Supporting Information

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SI Methods

Stereotaxic Surgery. Three groups were generated and studied. One group did not undergo any surgical procedure and was not injected with any chemicals; we refer to these animals as nondiabetic mice. The second group consisted of mice that received two i.p. injections of STZ (150 mg/kg of body weight; Sigma Aldrich): the first at 8 wk of age and the second at 9 wk of age. Five days after the second injection, serum insulin levels were measured, and only mice displaying undetectable insulin were used further in the study. These mice underwent the following surgical procedure at the age of 10 wk. A cannula was positioned stereotaxically into the cerebral lateral ventricles (−0.34 mm from the bregma, ±1 mm lateral, −2.5 mm from the skull), and a small osmotic minipump (model 1004; Alzet) implanted s.c. was attached via a catheter to the cannula for icv infusion. The catheter was 25% longer than the distance between the site of placement of the minipump and the cannula to allow free movement of the neck. In these mice, the minipump was filled with a sterile PBS (pH = 7.4) solution (Invitrogen), and we refer to these animals as T1D-icv-PBS mice. The third group was treated like the second group; however, the minipump implanted in these mice was filled with leptin dissolved in PBS, and we refer to these animals as to T1D-icv-Leptin mice. There were four different subgroups of T1D-icv-Leptin mice—T1D-icv-Leptin-50, T1D-icv-Leptin-25, T1D-icv-Leptin-10, and T1D-icv-Leptin-5—in which the minipump was filled with leptin at a final concentration of 454, 227, 90, and 45 ng/μL, respectively. Because a volume of 0.11 μL/h was infused, these mice received 50, 25, 10, or 5 ng of icv leptin per hour, respectively. Twenty-five days after surgery, leptin delivery was interrupted in some of these mice by cutting and sealing the catheter with loctite 454 (Alzet); this procedure was performed under nonanesthetized conditions. For the pair-feeding experiment, a group of T1D-icv-PBS mice was allowed to eat the same amount of food that T1D-icv-Leptin-50 mice ate.

Assessment of Energy Substrates and Hormone Levels in the Blood. Tail vein blood was collected between 2:00 and 4:00 PM from mice that were fed ad libitum but did not have access to food only for the 2 h before the blood collection procedure. Serum blood was isolated after centrifugation (3,000 × *g* for 20 min) and stored at −80 °C. Ten days after surgery, mice were killed between 2:00 and 4:00 PM (after fasting for 2 h), with deep anesthesia provided by i.p. injection of chloral hydrate (350 mg/kg). Blood was collected into a chilled tube containing 5 μmol EDTA and 1 mmol p-hydroxymercuribenzoate per 1 mL of blood. Plasma was isolated by centrifugation (3,000 × *g* for 20 min) and then stored at −80 °C. Glucose, nonesterified fatty acid, ketone bodies, and triglycerides were measured by commercially available kits (Wako USA). Leptin and insulin were measured using ELISA kits (Crystal Chem., Inc.). Glucagon was measured using a RIA kit (Millipore).

