Supplementary Material

S1. Methodology

Instrumentation

Waters Acquity UPLC class I system coupled to a Xevo TQ-S electrospray-ionization triple-quadrupole mass spectrometer (Waters Milford, MA, USA) was used to perform the mass spectrometric operations. The chromatographic and MS conditions are detailed below.

INSTRUMENT CONDITIONS		SET PARAMETERS			
Ionization mode		ESI +			
Column		Waters x Bridge C18, 4.6 × 50mm, 3.5µm			
Column temperature		40ºC			
Mobile phase		A- 4mM ammonium acetate, B- 100% acetonitrile			
Gradient Table		Time (min)	Flow (ml/min)	% A	% B
		Initial	0.350	90.0	10.0
		0.5	0.350	90.0	10.0
		2.5	0.350	20.0	80.0
		3	0.350	90.0	10.0
		5	0.350	90.0	10.0
Source temperature		150°C	Desolvation temperature		600°C
Cone gas flow		150 L/hour	Desolvation gas flow		1000 L/hour
Collision gas flow		0.15 mL/min			
Analyte related parameters	Compound	Parent Transition of analyte (m/z)	Product Ion Transition of analyte	Cone Voltage (V)	Collision Energy (eV)
	ATP	507.83	136.03	2	32
	ADP	427.91	136.03	2	22
	AMP	347.93	136.03	2	16
	13C10ATP (IS)	517.89	141.03	2	28

Standard preparation, calibration curve, and quantification:

A stable-isotope-labeled Adenosine 13C10 ATP was used as an internal standard. Standards of ATP, ADP and AMP were weighed accurately and dissolved in LC MS grade water to prepare stock solutions at 1.0 mg/mL. The concentration of nucleotides was calculated from the calibration curves constructed in the range of 0.1 µg/mL to 20 µg/mL for all 3 compounds, by adding various concentrations of standards and fixed concentration of IS to the pooled DBS, liver and brain tissue homogenates. The mixtures were then processed as per the tissue processing protocol. The chromatogram obtained was background-subtracted using the respective blank. Values for the

slope, intercept and correlation coefficient were obtained by linear-regression analysis of the calibration curves constructed by plotting analyte/internal-standard peak-area ratios versus concentration. The curves were fitted using a linear regression with 1/x weighting. The calibration curve was used for measuring analyte concentration using the software Mass Lynx version 4.1. The cellular adenylate energy charge (AEC) was calculated as per the following formula [24]:

