iScience, Volume *26*

Supplemental information

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Mechanism of Hyperproteinemia-Induced Damage to Female Reproduction in a Genetic Silkworm Model

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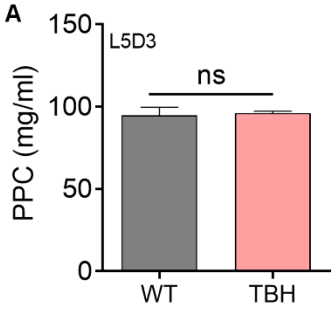
Supplemental information

This file includes:

Methods S1: Detection of free amino acid, related to Figure S4.

An equal volume of 10% trichloroacetic acid was added to the hemolymph samples of the P2-P6 stage. The mixture was mixed well and centrifuged for 10 min at 13000 *g* after being placed at room temperature for 2 h to collect the supernatants for testing. The detection was performed using high performance liquid chromatography. The chromatographic column used was ODS HYPERSIL (250 × 4.6 mm, 5 μm). For Mobile phase A, 225 μL of triethylamine was added to a 0.65% (w/v) sodium acetate, and the PH was adjusted to 7.20 ± 0.05 using 5% acetic acid. Finally, 5 mL of tetrahydrofuran was added. For Mobile phase B, 2% acetic acid was used to adjust the PH of a 3.25% (w/v) sodium acetate solution to 7.20 ± 0.05, and then 400 mL of acetonitrile and 400 mL of methanol was added. The flow rate was set at 1.0 mL/min, and the detection wavelengths were 338 nm and 262 nm, respectively.

A



B

	the number of laid egg	the number of EP	the number of no-EP	Percent (EP/laid egg)	Percent (no-EP/laid egg)
WT	534	483	51	90.4%	9.6%
	511	466	45	91.2%	8.8%
	526	496	30	94.3%	5.7%
	512	491	21	95.9%	4.1%
	561	507	54	90.4%	9.6%
	518	492	26	95.0%	5.0%
TBH	373	241	132	64.6%	35.4%
	483	426	57	88.2%	11.8%
	391	275	116	70.3%	29.7%
	461	371	90	80.5%	19.5%
	449	348	101	77.5%	22.5%
	357	303	54	84.9%	15.1%

Figure S1: Impact of high PPC on the quality of silkworm eggs, related to Figure 1. (A) PPC. L5D3, the third day of the fifth-instar larval stage, n = 3. **(B) EP rate.** EP, eggs in the body pigmentation stage. no-EP, eggs in the body no-pigmentation stage, n = 6. The data were mean ± standard deviation (SD), and the significance of the difference in the student's *t*-test was not significant (ns), with $P > 0.05$.

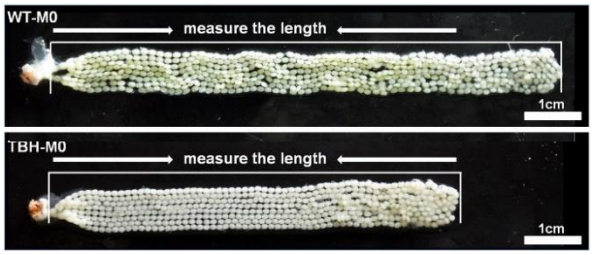


Figure S2: Schematic diagram of ovarian length measurement, related to Figure 2. M0, newly molted adult stage.

