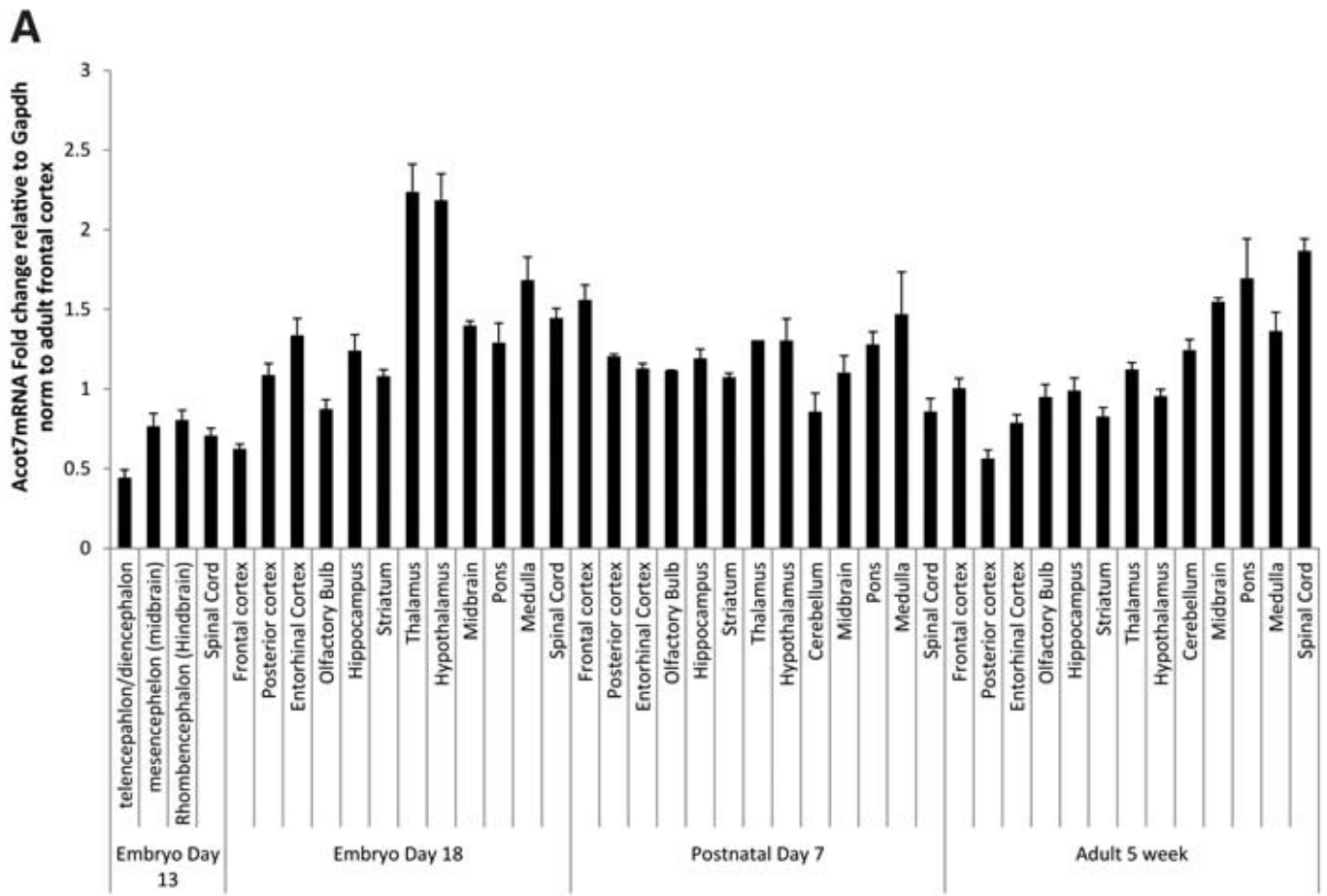
SUPPLEMENTAL INFORMATION

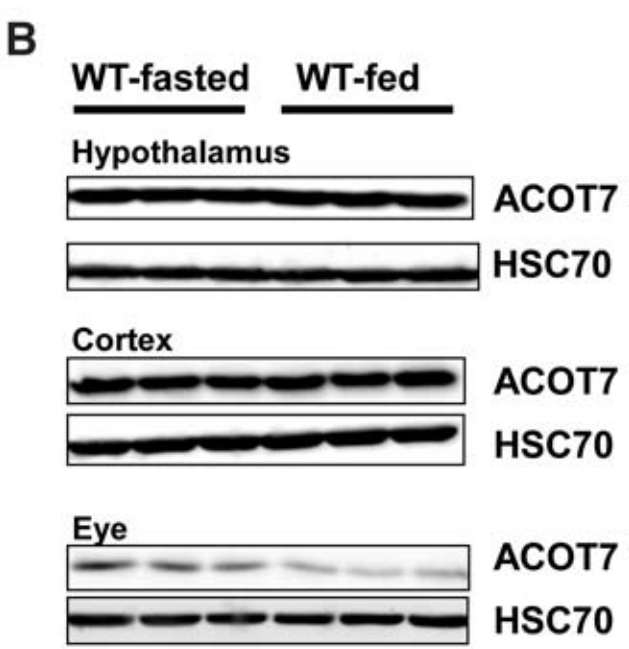
Supplemental Figure 1.
A) Mouse Acot7 mRNA abundance across different brain regions across development. **B**) Western blot images of ACOT7 in brain regions from fed or overnight fasted mice, n=3. **C**) Western blot images of cytosol and total membrane fractions isolated from mouse brain and probed for ACOT7 or VDAC as a membrane protein control, n=3. Data represent average ± standard error of the mean.

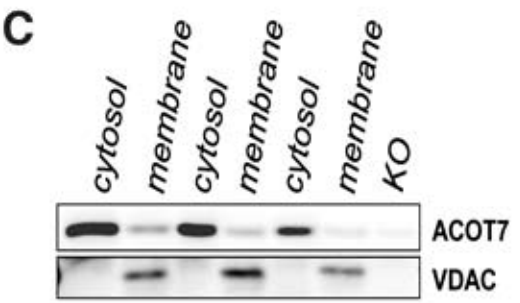
Supplemental Figure 2.
