

The “drug” transporter OAT3 regulates endogenous metabolite flow through the gut-liver-kidney axis

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SUPPLEMENTAL TABLE 1
Metabolites Increased in Plasma of *Oat3KO* Mice

Pathway	Biochemical Name	Fold Change	p-value	q-value	KEGG	HMDB
Aminosugar Metabolism	Glucuronate	1.49	0.0195	0.0810	C00191	HMDB00127
Benzoate Metabolism	catechol sulfate	3.92	0.0023	0.0233	C00090	HMDB59724
	O-methylcatechol sulfate	5.67	0.0163	0.0733		
	4-vinylphenol sulfate	5.34	0.0180	0.0777	C05627	HMDB04072
	p-hydroxybenzaldehyde	3.43	0.0612	0.1593	C00633	HMDB11718
Carnitine Metabolism	deoxycarnitine	1.46	0.0295	0.1042	C01181	HMDB01161
Chemical	2-aminophenol sulfate	3.21	0.0082	0.0461		HMDB61116
	6-hydroxyindole sulfate	6.08	0.0003	0.0083		
Drug	Salicylate	7.21	0.0045	0.0322	C00805	HMDB01895
	hydroquinone sulfate	2.63	0.0555	0.1557	C00530	HMDB02434
Fatty Acid, Dicarboxylate	sebacate (decanedioate)	1.90	0.0166	0.0737	C08277	HMDB00792
	tetradecanedioate	2.05	0.0037	0.0289		HMDB00872
	azelate (nonanedioate)	1.71	0.0754	0.1803	C08261	HMDB00784
	3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	2.58	0.0829	0.1915		HMDB61112
Fatty Acid, Monohydroxy	2-hydroxydecanoate	2.06	0.0143	0.0665		
	3-hydroxyhexanoate	2.12	0.0127	0.0610		
Food Component	2-hydroxyoctanoate	2.82	0.0606	0.1593		HMDB02264
	2-oxindole-3-acetate	3.94	0.0028	0.0253		
	equol glucuronide	42.32	0.0000	0.0000		
	equol sulfate	7.81	0.0000	0.0000		
	homostachydrine*	1.97	0.0233	0.0891	C08283	HMDB33433
	indoleacrylate	4.82	0.0062	0.0395		HMDB00734
	indolin-2-one	8.81	0.0040	0.0302	C12312	
	Stachydrine	1.86	0.0360	0.1182	C10172	HMDB04827
	4-allylphenol sulfate	6.26	0.0262	0.0959		
	Pyraline	1.60	0.0942	0.2029		
	N-acetylpyraline	2.08	0.0967	0.2055		
	daidzein sulfate (1)	5.99	0.0683	0.1712		
	Fructose, Mannose and Galactose Metabolism	mannitol/sorbitol	1.74	0.0473	0.1461	C01507
Galactonate		1.62	0.0621	0.1605	C00880	HMDB00565
Glutathione Metabolism	S-methylglutathione	2.30	0.0585	0.1575	C11347	
Glycine, Serine and Threonine Metabolism	O-acetylhomoserine	3.06	0.0501	0.1501	C01077	
Histidine Metabolism	imidazole propionate	4.46	0.0070	0.0419		HMDB02271
	1-methylimidazoleacetate	1.54	0.0188	0.0795	C05828	HMDB02820
Long Chain Fatty Acid	myristate (14:0)	1.78	0.0573	0.1575	C06424	HMDB00806
Lysoplasmalogen	1-(1-enyl-oleoyl)-2-linoleoyl-GPE (P-18:1/18:2)*	3.64	0.0522	0.1536		
Medium Chain Fatty Acid	caproate (6:0)	1.46	0.0939	0.2029	C01585	HMDB00535
Methionine, Cysteine, SAM and Taurine Metabolism	Hypotaurine	3.19	0.0413	0.1325	C00519	HMDB00965
	methionine sulfone	2.21	0.0676	0.1704		
	cysteine s-sulfate	1.97	0.0548	0.1551	C05824	HMDB00731
Mevalonate Metabolism	Mevalonate	1.67	0.0188	0.0795	C02104	HMDB00227
Nicotinate and Nicotinamide Metabolism	trigonelline (N'-methylnicotinate)	1.86	0.0351	0.1161	C01004	HMDB00875
Pentose Metabolism	sedoheptulose	1.73	0.0529	0.1536		HMDB03219
	phenyllactate (PLA)	3.20	0.0000	0.0019	C05607	HMDB00779
Phenylalanine and Tyrosine Metabolism	phenol sulfate	2.84	0.0035	0.0289	C02180	HMDB60015
	p-cresol sulfate	3.83	0.0154	0.0700	C01468	HMDB11635
	Gentisate	5.99	0.0009	0.0128	C00628	HMDB00152
	3-(3-hydroxyphenyl)propionate sulfate	8.03	0.0068	0.0414		
	p-cresol-glucuronide*	3.72	0.0198	0.0810		HMDB11686
	3-(3-hydroxyphenyl)propionate	4.08	0.0839	0.1927	C11457	HMDB00375
	2-hydroxyphenylacetate	2.98	0.0505	0.1503	C05852	HMDB00669
	4-hydroxycinnamate sulfate	3.13	0.0752	0.1803		
Phospholipid Metabolism	trimethylamine N-oxide	4.54	0.0002	0.0080	C01104	HMDB00925
Polypeptide	bradykinin, des-arg(9)	3.26	0.0809	0.1880	C00306	HMDB04246
Polyunsaturated Fatty Acid (n3 and n6)	docosapentaenoate (n6 DPA; 22:5n6)	1.38	0.0794	0.1865	C16513	HMDB01976
Bile Acid Metabolism	Cholate	158.95	0.0018	0.0202	C00695	HMDB00619
	beta-muricholate	24.87	0.0037	0.0289	C17726	HMDB00415
	deoxycholate	8.59	0.0255	0.0959	C04483	HMDB00626
	ursodeoxycholate	14.60	0.0086	0.0466	C07880	HMDB00946
	7-ketodeoxycholate	39.10	0.0091	0.0482		HMDB00391
	Hyocholate	3.50	0.0694	0.1725	C17649	HMDB00760
Purine Metabolism, Guanine containing	7-methylguanine	1.51	0.0701	0.1725	C02242	HMDB00897
Pyrimidine Metabolism, Uracil containing	5,6-dihydrouracil	1.87	0.0969	0.2055	C00429	HMDB00076
Sterol	4-cholesten-3-one	1.62	0.0922	0.2027	C00599	HMDB00921
TCA Cycle	succinylcarnitine (C4-DC)	4.44	0.0332	0.1124		
Tryptophan Metabolism	N-acetyltryptophan	2.74	0.0175	0.0764	C03137	HMDB13713
	indolelactate	4.63	0.0000	0.0000	C02043	HMDB00671
	indoleacetate	2.80	0.0088	0.0475	C00954	HMDB00197
	3-indoxyl sulfate	3.83	0.0008	0.0128		HMDB00682
	Serotonin	1.52	0.0065	0.0405	C00780	HMDB00259
	indole-3-carboxylic acid	2.85	0.0060	0.0390	C19837	HMDB03320
	N-acetylkynurenine (2)	3.05	0.0196	0.0810		
	Picolinate	1.98	0.0903	0.2005	C10164	HMDB02243
	5-hydroxyindoleacetate	1.61	0.0947	0.2029	C05635	HMDB00763
	indoleacetylglutamine	2.03	0.0575	0.1575		
Urea cycle; Arginine and Proline Metabolism	Citrulline	1.28	0.0413	0.1325	C00327	HMDB00904
	N-delta-acetylornithine	1.58	0.0486	0.1468		
	N2,N5-diacetylornithine	1.75	0.0538	0.1546		
Vitamin B6 Metabolism	Pyridoxal	2.41	0.0330	0.1124	C00250	HMDB01545
	Pyridoxate	1.92	0.0381	0.1240	C00847	HMDB00017