$$AEC = \frac{ATP + (0.5 \times ADP)}{ATP + ADP + AMP}$$

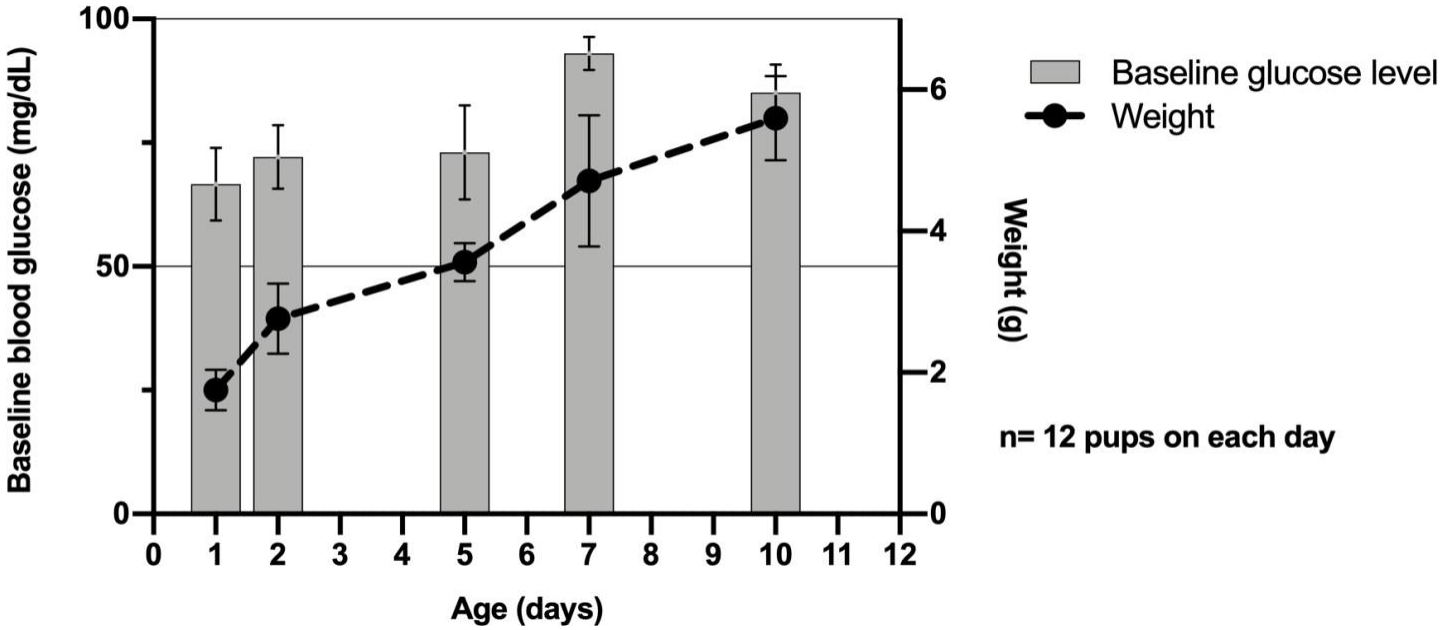
Table S1. Weight and initial blood glucose concentrations in BALB/c mice pups						
		Day 1	**Day 2**	**Day 5**	**Day 7**	**Day 10**
Number of pups		12	12	12	12	12
Weight (g)	Control	2.10 ± 0.10	3.12 ± 0.16	3.44 ± 0.15	5.51 ± 0.72	5.78 ± 0.61
	Treated	1.75 ± 0.29	2.76 ± 0.50	3.56 ± 0.27	4.71 ± 0.93	5.60 ± 0.60
Initial blood glucose (mg/dL)	Control	71.33 ± 3.20	69.33 ± 6.86	74.50 ± 3.56	89.17 ± 3.37	93.00 ± 3.41
	Treated	66.6 ± 7.33	72.16 ± 6.46	73.0 ± 9.50	93.0 ± 3.40	85.16 ± 5.70
Administration of insulin		6 pups	6 pups	6 pups	6 pups	6 pups
Blood glucose (mg/dL) after	5 minutes	60.16 ± 3.37	59.5 ± 3.01	63.0 ± 3.84	84.5 ± 2.66	72.5 ± 1.64
	10 minutes	56.33 ± 2.33	55.16 ± 9.30	53.5 ± 1.64	61.83 ± 2.92	69.33 ± 5.88
	15 minutes	44.16 ± 1.17	37.5 ± 6.24	37.66 ± 9.95	57.5 ± 2.25	57.0 ± 3.22
	20 minutes	NA	NA	NA	41.5 ± 5.24	41.0 ± 2.45
Time of sacrifice *(number of insulin injections required)*		All 6 pups sacrificed at 15 min *(2)*	2 pups sacrificed at 10 min (*1*) and 4 pups at 15 min *(2)*	All 6 pups sacrificed at 15 min (*2*)	All 6 pups sacrificed at 20 min (*3*)	All 6 pups sacrificed at 20 min (*3*)
Values are expressed as mean ± standard deviation. Blood glucose levels of mice at different age were documented before and after administration of insulin, and the pups were sacrificed when hypoglycemia confirmed (blood glucose <50mg/dL or 2.8 mmol/L)						

Table S2. Adenine nucleotides and cellular adenylate energy charge(AEC) measurements in brain tissues over 10 days in 6 controls and 6 hypoglycemic BALB/c mice pups

	Age (days)		ATP (µg/mL)	ADP (µg/mL)	AMP (µg/mL)	AEC
Parietal	1	Control	0.036 ± 0.03	0 ± 0	0 ± 0	1.0 ± 0
		Hypoglycemia	0.29 ± 0.24	0.37 ± 0.30	0.72 ± 0.82	0.46 ± 0.26
	2	Control	0.19 ± 0.10	0 ± 0	0 ± 0	1.0 ± 0
		Hypoglycemia	0.37 ± 0.24	0.53 ± 0.34	0.77 ± 0.85	0.517 ± 0.23
	5	Control	0.13 ± 0.15	0 ± 0	0 ± 0	1.0 ± 0
		Hypoglycemia	0.18 ± 0.14	0.21 ± 0.19	0.003 ± 0.007	0.71 ± 0.07
	7	Control	0.34 ± 0.19	0.09 ± 0.23	0 ± 0	0.96 ± 0.09
		Hypoglycemia	0.81 ± 0.53	1.16 ± 0.58	2.95 ± 1.44	0.27 ± 0.06
	10	Control	0.27 ± 0.17	0.06 ± 0.1	0 ± 0	0.95 ± 0.06
		Hypoglycemia	0.51 ± 0.39	0.97 ± 0.57	2.34 ± 2.0	0.34 ± 0.21
Occipital	1	Control	0.54 ± 0.02	0.03 ± 0.09	0 ± 0	0.97 ± 0.06
		Hypoglycemia	1.25 ± 0.04	0.43 ± 0.05	0.31 ± 0.20	0.74 ± 0.08
	2	Control	1.2 ± 0.08	0.17 ± 0.19	0.21 ± 0.51	0.88 ± 0.18
		Hypoglycemia	0.37 ± 0.05	0.02 ± 0.05	1.05 ± 0.38	0.28 ± 0.07
	5	Control	0.07 ± 0.01	0 ± 0	0 ± 0	1.0 ± 0
		Hypoglycemia	0.04 ± 0.02	0 ± 0	1.74 ± 0.23	0.02 ± 0.009
	7	Control	0.25 ± 0.09	0.11 ± 0.25	0.35 ± 0.64	0.70 ± 0.33
		Hypoglycemia	0.19 ± 0.03	0 ± 0	1.30 ± 0.22	0.13 ± 0.01
	10	Control	0.20 ± 0.20	0.28 ± 0.37	1.9 ± 1.6	0.13 ± 0.09
		Hypoglycemia	0.26 ± 0.29	0.31 ± 0.46	4.53 ± 0.73	0.07 ± 0.08

