Supporting Information

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SI Materials and Methods

Materials. We obtained Contour blood glucose test strips from Bayer, protease inhibitor mixture tablets from Roche, phosphatase inhibitor mixtures I and II from Calbiochem, and leupeptin from Sigma-Aldrich. All other chemicals were obtained from Sigma-Aldrich unless specified cotherwise.

Mice. *Goat*$^{-/-}$ mice were generated as described previously (1). All mice were housed in colony cages under a 12-h light/12-h dark cycle. The dark cycle began at 9 or 10:00 PM. The chow diet (Teklad Mouse/Rat Diet 7002; Harlan Teklad Global Diets) contains 3.0 kcal/g of metabolizable energy, of which 18% of calories are from fat, 49% are from carbohydrates, and 33% are from protein. All animal experiments were performed with approval of the University of Texas Southwestern Medical Center's Institutional Animal Care and Research Advisory Committee.

Calorie Restriction. Calorie restriction was implemented as described previously (1). At 1 wk before the initiation of calorie restriction, 8-wk-old male WT and *Goat*$^{-/-}$ littermates were placed in individual cages and fed the chow diet ad libitum. During this week of acclimation, food intake was monitored to determine the average amount of food consumed daily by each mouse. Thereafter, the mice were subjected to 60% calorie restriction, such that each mouse was fed at 6:00 PM every day with an amount of food equal to 40% of the daily amount consumed by the same mouse during the week of acclimation.

Infusions and Injections. Infusion of GH was carried out as described previously (1). An Alzet osmotic pump (catalog no. 1002; DURECT) was inserted s.c. in the interscapular region of each mouse at 3 d before the initiation of calorie restriction. Each pump was filled with vehicle (70 mM sodium bicarbonate pH 9.5, 137 mM NaCl, and 100 μg/mL rat albumin) or vehicle containing 2.5 mg/mL recombinant rat GH (obtained from the National Hormone and Peptide Program of the National Institute of Diabetes and Digestive and Kidney Disorders through A. F. Parlow). GH was delivered at a rate of 0.25 μL/h (15 μg/24 h). For GH injection, GH (30 μg/mouse) or vehicle was injected s.c. at 2:30 PM, 3:30 PM, and 4:30 PM on day 7 of calorie restriction.

Metabolic Parameters. Blood glucose was measured from tail-vein blood using a Bayer blood glucose meter. For measurement of plasma GH, blood was drawn from the retro-orbital sinus and collected in EDTA-coated tubes. Plasma was separated, and GH plasma levels were measured with a commercial kit (Cayman; catalog no. 589601). For liver ATP and ADP measurements, the liver was freeze-clamped immediately after dissection. Approximately 50 mg of tissue was homogenized in 0.5 mL of ice-cold 3.5% perchloric acid and neutralized with 0.25 mL of 2.2 M potassium bicarbonate. The supernatant was used to measure ATP and ADP with commercial kits (Biovision; catalog nos. K354-100 and K355-100).

Electron Microscopy for Quantifying Autolysosomes. For each experiment, two WT and two *Goat*$^{-/-}$ mice were anesthesized with pentobarbital at 9:30 AM or 5:30 PM on day 8 of calorie restriction. Their bodies were perfused with 25 mL of solution containing 4% (vol/vol) paraformaldehyde, 1% (wt/vol) glutaraldehyde, and 250 mM sucrose in 0.1 M cacodylate buffer (pH 7.4) injected into the left ventricle of the heart. Mice were then dissected, and tissues were fixed in a solution containing 0.1 M cacodylate (pH 7.4) and 2.5% glutaraldehyde. The samples were postfixed with 1% (wt/vol) OsO_4, embedded, and sectioned. Specimens were visualized on a JEOL 1200 EX transmission electron microscope. Twenty digital electron micrographs (10 from each mouse) were obtained from random areas at a magnification of 5,000×. An autolysosome was defined as a single membranous vacuole containing amorphous and multilammelar structures. The number of autolysosomes per unit area (36 × 26 μm) were counted by three examiners who were blinded to the experimental parameters. Their results varied by <2%. Each value represents mean ± SEM of data obtained from 20 images of WT or *Goat*$^{-/-}$ mice.