SUPPLEMENTAL TABLE 2			
Re-Clusterization of Cluster II In Vivo OAT3 Knockout Metabolites Together with Metabolites For Which In Vitro Data Exists for Interaction with OAT3			
Biochemical Name	Significantly Increased in Serum of <i>Oat3KO</i>	Existence of In Vitro Support for Interaction with OAT3	In Vitro Reference (PMID)
Cholate	✓ ^a	✓	18480179
beta-muricholate	✓ ^a		
deoxycholate	✓ ^a	✓	18480179
ursodeoxycholate	✓ ^a		
7-ketodeoxycholate	✓ ^a		
chenodeoxycholate		✓	18480179
glycocholate		✓	18480179
glycochenodeoxycholate		✓	18480179
Taurochenodeoxycholate		✓	18480179
Taurocholate		✓	11408557
Prostaglandin F2a		✓	11907186
Prostaglandin E2		✓	11907186
Androsterone sulfate		✓	18508962
Dehydroepiandrosterone sulfate	✓ ^b	✓	17255469
Progesterone		✓	18508962
Cortisol		✓	15864504
Aldosterone		✓	18508962
Deoxycorticosterone		✓	18508962
Corticosterone		✓	18508962

^aData from this study; ^bDelayed efflux from brain of *Oat3KO* (PMID: 21325432)

