For immunoblot analysis of hemolymph AGEs, silkworms were fed a normal diet or a 10% (w/w) glucose diet for 4 days. Aminoguanidine was injected into the hemolymph of the silkworms at 12-h intervals. The AGEs in hemolymph were detected by immunoblot analysis using anti-AGEs antibody and proteins were stained with Coomassie brilliant blue.

For immunoblot analysis of GLUT2 in mouse liver, the mouse liver membrane fraction was prepared as follows. Approximately 10 mg of mouse liver was harvested and cut into small pieces using scissors in Tris B (10 mM Tris/HCl (pH 7.4), 10 mM NaCl, 1.5 mM $MgCl_2$) then centrifuged at 3300 rpm for 10 min. The supernatant was collected as liver extract. The extract was further centrifuged at 45,000 rpm for 1 h, and the resulting precipitate was dissolved by adding 50 µl of 1 M Tris base and used as the membrane fraction. GLUT2 and Na, K-ATPase in the membrane fraction of mouse liver were detected by immunoblot analysis using anti-GLUT2 and anti-Na, K-ATPase antibody.

Quantification of the amount of phosphorylated Akt or phosphorylated AMPK or GLUT2 was performed by densitometric scanning with Image Gauge software. The relative amount of phosphorylated Akt or phosphorylated AMPK or GLUT2 on total Akt or total AMPK or Na, K-ATPase was determined. The amount of AGEs was normalized to the lysate protein concentration.

### TLC analysis

Jiou extract (0.4 mg) was mixed with TFA solution (final 2 M) and incubated at 96°C for 2 h. The sample was dried by evaporation and dissolved in 50 µl water. The sample (5 µl) was spotted on a silica gel plate (Silica gel 60F254, Merck) and developed with a propanol solution (1-propanol:water = 85:15). The plate was sprayed with 10% sulfuric acid solution (sulfuric acid:ethanol = 10:90) and heated to detect the spots.

### Streptozotocin-induced diabetic mouse

Mature male C57BL6/J mice (8 weeks of age) were purchased from SLC. Diabetes was induced by a single intraperitoneal injection of streptozotocin (150 mg/kg)[27]. Blood samples were collected from the tail vein 4–7 days after injection of streptozotocin and the blood glucose concentration was determined using a glucometer (Accu-Chek Aviva, Roche). Mice with a blood glucose level of 250 to 450 mg/dl were used to evaluate the hypoglycemic effects of test samples.

### Ethics Statement

All mouse protocols followed the Regulations for Animal Care and Use of the University of Tokyo and were approved by the Animal Use Committee at the Graduate School of Pharmaceutical Science at the University of Tokyo (approval number: P21-12).

### Statistical Analysis

Data are shown as means ± SD. Statistical significance between groups was evaluated using a two-tailed Student's *t* test. A p-value of less than 0.05 was considered statistically significant.

## Supporting Information

**Figure S1 Schematic illustration of the strategy for screening anti-diabetic agents using silkworms.**
(TIF)

**Figure S2 Increased hemolymph sugar levels in silkworms fed a normal diet followed by a decrease in hemolymph sugar levels induced by subsequent fasting.** Silkworms were fed a normal diet for 24 h (shown in gray), then fasted. The hemolymph sugar level of silkworms before feeding, 12 or 24 h after feeding, or fasted for 12 or 24 h was determined. n = 5 per group. Data represents means ± SD.
(TIF)

**Figure S3 Growth inhibition by feeding a high glucose diet in male silkworms.** (A–D) Male silkworms were fed a normal diet (N.D.), a 5%, 10%, 15%, 30% (w/w) glucose diet (G.D.), or fasted for 3 days. Body size (A, B), body weight (C), and sugar level in hemolymph (D) were determined. n = 7–10 per group. Data represents mean±SD. *$p<0.0001$ versus saline injected silkworms fed a normal diet (N.D.).
(TIF)

**Figure S4 Decrease in total sugar in hemolymph after injection of human insulin.** (A) Silkworms were fed a 10% (w/w) glucose diet (G.D.) for 60 min (indicated by gray background) then fasted. 50 µl of human insulin (2 mg/ml) was injected into the hemolymph of the hyperglycemic silkworms, and hemolymph sugar levels were measured 0, 1, 3, and 6 h after injection. n = 5–7 per group. Data represents mean ± standard deviation. *$p<0.05$ versus saline injected silkworms fed a glucose diet (G.D.). (B) Silkworms were fed a 10% (w/w) glucose diet for 60 min. After cessation of the diet, serially diluted human insulin (0.005–0.5 mg/g larva) was injected into the hemolymph of the hyperglycemic silkworms. Hemolymph sugar levels were measured 6 h after injection. n = 8–10 per group.
(TIF)

**Figure S5 Stimulation of AMPK phosphorylation in the fat body by AICAR.** Isolated fat bodies from silkworm were cultured with AICAR (final conc. 0.8 mg/ml) in Grace's insect medium for 0, 60, or 120 min. Fat bodies were homogenized and extracts were prepared. Total AMPK and phosphorylated AMPK were detected by immunoblot analysis.
(TIF)

**Figure S6 Effect of glucose concentration in the culture medium on total sugar in the fat body.** Isolated fat body from silkworms was cultured in Grace's insect medium containing 0%, 0.5%, 1.0%, or 2.5% glucose for 3 h, and the amount of sugar in the fat body was measured.
(TIF)

**Figure S7 Effect of a high fat diet in silkworms.** (A–C) Silkworms were fed a normal diet (N.D.); a 7.5%, 15%, or 30% (w/w) olive oil-containing diet; or a 7.5% or 15% (w/w) oleic acid containing diet for 1 day. Sugar levels in the hemolymph (A), body weight (B), and food intake (C) were determined. n = 5 per group. Data represents mean±SD. The statistical significance of the difference was evaluated using Student's *t* test. p: P value versus silkworms fed a normal diet (N.D.).
(TIF)

## Author Contributions

Conceived and designed the experiments: YM ES. Performed the experiments: YM ES TS. Analyzed the data: YM ES TS KS. Wrote the paper: YM ES KS.

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