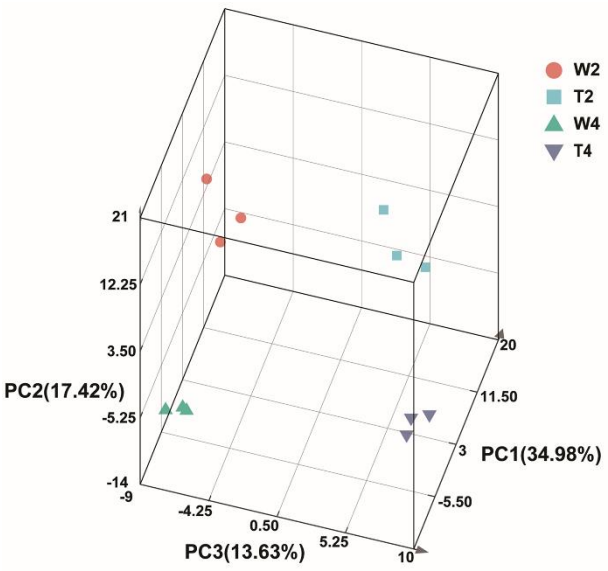


Figure S3: PCA, related to Figure 3. T2 and T4 represent TBH in P2 and P4 respectively. W2 and W4 represent WT in P2 and P4 respectively.

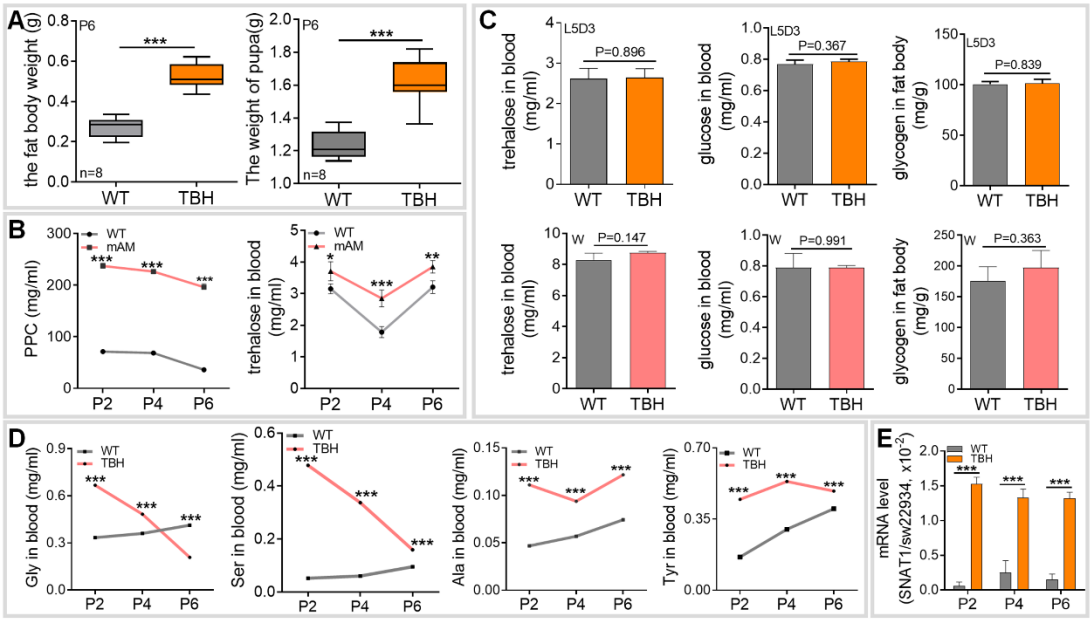


Figure S4: Levels of blood sugar and amino acid, related to Figure 4. (A) Fat body weight and pupa weight (n = 8). (B) PPC and trehalose levels in hemolymph of mAM (n = 3). (C) Levels of trehalose, glucose, and glycogen in L5D3 and W stage (n = 3). (D) Amino acid level (n = 3). Gly, glycine; Ser, serine; Ala, Alanine; Tyr, tyrosine. **(E) The transcriptional level of *SNAT1* (n = 3).** *SNAT1*, sodium-dependent nutrient amino acid transporter 1. *sw22934* was determined as the reference gene for qRT-PCR. The data were mean ± SD, and the significance of the difference in the student's *t*-test was ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