Long chain acyl-CoA content in control and *Acot7*$^{N-/-}$ mice fed or overnight fasted in the **A**) hypothalamus, **B**) cortex, and **C**) cerebellum, n=5-7. Data represent average ± standard error of the mean. * indicates $p \leq 0.05$ by two-tail Student's t-test comparing *Acot7*$^{N-/-}$ to controls.

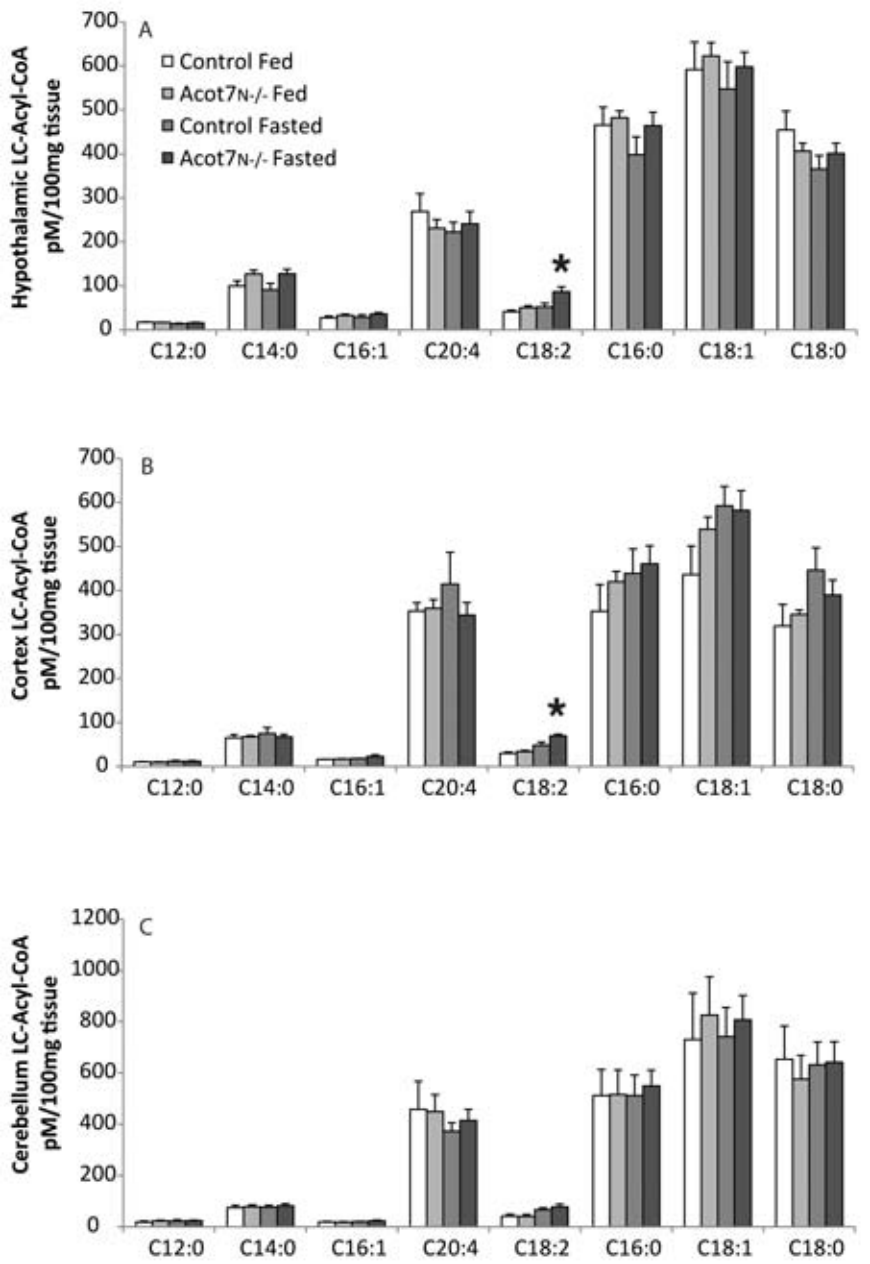
Supplemental Figure 3.
A) Mitochondrial DNA abundance relative to nuclear DNA in control and *Acot7*$^{N-/-}$ livers, n=5-7. **B**) Brown adipose mRNA abundance in control and *Acot7*$^{N-/-}$ mice overnight fasted and with or without a 6 hour cold exposure, n=5-6. C) Hypothalamus mRNA abundance in control and *Acot7*$^{N-/-}$ mice, n=6. Abbreviations: mitochondrial cytochrome *c* oxidase 1 (mtCo), mitochondrial cytochrome *b* (mtCy), mitochondrial NADH dehydrogenase subunit 1 (mtNd1), uncoupling protein 1 (Ucp1), deiodinase 2 (dio2), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (Pgc1a), Agouti-related protein (AgRP), neuropeptide Y (Npy), cocaine-and-amphetamine-regulated transcript (Cart), and pro-opiomelanocortin (Pomc). Data represent average ± standard error of the mean. * indicates $p \leq 0.05$ by two-tail Student's t-test comparing *Acot7*$^{N-/-}$ to controls.

