Detailed Methods and Material

Mouse breeding and husbandry: All procedures were in accordance with current National Institutes of Health guidelines and were approved by the Columbia University Institutional Animal Care and Use Committee. Wild type C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Muscle-specific LpL-expressing transgenic mice (MCK-LpL) were generated as described[1]. Heterozygous LpL knockout mice (*Lpl*$^{+/-}$) were generated by crossing LpL knockout mice rescued with LpL expression in skeletal muscle (MCK-LpL/*Lpl*$^{-/-}$ mice) with wild type C57BL/6 mice. Mice were maintained in a temperature-controlled (25°C) facility with a 12-h light/dark cycle and given free access to water and food, except when fasting blood specimens were obtained. Mice were fed a laboratory rodent chow diet. After 4 hour-fasting blood samples were collected using heparinized capillary tubes and then placed into 1 mM EDTA-containing Eppendorf tubes. Mice were allowed 7 days to recover between phlebotomies.

Genotyping: LpL knockout mice were screened with primers 5'-GCGGGGGCGGGGGGGAACTTCCTGACTAGGGG-3, 5-CTCGCTGGCACCGTTGAG CCTCGTTACCG-3' and 5'-ACTGGAGCGCGGTGGAGCGCCGTAGGGCA-3'. PCR amplification was performed using 30 cycles at 94°C for 1 min and 60°C for 2 min and 72°C for 3 min. Genotypes for MCK–human LpL mice were determined by PCR as described below. Human LpL specific primers used were as follows: 5´- CCA GTG AGC AAG TCA GCC CT – 3' and 5'- CAC ACG GCC AGA GTC AGC AC – 3'. The protocol involved 20 cycles of 94°C for 30 sec, 68°-0.5°C/cycle for 1 min, and 72°C for 1 min followed by 10 cycles of 94°C for 30 sec, 58°C for 1 min and 72°C for 60 sec. PCR products were resolved by gel electrophoresis, and were visualized by ethidium bromide staining.

Induction of insulin deficiency: Mice were made diabetic by streptozotocin (STZ) treatment similar to that described by Kunjathoor, Wilson, and LeBoeuf[2] and adopted by the Diabetes Complications Consortium[3]. Mice were divided into two groups; one group was treated with STZ (Sigma Chemical Co., St. Louis, MO), the other group was treated with vehicle. STZ was dissolved in sterile citrate buffer (pH 4.5) and used within 20 min of preparation. The solution was injected intraperitoneally (i.p.) into mice (50 mg/kg, ~200 µl) for five consecutive days. 10 days after the last STZ injection 4-hours fasting glucose levels were measured. STZ-injected mice with glucose levels >13.9 mmol/l (>250mg/dl) were considered to be diabetic.

Plasma glucose, plasma insulin, lipid, and lipoprotein determinations: Glucose was measured using a glucose meter (OneTouch Ultra2, LifeScan, Milpitas, CA). Plasma insulin levels were measured using the Mouse Insulin ELISA by Merck Millipore (# EZRMI-13K, Billerica, MA). Lipids were measured using Infinity Triglyceride Reagent and Infinity Total Cholesterol Reagent (#TR22321 and #TR13521 respectively, Thermo Scientific, Waltham, MA). Lipoproteins, VLDL (d < 1.006 g/ml), IDL+LDL (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml), were separated by sequential density ultracentrifugation of mouse plasma in a TLA 100 rotor (Beckmann Instruments, Palo Alto, CA). Free fatty acids (FFA) were measured using the Wako HR series kit NEFA (#99934691, Wako Life Sciences, Richmond, VA). All of the above parameters were measured after 4 hours of fasting (8am -12 am).

Tissue collection: After 4 hour-fasting mice were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg and then perfused by heart puncture with 10 ml of PBS or until the livers blanched. Tissues were rapidly excised and snap frozen in liquid nitrogen unless otherwise noted.

Gene expression by quantitative real time PCR: Total RNA was obtained from tissues homogenized in TRIzol reagent (#15596-018, Ambion, Austin, Tx). The PureLink RNA mini (#12183018A, Ambion, Austin, Tx) kit was used for the RNA purification. The RNA was then

reverse-transcribed by ThermoScript RT-PCR System (#11146-057, Invitrogen, Carlsbad, CA), and quantitative real time PCR was performed with Stratagene Mx3005 using SYBR Green PCR master mix (Applied Biosystems, Foster City, CA). All genes were normalized to 18s rNA expression. All primer sequences are listed in Supplement Table I.

