m) + 4 H+(PG)(m) → Ubiquinone(m) + 4 H+(PG)(c) + 2 Ferrocytochrome C(m)
r0508	0.14	-0.32	-0.14	5.53	5.39	0.03	0.14	-0.32	-0.34	5.46	5.12	0.07	5.73	2.39	Ubiquinone(m) + NADH(m) + 4 H+(PG)(m) → Ubiquinol(m) + NAD+(m) + 4 H+(PG)(c)
r0607	0.92	0.26	-0.67	1.8	1.12	0.12	0.98	0.28	-0.78	1.67	0.89	0.17	0.16	0.39	Squalene 2,3-oxide(r) ⇌ Lanosterol(r)
r1135	0.82	0.21	-0.57	1.42	0.85	0.11	0.95	0.27	-0.6	1.33	0.73	0.13	0.16	0.39	3-Keto-4-methylzymosterol(r) + NADP+(r) ⇌ NADPH(r) + 4alpha-Methylzymosterol(r)
r0104	0.87	0.37	-1.47	9.95	8.48	0.27	0.46	0.11	-2.76	9.82	7.06	0.6	0.37	0.61	NAD+(c) + Malate(c) ⇌ OAA(c) + NADH(c)
r1047	0.13	-0.22	1.08	16.7	17.8	-0.2	0.11	-0.24	2.19	16.7	18.8	-0.48	11	1.48	Pi(c) + H+(PG)(c) → Pi(m) + H+(PG)(m)
r0007	0.15	-0.23	-0.31	3.88	3.58	0.06	0.15	-0.23	-0.37	3.96	3.59	0.08	3.19	1.79	PPi(c) + H2O(c) → 2 Pi(c)
r2524	0.13	-0.22	0.01	0.45	0.46	-0.002	0.14	-0.23	-0.01	0.46	0.45	0.001	2.65	1.63	Alanine(s) ⇌ Alanine(c)
r0030	0.14	-0.22	-0.05	1.21	1.16	0.01	0.14	-0.23	-0.01	1.19	1.18	0.002	2.57	1.6	ATP(c) + AMP(c) ⇌ 2 ADP(c)
r0781	0.84	0.39	-1.09	2.48	1.39	0.2	0.34	0.04	-1.24	2.29	1.05	0.27	0.16	0.68	3 O2(r) + 3 NADPH(r) + Lanosterol(r) ⇌ 4,4-Dimethyl-5alpha-cholesta-8,14,24-trien-3beta-ol(r) + Formate(r) + 4 H2O(r) + 3 NADP+(r)
r0414	0.64	0.14	-0.44	2.14	1.7	0.08	0.99	0.28	-0.66	2.05	1.39	0.14	0.16	0.39	ATP(m) + Propanoyl-CoA(m) + HCO3-(m) → ADP(m) + Pi(m) + Methylmalonyl-CoA(m)
r0872	0.64	0.18	-0.68	1.26	0.58	0.13	0.77	0.24	-0.7	1.2	0.5	0.15	0.24	0.49	Pyruvate(c) + H+(PG)(c) → Pyruvate(m) + H+(PG)(m)
r0056	0.15	-0.21	-0.32	3.46	3.14	0.06	0.16	-0.2	-0.54	3.33	2.79	0.12	2.81	1.67	Pyruvate(m) + CoA(m) + NAD+(m) → Acetyl-CoA(m) + CO2(m) + NADH(m)
r2383	0.39	0.08	-0.64	5.95	5.32	0.12	0.81	0.33	-1.15	6.19	5.04	0.25	0.37	0.61	Citrate(c) + H+(PG)(c) + Malate(m) → Citrate(m) + H+(PG)(m) + Malate(c)
r0406	0.03	-0.2	1.17	4.5	5.67	-0.22	0.02	-0.2	1.14	4.46	5.6	-0.25	0.65	0.8	Sedoheptulose-7P(c) + GAP(c) ⇌ Fructose-6P(c) + Erythrose-4P(c)
r0227	0.92	0.36	-1.35	3.03	1.68	0.25	0.21	-0.04	-0.17	2.78	2.61	0.04	0.31	0.56	10-Formyl-THF(c) + H2O(c) + NADP+(c) → THF(c) + CO2(c) + NADPH(c)
r0783	0.54	0.1	-0.37	1.76	1.39	0.07	0.99	0.28	-0.66	1.83	1.16	0.14	0.16	0.39	5alpha-Cholesta-7,24-dien-3beta-ol(r) + NADPH(r) ⇌ Lathosterol(r) + NADP+(r)
r0108	0.11	-0.19	0.41	2.66	3.07	-0.08	0.11	-0.19	0.32	2.66	2.98	-0.07	1.28	1.13	ATP(c) + Citrate(c) + CoA(c) → ADP(c) + Pi(c) + Acetyl-CoA(c) + OAA(c)
r0197	0.66	0.15	-0.46	2.29	1.83	0.08	0.84	0.22	-0.5	2.14	1.64	0.11	0.16	0.39	(R)-Methylmalonyl-CoA(m) ⇌ Succinyl-CoA(m)
r0821	0.12	-0.19	0.25	1.1	1.35	-0.05	0.14	-0.18	-0.03	1.13	1.1	0.01	1.65	1.28	Fumarate(c) + Succinate(m) ⇌ Fumarate(m) + Succinate(c)
r0256	0.67	0.32	-5.07	8.86	3.79	0.94	0.22	-0.05	-5.58	8.25	2.67	1.22	0.65	0.8	DHAP(c) + GAP(c) ⇌ Fructose-1,6PP(c)
r1134	0.02	-0.18	-2.06	5.16	3.1	0.38	0.0002	-0.19	-2.19	4.53	2.34	0.48	0.16	0.68	14-Demethyllanosterol(r) + 3 NADPH(r) + 3 O2(r) ⇌ 4 H2O(r) + 4alpha-Methylzymosterol-4-carboxylate(r) + 3 NADP+(r)
r0230	0.93	0.26	-1	3.15	2.15	0.18	0.03	-0.1	-1.67	3.06	1.39	0.36	0.16	0.39	THF(c) + Serine(c) ⇌ 5,10-Methylene-THF(c) + Glycine(c) + H2O(c)
r0344	0.12	-0.17	0.2	1.35	1.55	-0.04	0.11	-0.19	0.26	1.32	1.58	-0.06	1.3	1.14	Ribulose-5P(c) ⇌ D-Xylulose-5P(c)
r0896	0.14	-0.17	-0.02	0.4	0.38	0.003	0.14	-0.18	-0.02	0.4	0.38	0.003	1.65	1.28	Urea(c) ⇌ Urea(s)
r0243	0.43	0.13	-5.77	18.5	12.7	1.07	0	-0.22	-10.2	17.4	7.21	2.23	0.65	0.8	GAP(c) ⇌ DHAP(c)
r0947	0.14	-0.17	-0.02	0.45	0.43	0.004	0.14	-0.18	-0.03	0.47	0.43	0.01	1.65	1.28	Citrulline(m) + Ornithine(c) ⇌ Citrulline(c) + Ornithine(m)
r0069	0.62	0.14	-0.47	1.66	1.18	0.09	0.76	0.2	-0.5	1.61	1.11	0.11	0.17	0.42	2 Acetyl-CoA(c) ⇌ CoA(c) + Acetoacetyl-CoA(c)
r0059	0.14	-0.16	-0.02	0.23	0.21	0.004	0.14	-0.17	-0.01	0.23	0.22	0.002	1.44	1.2	Malate(m) + NADP+(m) ⇌ Pyruvate(m) + CO2(m) + NADPH(m)
r0672	0.81	0.21	-1.11	5.45	4.33	0.21	0.0004	-0.11	0.71	5.66	6.37	-0.15	0.16	0.39	7alpha-Hydroxycholesterol(r) + NADP+(r) ⇌ 7alpha-Hydroxycholest-4-en-3-one(r) + NADPH(r)
r0478	0.42	0.13	-1.44	6.54	5.1	0.27	0.06	-0.19	0.66	6.58	7.24	-0.14	0.78	0.88	Glucono-1,5-lactone-6P(r) + H2O(r) ⇌ 6-Phospho-D-gluconate(r)
r0342	0.08	-0.17	0.54	9.42	9.96	-0.1	0.44	0.14	-1.29	9.67	8.39	0.28	0.78	0.88	6-Phospho-D-gluconate(r) + NADP+(r) ⇌ NADPH(r) + CO2(r) + Ribulose-5P(r)
r0259	0.14	-0.18	-0.02	4.76	4.73	0.004	0.18	-0.12	-0.67	4.81	4.13	0.15	1.8	1.34	Fumarate(m) + H2O(m) ⇌ Malate(m)