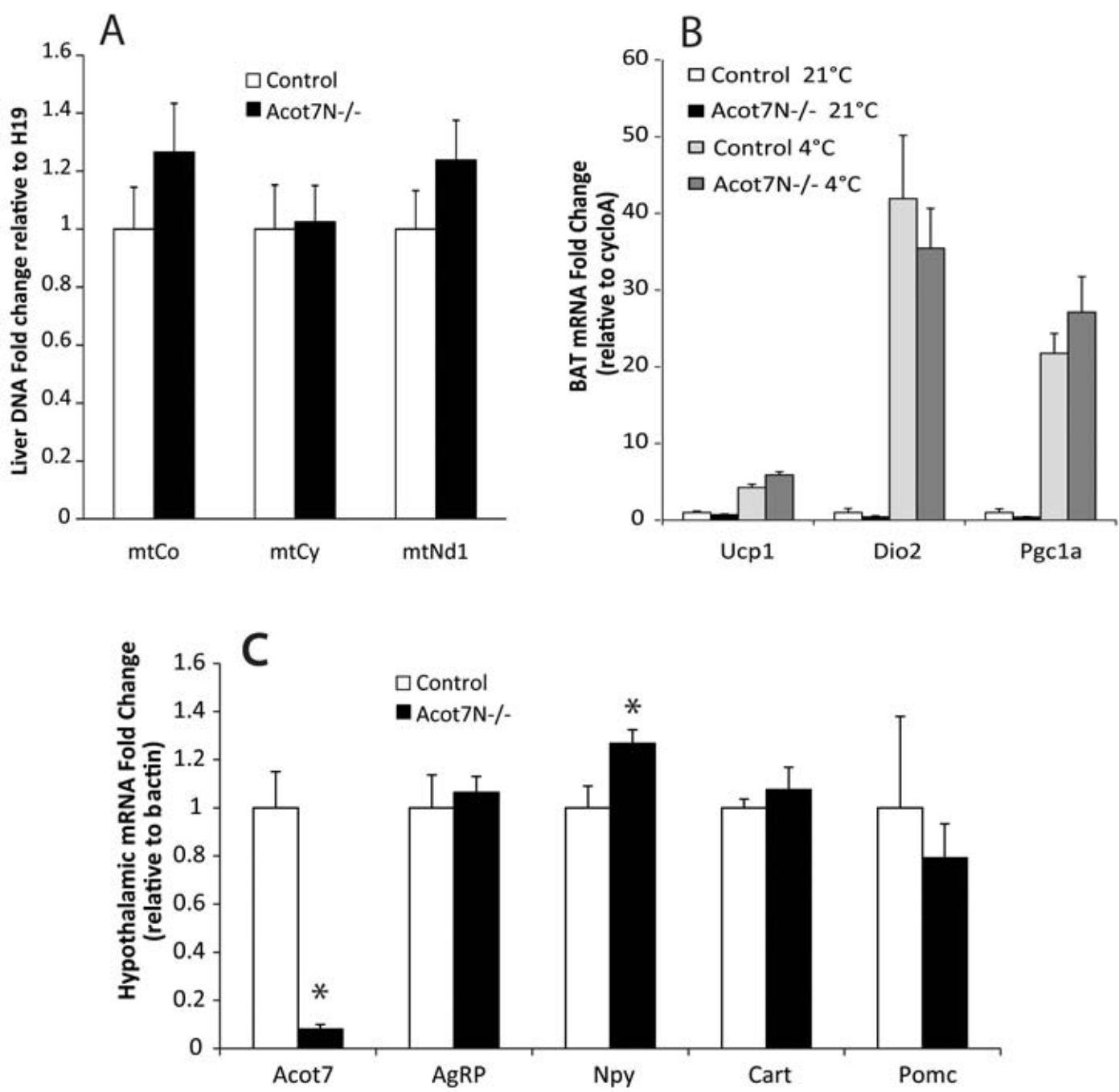
Supplemental Table 1.
Average metabolite values from control and *Acot7*$^{N-/-}$ brains expressed as fold-change relative to controls. P-values indicate values determined by Welch's t-test.


