

Supplementary Material

Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome and metabolome

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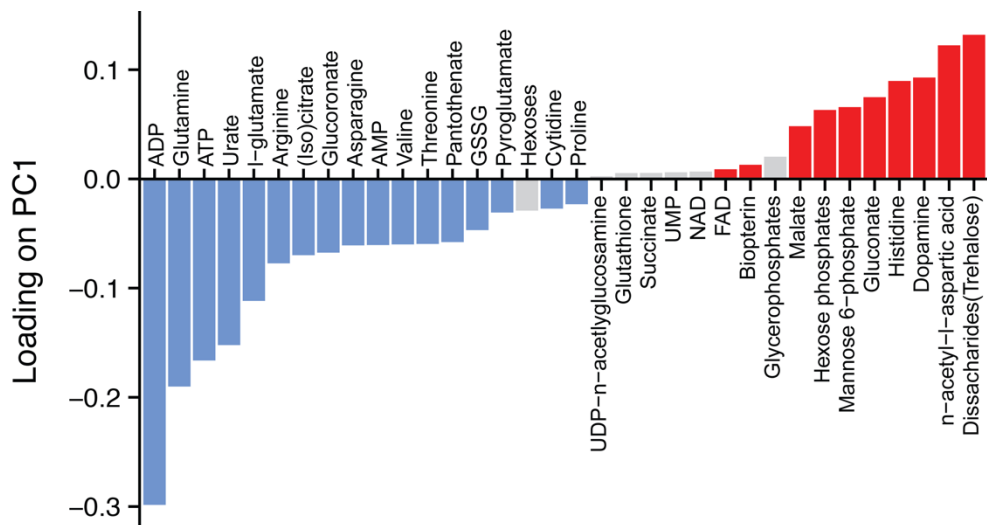


Figure S1. Extended results of principal components analysis of metabolite abundance in whole-body homogenates of *D. melanogaster* males, acclimated as adults to 21.5°C or 6°C. Loadings of identified metabolites along principal component 1 (PC1). Metabolites that were significantly more abundant in cold-acclimated flies or warm acclimated flies are shown as blue or red bars, respectively. Metabolites that did not differ in abundance between the acclimation groups are shown as grey bars.