shortened

Predictions

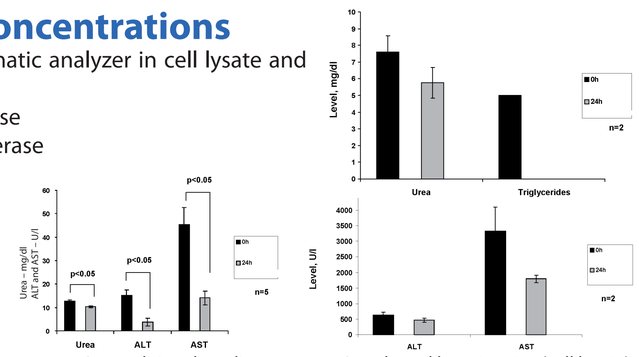
(i) Cell culture leads to down regulation of the metabolism as a whole which is accelerated by TGFβ.
(ii) The down regulation is especially pronounced at glycolysis to lactate, gluconeogenesis, β-oxidation of fatty acids, bile acid production, and bilirubin conversion, and amplified in TGFβ experiments.
(iii) Ethanol degradation is down regulated in the TGFβ experiment but not spontaneously. In contrast, triacylglycerol synthesis is down regulated in the control experiment but not in the TGFβ treated samples.
(iv) Glyconeogenesis' and β-oxidation's down regulation is more pronounced in the later stage in both experiments.
(v) In TGFβ treated samples, the down regulation of glycolysis mainly occurs before 6h, while in the control experiment it appears after the 6h time point. In contrast, decreased urea production in TGFβ treated samples mainly occurs after 6h while in the control experiment it shows before 6h.

Hepatocye morphology



First measured concentrations

Measured with Hitachi automatic analyzer in cell lysate and conditional media.
ALT – Alanine-aminotransferase
AST – Aspartate-aminotransferase



Large image: Flux distribution of the synthesis of Glycochenodeoxycholate. Control sample. Color codes the expression value of 24h sample with respect to 1h sample. red — upregulation, green — downregulation, yellow — constant. FLux distribution comprises of 139 reactions 103 of which are assigned to an RNA level.

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virtual liver network
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