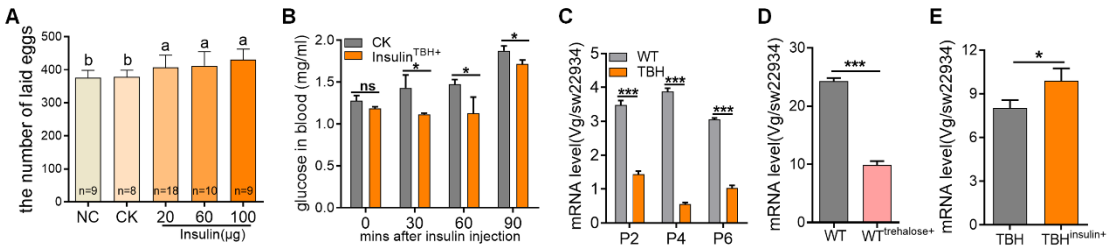


Figure S5: Egg numbers and expression level of *Vg* gene, related to Figure 5. (A) Number of laid eggs. Bovine insulin was injected into the body fluid at the P1 stage when PPC just began to rise, at doses of 20, 60, and 100 μg per individual, and the number of eggs was counted at the adult stage. **(B) Glucose content (n = 3).** Hemolymph samples were collected at 0, 30, 60, and 90 min after 100 μg bovine insulin injection. **qRT-PCR analysis of *Vg* in TBH (C), WT injected with trehalose (D) and TBH injected with bovine insulin (E).** *sw22934* was determined as the reference gene for qRT-PCR, n = 3. The data in the figures were the mean ± SD. The data in figure A were analyzed by the ANOVA, and the different letters among groups indicated a significant difference. The data in figure B-E were analyzed by the student's *t*-test and the significance of the difference was ns, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$.

Table S1: Primers used in qRT-PCR, related to Figures 1, 3, 4, S4 and S5.

Genes	Primers	Forward (5′ to 3′)	Reverse (5′ to 3′)
Fibroin heavy chain protein-like	*Hpl*	GCGACGAAAATGGAGGATAGGG	CGCTGGACCGCTTTGACTTC
Facilitated trehalose transporter1	*Tret1*	CGGATACCCGCTGAAGT	TCGGATAGCAACACCCAC
Trehalase2	*Treh2*	GTTACAGATTACGGCGAACTTC	CTTCTTCATCCCATAACGCTTG
Alanine aminotransferase	*ALT*	GCTGCGACCTGTTGTGAG	TCCCGACCTCAAGCCACA
Glutamate dehydrogenase	*m-GDH*	CTACCCGAACGCCAAAGA	GCTGCGACCTGTTGTGAG
Serine hydroxymethyl transferase	*SHMT*	TCGCAGACGCAAATGGA	CGAACGGGCTCGGTATT
Fuarylacetoacetehydrolase	*FAH*	GGACGGTGAAACAGCAAC	GTGCCCTTCCAGGACAAT
Pyruvate carboxylase	*PC*	GTGCCCTTCCAGGACAAT	CGAGCGAGTACAGGTTGTTGA AG
Phosphoenolpyruvate carboxykinase	*PEPCK*	ACTAACGTGGCGGAAACA	ACTAACGTGGCGGAAACA
Trehalose-6-phosphate synthase	*TPS*	TGAGGACAGCATTCGGTTTG	CATCGCCAGTAAAGAGTCGG
Trehalose synthase	*Tres*	TACCATGCAACCAGTCACTATT	ATGATGGCTATGTACACATCGG
Glycogen branching enzyme	*Gbe*	CCCGTGTCCAAATCATAG	AGGGCTGTTCTGGCTTAT
Glycogen synthase	*Gys*	GGGCTCATCGTTCTAAGGGTA	CCATGCAGTAGCGGTGGTAT
Glycogen synthase kinase	*Gsk*	GTTAAACGCAATGCAGACCC	AAAGAATGAATGTACGCCAGA
Sodium-dependent nutrient amino acid transporter 1	*SNAT1*	CGCCCACAACAAATACAAAG	GGAACTTCTCTTCTGGAACCTT
Vitellogenin	*Vg*	CTTGTGCCATCGATAGAACAG	GTCGATATTGCATCCCCATC
Sw22934	*sw22934*	TTCGTACTGCTCTTCTCG	CAAAGTTGATAGCAATTCCCT
RpL32	*Rp49*	CAGGCGGTTCAAGGGTCAATAC	TACGGAATCCATTTGGGAGCAT