A
Acot7mRNA Fold change relative to Gapdh norm to adult frontal cortex
3
2.5
2
1.5
1
0.5
0
telencepahlon/diencephalon
mesencephelon (midbrain)
Rhombencephalon (Hindbrain)
Spinal Cord
Frontal cortex
Posterior cortex
Entorhinal Cortex
Olfactory Bulb
Hippocampus
Striatum
Thalamus
Hypothalamus
Midbrain
Pons
Medulla
Spinal Cord
Frontal cortex
Posterior cortex
Entorhinal Cortex
Olfactory Bulb
Hippocampus
Striatum
Thalamus
Hypothalamus
Cerebellum
Midbrain
Pons
Medulla
Spinal Cord
Frontal cortex
Posterior cortex
Entorhinal Cortex
Olfactory Bulb
Hippocampus
Striatum
Thalamus
Hypothalamus
Cerebellum
Midbrain
Pons
Medulla
Spinal Cord
Embryo Day 13
Embryo Day 18
Postnatal Day 7
Adult 5 week


B
WT-fasted
WT-fed
Hypothalamus
ACOT7
HSC70
Cortex
ACOT7
HSC70
Eye
ACOT7
HSC70


C
cytosol
membrane
cytosol
membrane
cytosol
membrane
KO
ACOT7
VDAC


A
Control Fed
Acot7N-/- Fed
Control Fasted
Acot7N-/- Fasted
Hypothalamic LC-Acyl-CoA pM/100mg tissue
700
600
500
400
300
200
100
0
*
C12:0
C14:0
C16:1
C20:4
C18:2
C16:0
C18:1
C18:0
B
Cortex LC-Acyl-CoA pM/100mg tissue
700
600
500
400
300
200
100
0
*
C12:0
C14:0
C16:1
C20:4
C18:2
C16:0
C18:1
C18:0
C
Cerebellum LC-Acyl-CoA pM/100mg tissue
1200
1000
800
600
400
200
0
C12:0
C14:0
C16:1
C20:4
C18:2
C16:0
C18:1
C18:0


A
Liver DNA Fold change relative to H19
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Acot7N-/-
mtCo
mtCy
mtNd1
B
BAT mRNA Fold Change (relative to cycloA)
60
50
40
30
20
10
0
Control 21°C
Acot7N-/- 21°C
Control 4°C
Acot7N-/- 4°C
Ucp1
Dio2
Pgc1a
C
Hypothalamic mRNA Fold Change (relative to bactin)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Acot7N-/-
*
*
Acot7
AgRP
Npy
Cart
Pomc

SUPER PATHWAY	BIOCHEMICAL NAME	Control AVE	Acot7N-/- AVE	*p*-Value
Amino acid	glycine	1.00	1.01	0.95
	serine	1.00	0.97	0.63
	N-acetylserine	1.00	1.01	0.79
	homoserine	1.00	0.89	0.11
	3-phosphoserine	1.00	0.94	0.54
	threonine	1.00	0.89	**0.03**
	allo-threonine	1.00	0.80	0.06
	betaine	1.00	0.87	0.09
	alanine	1.00	0.99	0.91
	beta-alanine	1.00	1.00	0.82
	N-acetylalanine	1.00	1.06	0.39
	aspartate	1.00	0.95	0.20
	N-acetylaspartate (NAA)	1.00	0.95	0.38
	glutamate	1.00	0.86	**0.03**
	glutamine	1.00	0.92	**0.03**
	gamma-aminobutyrate (GABA)	1.00	0.94	0.52
	N-acetylglutamate	1.00	0.98	0.84
	N-acetyl-aspartyl-glutamate (NAAG)	1.00	1.04	0.15
	N-acetylglutamine	1.00	0.86	0.10
	histidine	1.00	0.91	0.33
	lysine	1.00	1.03	0.54
	2-aminoadipate	1.00	0.74	**0.00**
	pipecolate	1.00	1.42	0.14
	glutaroyl carnitine	1.00	0.67	**0.00**
	phenylalanine	1.00	1.04	0.42
	tyrosine	1.00	1.08	0.21