Supplemental Table 3			
Transporter Preference of Endogenous Metabolites For Which Interaction Data (Km, Ki, IC50) Exists			
Metabolite	Ratio OAT1/OAT3* In Vitro Assay		Preference
	Ratio	PMID	
Indoleacetate	0.04	14675047	OAT1 Preference
3-Methoxy-4-hydroxymandelate	0.06	14737013	
Glutarate	0.04	21865262	
Homovanillate	0.09	14737013	
Indoxyl sulfate	0.07	14675047	
5-Hydroxyindoleacetate	0.12	14737013	
5-Methoxyindoleacetate	0.42	14737013	
2-nonenal	0.32	27467266	
Phenylacetic acid	0.42	27467266	
3,4-Dihydroxymandelate	0.44	14737013	
Prostaglandin F2alpha	0.52	11907186	No or Slight Preference
3,4-Dihydroxyphenylacetate	0.57	14737013	
4-decenal	0.72	27467266	
Hippurate	0.61	14675047	
N-Acetylserotonin	0.90	14737013	
p-cresol sulfate	1.05	27467266	
5-Methoxytryptamine	1.70	14737013	OAT3 Preference
Prostaglandin E2	2.81	11907186	
3-Carboxy-4-methyl-5propyl-2-furanpropionate	5.32	14675047	
Urate	3.28	27467266	
5-Methoxytryptophol	3.30	14737013	
Taurocholate	3.51	11408557	
4-Hydroxy-2-quinolinecarboxylic acid	4.25	15944205	
Melatonin	18.2	14675047	
*In the case of some metabolites, multiple values (e.g., Km, Ki, IC50) are found in different publications. Here, only those values for which in vitro assays were described in the same paper were chosen for comparison. Thus, the experiments were performed in the same laboratory.			

Supplemental Table 4
List of OAT1 and OAT3 in vivo Endogenous Metabolites from This and Previous Studies

	Metabolite	PMID	Metabolite	PMID
	Oat1	2-hydroxy-3-methylvalerate	16354673	indole lactic acid
2-hydroxyisovalerate		16354673	indoxyl sulfate	21476605
2-oxo-3-methylvalerate		16354673	kynurenine	21476605
3-hydroxybutyrate		16354673	methionine	21476605
3-hydroxyisobutyrate		16354673	N2-N2-dimethyl guanosine	21476605
3-hydroxypropionate		16354673	n-acetylaspartate	16354673
3-hydroxyvalerate		16354673	n-acetylglycine	21476605
3-methylcrotonylglycine		16354673	orotate	16354673
4-hydroxyphenylacetate		16354673	pantothenic acid	21476605
4-hydroxyphenyllactate		16354673	phenyl sulfate	21476605
4-hydroxyphenylpyruvate		16354673	phenylacetyl glycine	21476605
4-pyridoxic acid		21476605	propionylglycine	16354673
a-ketoglutarate		16354673	thymidine	21476605
Benzoate		16354673	uracil	16354673
Creatinine		22338083	urate	18270321 21476605
hexanoylglycine		16354673	xanthurenic acid	21476605
Oat3		Metabolite	PMID	Metabolite
	1-methylguanosine	23920220	Glucuronate	This study
	1-methylimidazoleacetate	This study	Homostachydrine	This study
	2-aminophenol sulfate	This study	Hypotaurine	This study
	2-hydroxydecanoate	This study	Imidazole propionate	This study
	2-oxindole-3-acetate	This study	Indole-3-carboxylic acid	This study
	2-oxo-9-methylthionoanoic acid	23920220	Indoleacetate	This study
	3-(3-hydroxyphenyl)propionate sulfate	This study	Indoleacrylate	This study
	3-hydroxyhexanoate	This study	Indolelactate	This study
	3-indoxyl sulfate	This study	indolin-2-one	This study
	4-allylphenol sulfate	This study	Licuroside	23920220
	4-hydroxyphenylacetate	23920220	mannitol/sorbitol	This study
	4-vinylphenol sulfate	This study	Mevalonate	This study
	7-hydroxyfluphenazine	23920220	N-acetyltryptophan	This study
	7-ketodeoxycholate	This study	N-delta-acetylornithine	This study
	7-methylguanosine	23920220	O-methylcatechol sulfate	This study
	9-amino-nonanoic acid	23920220	p-cresol sulfate	This study
	9-O-acetylneuraminic acid	23920220	p-cresol-glucuronide	This study
	beta-muricholate	This study	Phenol sulfate	This study
	Catechol sulfate	This study	Phenyllactate (PLA)	This study
	Cholate	This study	pongamoside A	23920220
	Citrate	23920220	Pyridoxal	This study
	Citrulline	This study	Pyridoxate	This study
	Creatinine	22338083	Salicylate	This study
	Dehydroascorbic acid	23920220	Sebacate (decanedioate)	This study
	Dehydroepiandrosterone sulfate	21325432	Serotonin	This study
	Deoxycarnitine	This study	Stachydrine	This study
	Deoxycholate	This study	succinylcarnitine (C4-DC)	This study
	Epicatechin 3-O-(3-O-methylgallate)	23920220	Tetradecanedioate	This study
	Equol glucuronide	This study	Thymidine	18508962
	Equol sulfate	This study	trigonelline (N'-methylnicotinate)	This study
	Estrone sulfate	21325432 This study	Trimethylamine N-oxide	This study
Flavin mononucleotide	18508962	Urate	18270321	
Gentisate	This study	Ursodeoxycholate	This study	