LpL Activity Assay: Postheparin plasma LpL activity was determined as described by Hocquette *et al.* [4]. Postheparin plasma was obtained from 4h-fasted mice 7 min after femoral vein injection of 100 units of heparin/kg body weight. To measure total lipase activity, plasma was incubated with 10% Intralipid/[^{3}H]-triolein (#NET431L005MC, Perkin Elmer, MA) emulsion as substrate and human serum as the source of apoCII[5]. The contribution of hepatic lipase in the plasma was determined by including NaCl (final concentration 1M) in the assay and was subtracted from the total lipase activity to estimate specific LpL activity. Activity was expressed as µmol FFA/ml/h. Aliquots of human plasma were used for a standard curve in all the experiments. Heparin-releasable LpL activity in skeletal muscle, BAT and heart was measured following the method by Haugen *et al.* [6]. Briefly, freshly isolated tissues were minced in Krebs-Ringer phosphate buffer and incubated for 1 hour in a 25 °C water bath in the presence of 5U/ml heparin. 100 µl aliquots of the buffer were used for the lipase assay with 100 µl of 10% Intralipid/[^{3}H]-triolein (NET431L005MC, Perkin Elmer, MA) emulsion for 1 hour at 25°C.

Hepatic TG secretion: To measure hepatic TG production rate, mice were injected intraperitoneally with Poloxamer-407 (#16758, Sigma-Aldrich, St. Louis, MO) at 1 g/kg in saline after 4-h fasting[7]. Immediately prior to injection, and at 1, 2, and 6h following injection, blood samples were drawn in heparin capillary tubes, plasma was prepared, and TG concentrations were determined. The TG production rate was calculated from the difference in plasma TG levels over a given interval following detergent injection.

Olive oil gavage: After a 4 hour fast, mice were given olive oil by oral gavage at 10ml/kg BW (~250µl). Blood samples were drawn at baseline 2, 4 and 6 hours after receiving the olive oil gavage. For comparison TG levels were enzymatically measured using Infinity Triglyceride Reagent (#TR22321,Thermo Scientific, Waltham, MA).

Western Blot Analysis: Plasma samples of four control and four STZ-diabetic mice were used for Western Blot Analysis of ApoCIII. Twenty micrograms protein of each sample were resolved by SDS-PAGE and transferred onto PVDF membranes. Immunoblotting was carried out using a rabbit anti-ApoCIII antibody (kindly provided by ISIS Pharmaceuticals, Inc., Carlsbad, CA). Albumin was used as a loading control. Bands were quantified by densitometry using Molecular Analysis Software (Bio-Rad).

Lipid extraction and hepatic TG, total cholesterol and FFA content: The lipid extraction protocol was adapted from Folch et al.[8]. Approximately 100 mg of tissue in 1 mL of PBS were homogenized using stainless steel beads for 1 min in a bead beater homogenizer. From each sample, 50 µL were removed for protein analysis and 3 mL of 2:1 chloroform: methanol was added to the rest and vortexed. Samples were then centrifuged for 10 minutes at 3000 rpm at 4°C. The lower, organic phase was then collected and dried under nitrogen gas. The dried lipid was then dissolved in 500 µL of 1% Triton-X 100 in chloroform, further dried and then dissolved in 100µL of double distilled water. Hepatic TG, total cholesterol and FFA content were measured using Infinity Triglyceride Reagent, Infinity Total Cholesterol (both Thermo Scientific, Waltham, MA) and the Wako HR series kit NEFA (Wako Life Sciences, Richmond, VA), respectively.

Tissue lipid and protein measurements: The sample of tissue lysate retained from the lipid extraction protocol was assayed for protein content using Pierce BCA Protein kit (#23227 Thermo Scientific, Rockfort, IL) following the instructions of the manufacturer. Using the

tissue lipid extract, assays for TG were performed as previously described for plasma lipids. Lipid measurements were normalized to protein content.

Antisense oligonucleotide (ASO) treatment: In order to inhibit the sodium glucose co-transporter 2 (SGLT2), expression mice were injected with the ASO, ISIS 388625 (sequence 5-TGTTCCAGCCCA-3) at 20 mg/kg body weight 10 days after the last STZ injection. Control mice were injected with ASO, ISIS-141923 (5-CCTTCCCTGAAGGTTCCTCC-3), which does not have perfect complementarity to any known gene in public databases. All ASOs were dissolved in sterile saline for IP injection.

Statistical analyses: Data are presented as means ± SEM, where the means are based on at least three independent experiments. Statistical differences were assessed via a paired Student's t-test or a one-way ANOVA where appropriate. A p value less than 0.05 was considered as significant.

Supplemental References

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