	3-(4-hydroxyphenyl)lactate	1.00	1.11	0.14
	tryptophan	1.00	0.98	0.68
	C-glycosyltryptophan*	1.00	1.03	0.62
	5-hydroxyindoleacetate	1.00	1.02	0.87
	isoleucine	1.00	1.07	0.25
	leucine	1.00	1.07	0.28
	valine	1.00	1.02	0.68
	alpha-hydroxyisovalerate	1.00	1.56	0.65
	2-methylbutyroylcarnitine	1.00	1.01	0.93
	isovalerylcarnitine	1.00	0.99	0.92
	hydroxyisovaleroyl carnitine	1.00	0.92	0.20
	cysteine	1.00	0.95	0.33
	cystine	1.00	0.99	1.00
	taurine	1.00	1.04	0.72
	S-adenosylhomocysteine (SAH)	1.00	0.95	0.58
	methionine	1.00	1.02	0.65
	N-acetylmethionine	1.00	1.03	0.68
	2-hydroxybutyrate (AHB)	1.00	0.91	0.69
	arginine	1.00	1.03	0.51
	ornithine	1.00	0.95	0.50
	urea	1.00	1.16	0.29
	proline	1.00	1.05	0.39
	N-acetylornithine	1.00	1.79	**0.01**
	trans-4-hydroxyproline	1.00	0.96	0.60
	argininosuccinate	1.00	0.92	0.27
	creatine	1.00	1.02	0.56
	creatinine	1.00	0.89	0.37
	2-aminobutyrate	1.00	0.97	0.84
	5-methylthioadenosine (MTA)	1.00	1.03	0.50
	putrescine	1.00	0.93	0.57
	spermidine	1.00	0.88	0.22
	spermine	1.00	0.65	0.23
	4-guanidinobutanoate	1.00	1.13	0.13
	glutathione, reduced (GSH)	1.00	0.54	**0.03**
	5-oxoproline	1.00	1.00	0.91
	glutathione, oxidized (GSSG)	1.00	0.66	0.30
	cysteine-glutathione disulfide	1.00	0.82	0.20
Peptide	carnosine	1.00	1.07	0.55
	homocarnosine	1.00	0.86	0.14
	gamma-glutamylleucine	1.00	1.20	**0.02**
	gamma-glutamylglutamate	1.00	0.77	**0.04**
	gamma-glutamylglutamine	1.00	0.80	**0.01**
	gamma-glutamylphenylalanine	1.00	1.03	0.94
Carbohydrate	N-acetylglucosamine	1.00	0.98	0.80
	erythronate*	1.00	1.15	**0.04**
	N-acetylneuraminate	1.00	0.96	0.39
	fructose	1.00	0.94	0.68
	mannose	1.00	1.85	0.06
	mannose-6-phosphate	1.00	1.07	0.49
	sorbitol	1.00	0.78	0.44
	1,5-anhydroglucitol (1,5-AG)	1.00	1.10	0.83
	glycerate	1.00	0.92	0.34
	glucose-6-phosphate (G6P)	1.00	1.17	0.12
	glucose	1.00	1.83	**0.00**
	fructose-6-phosphate	1.00	1.14	0.48
	Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate	1.00	1.26	**0.04**
	3-phosphoglycerate	1.00	0.70	**0.02**
	dihydroxyacetone phosphate (DHAP)	1.00	1.11	0.32
	1,3-dihydroxyacetone	1.00	1.21	0.71
	pyruvate	1.00	0.92	0.51
	lactate	1.00	0.99	0.88
	arabitol	1.00	1.16	0.50
	ribitol	1.00	1.00	0.93
	sedoheptulose-7-phosphate	1.00	1.43	0.12
	ribose 5-phosphate	1.00	0.86	**0.05**
	Isobar: ribulose 5-phosphate, xylulose 5-phosphate	1.00	1.02	0.93
	arabinose	1.00	1.32	0.15
Energy	citrate	1.00	0.97	0.72
	alpha-ketoglutarate	1.00	0.71	0.18
	succinate	1.00	1.19	0.60
	fumarate	1.00	1.16	0.15
	malate	1.00	0.98	0.81
	phosphate	1.00	0.96	0.14
	pyrophosphate (PPi)	1.00	1.29	0.78
	linoleate (18:2n6)	1.00	1.60	**< 0.001**