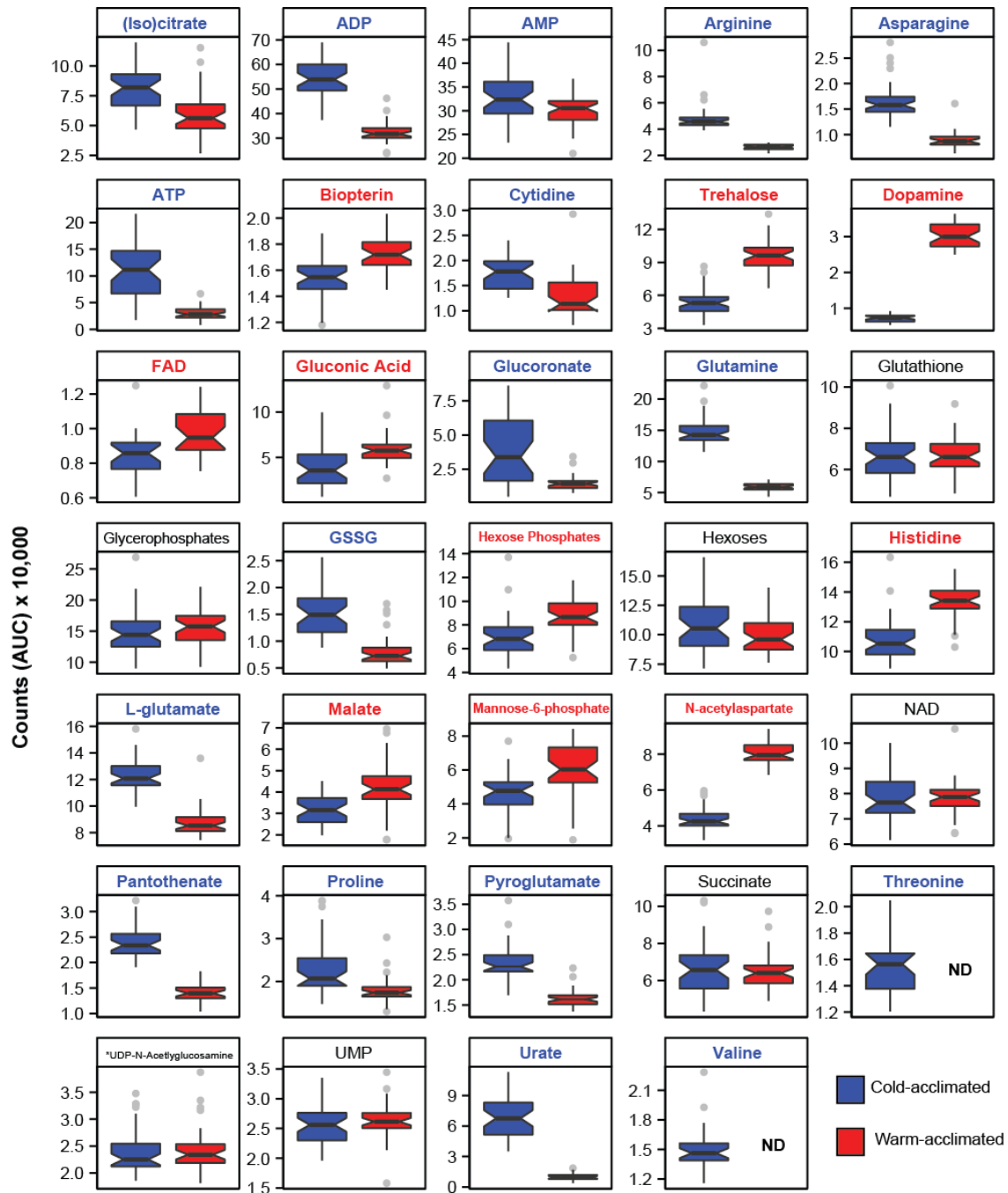


Figure S2: Boxplots of metabolite peak areas from whole-body homogenates of male *D. melanogaster* acclimated to 21.5°C (red) and 6°C (blue) as adults. Names of metabolites that were significantly more abundant in warm- or cold-acclimated flies are shown in red or blue, respectively. ND: not detected. Metabolite abundance is expressed as area under the curve (AUC). Notches in boxplots represent 95% confidence intervals.

Table S1. Metabolites detected in LC-MS analysis. Metabolites in bold typeface were significantly higher in warm- (red) or cold-acclimated (blue) flies following false discovery rate (FDR) correction.

Metabolite	<i>P</i> -value	Fold Change (Cold/Warm)	<i>Q</i> -value (FDR)
Threonine	Undetected in warm acclimated flies		
Valine	Undetected in warm acclimated flies		
Urate	<0.0001	6.67	<0.00001
Adenosine 5'-triphosphate (ATP)	<0.0001	3.57	<0.00001
Glucuronate	0.00001	2.56	0.00002
Glutamine	<0.0001	2.50	<0.00001
Oxidized Glutathione (GSSG)	<0.0001	1.92	<0.00001
Asparagine	<0.0001	1.82	<0.00001
Arginine	<0.0001	1.82	<0.00001
Pantothenate	<0.0001	1.69	<0.00001
Adenosine 5'-diphosphate (ADP)	<0.0001	1.67	<0.00001
Pyroglutamate	<0.0001	1.45	<0.00001
Glutamate	<0.0001	1.39	<0.00001
Cytidine	0.00001	1.37	0.00001
(Iso)citrate	0.00001	1.35	0.00002
Proline	0.00015	1.27	0.00032
Hexoses	0.02942	1.10	0.0502
Adenosine 5'-monophosphate (AMP)	0.00469	1.09	0.00872
Nicotinamide adenine dinucleotide (NAD)	0.50884	1.02	0.59304
Succinate	0.55667	1.02	0.62749
UDP-n-acetylglucosamine (UDP-glcNac)	0.81817	-1.01	0.83546
Glutathione (GSH)	0.87505	-1.01	0.88113
Uridine 5' monophosphate (UMP)	0.29389	-1.03	0.37707
Glycerophosphates	0.3377	-1.05	0.41852
Biopterin	<0.0001	-1.11	0.00001
Flavin adenine dinucleotide (FAD)	0.0009	-1.16	0.00183
Histidine	<0.0001	-1.24	<0.00001
Hexose phosphates	0.00001	-1.25	0.00003
Mannose 6-phosphate	0.00002	-1.3	0.00006
Malate	0.00003	-1.32	0.00006
Gluconate	0.00005	-1.52	0.00011
Trehalose	<0.0001	-1.78	<0.00001
N-acetyl-l-aspartate (NAA)	<0.0001	-1.84	<0.00001
Dopamine	<0.0001	-4.25	<0.00001

Table S2. Metabolite separation solvent table. Metabolites were separated using a multi-step, two-solvent gradient with diamylammonium acetate, pH 4.95, as solvent A and methanol as solvent B and a flow-rate of 0.4 mL/min.

Step	Total Time (min)	Solvent A (%)	Solvent B (%)
1	1.25	95	5
2	5.25	80	20
3	15.25	80	20
4	17.50	65	35
5	19.25	65	35
6	21.00	40	60
7	22.75	40	60
8	22.85	10	90
9	24.50	10	90
10	24.60	95	5
11	31.50	95	5