Assessment of mRNA and Protein Content. Mice were not allowed to eat for 2 h and were then killed 2 h before onset of the dark cycle (i.e., 4:00 PM). Tissues were quickly removed and frozen in liquid nitrogen and subsequently stored at −80 °C. RNAs were extracted using TRIzol reagent (Invitrogen). cDNA was generated by SuperScript II (Invitrogen) and used with SYBR Green PCR master mix (Applied Biosystems) for quantitative real-time PCR analysis. *Pomc*, *Agrp*, *Npy*, *Glut4*, *Cpt1-b*, *Hk*, *Pfkm*, *Aldoc*, *Ldha*, *preproinsulin*, and *preproglucagon* mRNA levels were normalized to *36B4* mRNA contents. Sequences of deoxy-oligonucleotides primers are outlined as follows: *Pomc* (5′gaggccactgaacatctttgtc and 5′gcagaggcaaacaagattgg), *Agrp* (5′cggccacgaacctctgtag and 5′ctcatcccctgcctttgc), *Npy* (5′ctactccgctctgcgacact and 5′agtgtctcagggctggatctc), *Glut4* (5′catggctgtcgctggtttct and 5′catactggaaacccatgccg), *Cpt1-b* (5′agaagtgtaggaccagcccg and 5′acttgcctttgtcccggaaat), *Hk* (5′cctttgtgaggtcaactccg and 5′ctgtcacccttactcggagcac), *Pfkm* (5′tgggtcagacttcagcatcg and 5′ggtcacaagtcgtgcagatgg), *Aldoc* (5′gcatcaaggttgacaagggtgt and 5′cttgagtggtggtttccccg), *Ldha* (5′gccctgctgccaagtggtac and 5′tgcagttggcagtgtgtctcag), *preproinsulin* (5′ggggagcgtggcttcttcta and 5′ggggacagaattcagtggca), *preproglucagon* (5′attcaccagcgactacagcaa and 5′tcatcaaccactgcacaaaatc), and *36B4* (5′cactggtctaggacccgagaag and 5′ggtgcctctgaagattttcg). All assays were performed at least three times using a Prism 7900HT sequence detection system (Applied Biosystems). Proteins were extracted by homogenizing samples in lysis buffer [20 mM Tris, 5 mM EDTA, 1% Nonidet P-40 (vol/vol), protease inhibitors (P2714-1BTL; Sigma)], resolved by SDS/PAGE, and finally transferred to a nitrocellulose membrane by electroblotting. The following primary antibodies were used for Western blot assays: rabbit polyclonal antisera against phosphoenolpyruvate carboxykinase 1 (Cayman Chemical Co.), rabbit polyclonal antisera against glycogen synthetase 2 (Abcam), mouse monoclonal antisera against STAT3 (Cell Signaling), and rabbit polyclonal antisera against Try^{705}-phosphorylated STAT3 (Cell Signaling). β-actin was used as a loading control and was detected with rabbit polyclonal antisera (Abcam). The following secondary antibodies were used: IRDye 680 Goat anti-Mouse IgG (Li-Cor Bioscience) and IRDye 800CW Goat anti-Rabbit IgG (Li-Cor Bioscience). The ODYSSEY infrared Imaging System (Li-Cor Bioscience) was used to detect signals according to the manufacturer's manual.

Assessment of Glycogen and Triglyceride Levels in the Liver. Glycogen levels were measured as previously described (1). Triglyceride levels were measured as previously described (1, 2), with the following modifications. Liver samples were weighed, homogenized in iced Folch solution (chloroform/methanol 2:1), and gently stirred for 16 h at 4 °C. Samples were centrifuged (3,500 × *g* for 60 min) at 4 °C. The chloroform phase was collected and completely dried out. Samples were resuspended in 1-Butanol/Methanol/Triton X-100 (9:4:2) solution (Sigma Aldrich), and triglyceride levels were measured using commercially available kits (Wako Chemicals).

Assessment of Pancreatic Insulin and Glucagon Levels. Insulin and glucagon were extracted from the pancreas using the acid ethanol method (3). Pancreata were removed and immediately put into acid ethanol solution (0.18 M HCl in 70% ethanol) and stored at −20 °C. After homogenization, supernatants were either used for measuring hormone levels or stored at −20 °C. Insulin levels were measured using a commercially available ELISA kit. Of note, for this measurement, samples from nondiabetic and diabetic mice were diluted 1:1,000 and 1:100, respectively. using the sample dilution solution provided by the kit. Glucagon levels were measured using a glucagon RIA kit. To measure glucagon, samples were diluted 1:200 using the sample dilution solution provided by the kit.

Immunohistochemistry. Mice were deeply anesthetized with i.p. injection of chloral hydrate and perfused as previously described (4, 5). Pancreatic glucagon and insulin distribution was determined as follows. Pancreata were fixed using OCT compound

(Sakura Finetek) and cut in 15-μm-thick sections using a cryostat. Sections were incubated with primary antibodies for 12 h at room temperature. Antibodies used were guinea pig anti-swine insulin (DAKO) and rabbit anti-human glucagon (Abcam). Secondary antibodies used were Alexa Fluor 488 donkey anti-rabbit IgG (Invitrogen) and Alexa Fluor 594 goat anti-guinea pig IgG (Invitrogen). Sections were analyzed with a Zeiss Axioplan light microscope, Zeiss Stemi 2000-C dissecting microscope, and Zeiss Apotome microscope.

Oral Glucose Tolerance Test. D-glucose was dissolved in distilled water [20% D-glucose (wt/vol)]. Mice were orally injected with D-glucose solution (2 g/kg) 2 h after food and water were removed. Blood samples were taken from the tail vein at indicated times.

Statistical Methods. Datasets were analyzed for statistical significance using PRISM (GraphPad) for a two-tailed unpaired Student's *t* test or one-way ANOVA (Tukey's posttest), as indicated in each of the figure legends.

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