Supplementary Material

Materials and Methods:

Monthly Hormone and Metabolic Profiles

Blood samples were collected from rats in both the short term and long term treatment groups once a month after an overnight (13 hour) fast and placed into EDTA treated tubes. The plasma was separated by centrifugation and assayed for glucose, insulin, glucagon, free fatty acids (FFA), triglycerides (TG), cholesterol, leptin and adiponectin. Whole blood samples were collected for the measurement of HbA1c. Plasma glucose, cholesterol, FFA and TG concentrations were measured using enzymatic colorimetric assays (Thermo DMA Louisville, CO). Leptin, glucagon and adiponectin were measured with rodent/rat specific RIAs (Millipore, St. Charles, MO). HbA1c was measured using an enzymatic colorimetric assay (Diazyme; Poway, CA).

Oral Glucose Tolerance Testing

An OGTT was performed after 3.5 months of treatment on animals from the short term and long term groups. Animals were fasted overnight and then received a 50% dextrose solution (1 g/kg BW) by oral gavage. Blood was collected from the tail for measurement of glucose and insulin concentrations. A second aliquot of blood was placed in tubes containing EDTA, aprotinin and a DPP-IV inhibitor and analyzed for total GLP-1. Serum glucose was measured using an enzymatic colorimetric assay for glucose (Thermo DMA Louisville, CO). Serum insulin and plasma GLP-1 were measured by sandwich electrochemiluminescence immunoassay (Meso Scale Discovery; Gaithersburg, MA).
The same procedure was followed for the performance of an acute study of the effects of alogliptin administration on circulating concentrations of active GLP-1. A separate set of male UCD-T2DM rats were fasted overnight and received an oral gavage of dextrose (1 g/kg) with or without alogliptin added to the gavage (1 mg alogliptin/kg body weight).

**Body Composition and Liver and Muscle Triglyceride Content**

After 4.5 months of treatment (6.5 months of age) animals in the short-term groups were euthanized with an overdose of pentobarbital (200 mg/kg i.p.) after an overnight fast. Subcutaneous, mesenteric, retroperitoneal and epididymal adipose depots and liver, heart, gastrocnemius muscle and kidney were dissected, weighed and flash frozen in liquid nitrogen and stored at -80 °C. Liver and skeletal muscle TG content were measured using the Folch method [33] for lipid extraction followed by spectrophotometric measurement of TG content (Thermo Electron, Louisville, CO).

**Islet Immunohistochemistry and Pancreatic Insulin Content**

Pancreas samples were collected and insulin and glucagon were extracted and analyzed as previously described [34]. Pancreas samples were also collected from a subset of animals for immunohistochemistry, as previously described [35]. Briefly, samples were fixed in 4% paraformaldehyde and embedded in paraffin. Ten sections (1µm) per pancreas were obtained. Sections were deparafinized in a xylene ethanol series and placed in Tris-EDTA buffer for antigen retrieval (10mM Tris, 1mM EDTA, 0.05% Tween, pH=9.0) and then blocked in 5% BSA. Sections were immunostained for insulin using a monoclonal anti-mouse antibody (1:100) and for glucagon using monoclonal anti-rabbit antibody (1:50) (Santa Cruz Biotechnology;
Detection of the primary antibodies was performed using Alexa Flour 488 anti-goat and Alexa Flour 633 anti-mouse secondary antibodies (1:200) (Invitrogen; Foster City, CA). Nuclei were detected using 4',6'-diamino-2-phenyl indole (DAPI), included in the mounting solution (Invitrogen; Foster City, CA). Ten sections per pancreas, taken throughout the pancreas, were imaged for quantification. Pancreatic β-cell area was analyzed using Image J software.

**rtPCR**

RNA was extracted from brown and subcutaneous white adipose tissue using TRIzol reagent (Invitrogen, CA). cDNA was generated using high-capacity cDNA Archive Kit (SuperScript™ III Reverse Transcriptase, Invitrogen). mRNA of UCP1, Cox8b and PGC1α was assessed by reverse transcription PCR (iCycler, BioRad) and normalized to β-actin. For RT-PCR, *Absolute blue* qPCR premix (Fisher Scientific) was mixed with each primer. UCP1 primers: 5’- ATACTGGCGAGATGACGTCCC -3’ (For.), 5’-ATCCGAGTCGAGAAAGAA-3’(Rev.); Cox8b primers: 5’- CCGAGAATCATGCCAAGGCT -3’ (For.), 5’-TCCTGCTGGAACCATGAAGC -3’ (Rev.); PGC1α primers: 5’-TAGCGGTCCTCACAGAGACA-3’ (For.), 5’- AGTGCTAAGACCGCTGCATT-3’ (Rev.); β-actin primers: 5’- CACGGCATTGTCACCAACTG-3’ (For.), 5’-GGGTTGTGAAGGTCTCAAA-3’ (Rev.).

**Immunoblotting**

Tissues were ground in the presence of liquid nitrogen and lysed using radio-immunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS], 1% Triton X-100, 1% sodium deoxycholate, 5 mM EDTA, 1 mM
NaF, 1 mM sodium orthovanadate and protease inhibitors). Lysates were clarified by centrifugation at 13,000 rpm for 10 min and protein concentrations were determined using bicinchoninic acid protein assay kit (Pierce Chemical, IL). Proteins (20-50ug) were resolved by SDS-PAGE (10-12%) and transferred to PVDF membranes. Immunoblots were performed with the relevant antibodies and proteins were visualized using Luminata™ Forte (Millipore; Billerica, MA). For quantitation purposes, pixel intensities of immuno-reactive bands from blots that were in the linear range of loading and exposure were quantified using FluorChem 9900 (Alpha Innotech, CA). Antibodies for FAS, PGC1α and Tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for acetyl-CoA carboxylase (ACC), pACC (Ser79), AMP-activated protein kinase (AMPK), pAMPK (Thr172), protein kinase B (AKT), pAKT (Ser473), extracellular-signal-regulated kinase1/2 (ERK1/2) and pERK1/2 (Thr202/Tyr204) were from Cell Signaling (Beverly, MA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from BioResources International (Carlsbad, CA).