	linolenate [alpha or gamma; (18:3n3 or 6)]	1.00	1.40	**0.01**
	dihomo-linolenate (20:3n3 or n6)	1.00	1.49	**0.00**
	eicosapentaenoate (EPA; 20:5n3)	1.00	1.20	0.11
	docosapentaenoate (n3 DPA; 22:5n3)	1.00	1.97	**< 0.001**
	docosapentaenoate (n6 DPA; 22:5n6)	1.00	0.93	0.46
	docosahexaenoate (DHA; 22:6n3)	1.00	1.39	**0.01**
	caproate (6:0)	1.00	0.91	0.41
	caprylate (8:0)	1.00	0.87	0.20
	pelargonate (9:0)	1.00	1.02	0.61
	laurate (12:0)	1.00	1.09	0.24
	myristate (14:0)	1.00	1.30	**0.01**
	myristoleate (14:1n5)	1.00	1.29	0.07

Lipid	**palmitate (16:0)**	1.00	1.28	**0.01**
	palmitoleate (16:1n7)	1.00	1.76	**< 0.001**
	margarate (17:0)	1.00	1.32	**0.00**
	10-heptadecenoate (17:1n7)	1.00	1.36	**0.00**
	stearate (18:0)	1.00	1.13	0.11
	oleate (18:1n9)	1.00	1.27	**0.02**
	10-nonadecenoate (19:1n9)	1.00	1.13	0.32
	eicosenoate (20:1n9 or 11)	1.00	1.09	0.51
	dihomo-linoleate (20:2n6)	1.00	1.25	0.07
	arachidonate (20:4n6)	1.00	1.23	**0.00**
	docosadienoate (22:2n6)	1.00	1.35	**0.04**
	adrenate (22:4n6)	1.00	1.12	0.45
	n-Butyl Oleate	1.00	0.83	**0.01**
	2-hydroxyglutarate	1.00	0.97	0.60
	oleamide	1.00	1.43	0.93
	stearamide	1.00	1.34	0.65
	prostaglandin D2	1.00	0.89	0.21
	prostaglandin E2	1.00	0.98	0.81
	5-HETE	1.00	1.42	**0.03**
	15-HETE	1.00	1.25	0.20
	palmitoyl ethanolamide	1.00	1.00	0.93
	propionylcarnitine	1.00	1.10	0.28
	carnitine	1.00	0.96	0.45
	3-dehydrocarnitine*	1.00	1.03	0.86
	acetylcarnitine	1.00	1.00	0.98
	oleoylcarnitine	1.00	2.91	**0.01**
	1-octadecanol	1.00	0.94	0.30
	choline phosphate	1.00	0.93	**0.03**
	ethanolamine	1.00	1.00	0.87
	phosphoethanolamine	1.00	0.91	0.35
	glycerol	1.00	1.01	0.74
	glycerol 3-phosphate (G3P)	1.00	0.99	0.94
	glycerophosphorylcholine (GPC)	1.00	0.94	0.62
	cytidine 5'-diphosphocholine	1.00	0.77	**0.01**
	myo-inositol	1.00	1.14	0.12
	chiro-inositol	1.00	1.57	0.20
	inositol 1-phosphate (I1P)	1.00	0.93	0.65
	scyllo-inositol	1.00	1.15	0.23
	3-hydroxybutyrate (BHBA)	1.00	1.59	**0.01**
	1-palmitoylglycerophosphoethanolamine	1.00	2.13	**0.01**
	2-palmitoylglycerophosphoethanolamine*	1.00	2.90	0.08
	1-stearoylglycerophosphoethanolamine	1.00	1.88	0.12
	1-oleoylglycerophosphoethanolamine	1.00	2.35	**0.02**
	2-oleoylglycerophosphoethanolamine*	1.00	1.65	0.10
	1-arachidonoylglycerophosphoethanolamine*	1.00	1.58	**0.04**
	2-arachidonoylglycerophosphoethanolamine*	1.00	3.25	0.11
	2-docosahexaenoylglycerophosphoethanolamine*	1.00	3.12	0.09
	1-palmitoylglycerophosphocholine	1.00	4.78	**0.05**
	2-palmitoylglycerophosphocholine*	1.00	5.19	**0.03**
	1-stearoylglycerophosphocholine	1.00	4.04	**0.04**
