Figure S1. Weight gain over time. Mean weight change from baseline shown for all the groups treated. Weight gain was significantly impaired in the TAC, SIR and TAC+SIR groups. MET did not alter weight gain over time. *p<0.05 compared to CTRL and CTRL+MET, α p < 0.05 compared to CTRL. Black triangle = CTRL; white triangle = CTRL+MET; black square = SIR; white square = SIR+MET; black circle = TAC; white circle = TAC+MET; black diamond = TAC+SIR; white diamond =TAC+SIR+MET.
**Figure S2. Glucose response to oral glucose challenge after 2 weeks treatment.**

TAC, SIR and TAC+SIR treatments impaired glucose tolerance compared to controls. MET treatment did not alter the glucose tolerance. Data are presented as mean ± S.E.M, n=6. * p < 0.05 compared to CTRL. Black triangle = CTRL; white triangle = CTRL+MET; black square = SIR; white square = SIR+MET; black circle = TAC; white circle = TAC+MET; black diamond = TAC+SIR; white diamond =TAC+SIR+MET.
**Figure S3. Insulin response to oral glucose challenge in the 2 week treatment group.** SIR treated rats had a higher insulin response than controls, which was not altered by MET treatment. * p < 0.05 compared to CTRL or CTRL+MET. Black triangle = CTRL; white triangle = CTRL+MET; black square = SIR; white square = SIR+MET; black circle = TAC; white circle = TAC+MET; black diamond = TAC+SIR; white diamond = TAC+SIR+MET.
Figure S4: Islet insulin content in the various groups. Immunofluorescence staining for islet insulin showed that insulin content was lower in the SIR+MET treated groups than the SIR treated groups alone (*p < 0.05). Insulin content was lower in TAC+SIR and TAC+SIR+MET compared to SIR (**p < 0.05). White bars = placebo (water); black bars = MET.

SDC, Materials and Methods

Experimental Design

Animals (n=6/group) were given one of eight daily treatment regimens that consisted of two subcutaneous injections and one oral gavage. Two groups received daily injections of tacrolimus (2 mg kg\(^{-1}\) d\(^{-1}\); LC Laboratories, Woburn, MA, USA) and diluent alone (10% ethanol in sunflower oil) subcutaneously for two weeks. Two groups
received daily sirolimus (1 mg kg\(^{-1}\) d\(^{-1}\); LC Laboratories, Woburn, MA, USA) and diluent alone subcutaneously for two weeks. Two groups received a daily subcutaneous injection of both tacrolimus (2 mg kg\(^{-1}\) d\(^{-1}\)) and sirolimus (1 mg kg\(^{-1}\) d\(^{-1}\)) for two weeks. Finally, two groups received two injections of diluent daily and served as the control group. These drug doses were chosen based on our previously published studies (24). Of the two groups receiving the same daily injections, one group received a daily oral gavage of metformin (200 mg/kg; Medisca Inc., Plattsburgh, NY, USA) while the other group received a daily oral gavage of water. Daily weights were obtained and non-fasting blood glucoses were measured by glucometer (FreeStyle Flash ®; Abbott Diabetes Care, Inc., Alameda, CA, USA) every other day.

After 2 weeks of treatment, an oral glucose challenge was performed on each group (1.5g/kg by gavage) prior to being killed. To obtain fasting samples, food was removed from the animals twelve hours before the oral glucose challenge. Baseline glucose and insulin were measured from blood taken by tail vein prior to administration of glucose. Blood was removed from the tail vein at 15, 30, 60 and 120 mins after glucose challenge to measure glucose and insulin levels. The groups of rats were then killed under anesthesia and cardiac blood, pancreas and liver, were harvested.

**Plasma insulin concentrations**

Plasma insulin concentration was measured using a high sensitivity radioimmunoassay (31, 32) (Linco Research, St. Charles, MO, USA, limit of sensitivity=0.02 ng/ml). The inter-assay variations were 3.8–10.8% and intra-assay variations were: 2.7–5.8 % for this assay.

**Islet insulin content**
Additional slides were stained for insulin and glucagon by immunohistochemistry by the Tissue Sciences Facility at the University of Nebraska Medical Center, using guinea pig anti-insulin antibody (Dako Cytomation, Carpinteria, CA, USA) and rabbit anti-glucagon antibody (Cell Signaling Technology, Danvers, MA, USA). Stained slides visualized using light microscopy and photographed at x 20 magnification. Image J software was used to quantify insulin and glucagon content and represented as the integrated density (34).

**Statistical Analysis**

Change in weight between groups over time was compared by two-way ANOVA. Bonferroni post-test was used for analysis. Glucose and insulin concentration responses to oral glucose load were calculated as area under the curve, and compared by one-way ANOVA with Tukey’s multiple comparison tests for posthoc differences.

Average integrated density values were calculated in square pixels and converted to square micrometers. Two-way ANOVA was used to compare insulin and glucagon integrated density between treatment groups and p values < 0.05 were considered significant for post-test differences. All data is represented by mean±SEM, unless otherwise specified. Graph pad Prism software was used for all the statistical analysis. N=6 in each group unless otherwise specified.