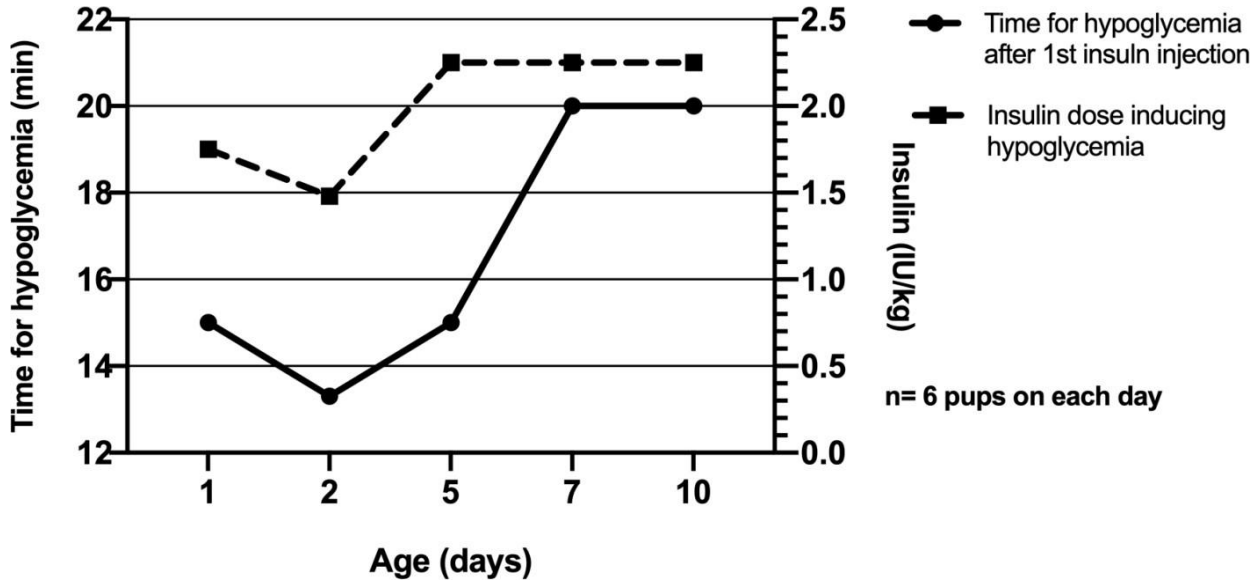
None of the adenine nucleotides on their own reflects the cellular adenylate energy charge (AEC). The brain parietal tissue in controls demonstrated an identical AEC of one unit in all age groups, with the adenylate pool consisting only of ATP. In contrast, the hypoglycemic pups showed a comparatively lower level of AEC < 0.7, with the adenylate pool consisting of ATP, ADP, and AMP. All hypoglycemic age groups demonstrated a comparatively higher or equal level of AMP. The occipital tissue in the controls had an AEC of 0.7 to 1.0 in the first seven days, with the adenylate pool consisting mainly of ATP. By contrast,on day 10, they had a very low AEC of 0.1 with the adenylate pool consisting mainly of AMP. At all ages,the hypoglycemic group's AEC was < 0.7, with their adenylate pool consisting mainly of AMP. On day 10, they showed a higher AMP level than in the controls.
Adenosine monophosphate (AMP); Adenosine diphosphate (ADP); Adenosine triphosphate (ATP)

Figure S1. Baseline blood glucose level and body weight by age in 60 BLB/c pups



Both baseline blood glucose level and body weight increased progressively in the first 10 days after birth.

Figure S2. Mean insulin dose inducing hypoglycemia and time for hypoglycemia after 1st injection, by age, in 30 BALB/c pups



Both the time for hypoglycemia, and the insulin dose required to induce it, increased steadily over the first 10 days after birth