	2-stearoylglycerophosphocholine*	1.00	1.58	0.29
	1-oleoylglycerophosphocholine	1.00	5.04	**0.02**
	2-oleoylglycerophosphocholine*	1.00	3.62	**0.04**
	1-arachidonoylglycerophosphocholine*	1.00	1.59	0.20
	2-arachidonoylglycerophosphocholine*	1.00	3.18	**0.05**
	1-docosahexaenoylglycerophosphocholine*	1.00	1.95	0.41
	2-docosahexaenoylglycerophosphocholine*	1.00	2.97	0.09
	1-palmitoylglycerophosphoinositol*	1.00	1.55	**0.02**
	1-stearoylglycerophosphoinositol	1.00	1.16	0.27
	1-arachidonoylglycerophosphoinositol*	1.00	1.04	0.87
	1-oleoylglycerophosphoserine	1.00	1.41	**0.01**
	2-oleoylglycerophosphoserine*	1.00	1.94	**0.02**
	1-palmitoylplasmenylethanolamine*	1.00	2.15	**0.03**
	1-palmitoylglycerol (1-monopalmitin)	1.00	1.22	0.13
	1-stearoylglycerol (1-monostearin)	1.00	1.06	0.82
	2-stearoylglycerol (2-monostearin)	1.00	1.02	0.83
	1-oleoylglycerol (1-monoolein)	1.00	1.41	0.12
	2-oleoylglycerol (2-monoolein)	1.00	1.00	0.83
	sphingosine	1.00	3.71	**0.02**
	palmitoyl sphingomyelin	1.00	1.06	0.61
	stearoyl sphingomyelin	1.00	0.87	0.15
	3-hydroxy-3-methylglutarate	1.00	0.35	**< 0.001**
	cholesterol	1.00	0.96	0.57
	7-alpha-hydroxycholesterol	1.00	1.11	0.47
	7-beta-hydroxycholesterol	1.00	1.11	0.41
	24(S)-hydroxycholesterol	1.00	0.94	0.70
	cortlcosterone	1.00	0.58	**0.02**
Nucleotide	xanthine	1.00	1.03	0.49
	hypoxanthine	1.00	1.03	0.38
	inosine	1.00	0.97	0.42
	adenine	1.00	0.92	0.22
	adenosine	1.00	1.01	0.96
	N1-methyladenosine	1.00	0.98	0.93
	adenosine 2'-monophosphate (2'-AMP)	1.00	1.00	
	adenosine 5'-monophosphate (AMP)	1.00	0.97	0.67
	guanosine	1.00	0.85	**0.05**
	urate	1.00	1.14	0.11
	allantoin	1.00	1.04	0.72
	cytidine	1.00	0.97	0.49
	cytidine 5'-monophosphate (5'-CMP)	1.00	0.86	**0.01**
	orotate	1.00	1.09	0.70
	uracil	1.00	1.17	**0.01**
	uridine	1.00	1.05	0.13
	pseudouridine	1.00	1.12	0.11
	methylphosphate	1.00	1.06	0.36
Cofactors and vitamins	ascorbate (Vitamin C)	1.00	0.95	0.80
	dehydroascorbate	1.00	0.88	0.66
	threonate	1.00	1.12	0.60
	heme*	1.00	1.63	0.11
	nicotinamide	1.00	0.96	0.51
	nicotinamide adenine dinucleotide (NAD+)	1.00	0.94	0.40
	pantothenate	1.00	1.05	0.63
	phosphopantetheine	1.00	0.76	0.08
	pyridoxal	1.00	1.05	0.77
	flavin adenine dinucleotide (FAD)	1.00	1.05	0.26
	riboflavin (Vitamin B2)	1.00	1.00	1.00
	flavin mononucleotide (FMN)	1.00	0.93	0.11
	alpha-tocopherol	1.00	0.86	0.55
Xenobiotics	glycolate (hydroxyacetate)	1.00	1.06	0.62
	glycerol 2-phosphate	1.00	1.05	0.56
	2-phenoxyethanol	1.00	0.84	0.18
	2-pyrrolidinone	1.00	1.18	0.96
	ergothioneine	1.00	1.08	0.48
	erythritol	1.00	1.03	0.81