Immunoblotting. For samples used for LC3 blotting, mice were injected i.p. with leupeptin (15 mg/kg) at 1 h before sacrifice (2). Frozen tissue samples (~50 mg) were homogenized on ice in 1 mL of lysis buffer [50 mM Tris·HCl pH 7.5, 150 mM NaCl, and 1% (vol/vol) Triton X-100] supplemented with protease inhibitor mixture and phosphatase inhibitor mixtures I and II. Homogenates were then incubated on ice for 20 min and then centrifuged at 20,000 × *g* for 20 min at 4 °C. Supernatants were collected, and protein concentrations were determined using the Pierce BCA Protein Assay Kit. Samples were then denatured in SDS buffer, boiled at 95 °C for 5 min, and loaded onto SDS gels for immunoblotting. The following antibodies were used: LC3 (1/500 dilution; Novus; catalog no. 100-2331), GAPDH (1/10,000; Cell Signaling; catalog no. 5174); t-STAT5 (1/1,000; Cell Signaling; catalog no. 9363); p-STAT5 (1/1,000; Cell Signaling; catalog no. 9351). Blots were exposed to film for 1–10 s. Films were scanned and quantified using ImageJ (rsbweb.nih.gov/ij/).

Quantitative Real-Time PCR. Total RNA was isolated, and real-time PCR measurements were performed on pooled RNA samples from four mice in each group. Primer sequences of LC3A (ID no. 23956148a1) and LC3B (ID no. 13385664a1) were obtained from Primerbank (pga.mgh.harvard.edu/primerbank). All reactions were done in triplicate, with a mean range of variation of 0.40 ± 0.03% for all values. The relative amount of all mRNAs was calculated using the comparative threshold cycle (C_T) method, with 36B4 mRNA as the invariant control.

Reproducibility. All results were confirmed in two or three independent experiments.

1. Zhao T-J, et al. (2010) Ghrelin *O*-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci USA* 107(16): 7467–7472.

2. Ezaki J, et al. (2011) Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. *Autophagy* 7(7):727–736.

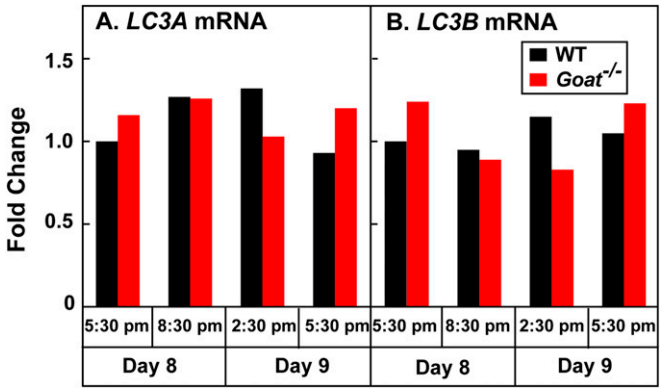

Fig. S1. Quantitative real-time PCR analysis of *LC3A* and *LC3B* mRNAs from calorie-restricted WT and *Goat*$^{-/-}$ mice. The mice used in this experiment are the same as those used in the experiment described in Fig. 1. Liver total RNA was extracted, and quantitative real-time PCR was performed as described in *Materials and Methods*. Each measurement was made on pooled mRNA samples from four mice in each group. Expression of *LC3A* or *LC3B* was normalized to its expression in WT at 5:30 PM on day 8. The C_T values of *LC3A* and *LC3B* in WT mice at 5:30 PM on day 8 were 22.4 and 20.8, respectively. Similar results were obtained in an independent experiment.