SUPPLEMENTARY DATA

Data Supplement

Animals.

Coronary artery disease (CAD) and metabolic syndrome (MetS) were induced in both treatment groups by feeding, once daily for 6 months, 1 kg of atherogenic diet, containing 16% kcal from protein, 41% kcal from complex carbohydrates, 19% kcal from fructose, and 43% kcal from fat, and supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (KT324, Purina Test Diet, Richmond, IN), as previously described (1-3). Following 6 months on this diet, swine were placed on placebo or AC3174 treatment twice daily and feeding was altered to 0.5 kg twice daily for an additional six months. Water was provided *ad libitum*. Six lean, age-matched Ossabaw miniature swine fed a standard chow diet (5L80, Purina Lab Diet, Richmond, IN) were a control group to verify presence of MetS. Coronary smooth muscle (CSM) cells were isolated from additional lean and MetS swine for acute, *in vitro* assessment of GLP-1 receptor agonist action on intracellular Ca^{2+} handling (see acute *in vitro* exenatide treatment methods).

Measurement of intracellular Ca^{2+} levels.

CSM were loaded with the fluorescent intracellular Ca^{2+} indicator, fura-2/AM. Fura-2 loaded cells were placed on a coverslip contained in a constant-flow superfusion chamber mounted on an inverted epifluorescence microscope (model TMS-F, Nikon, Melville, NY), with flow maintained at 1-2 ml/min. Whole cell intracellular Ca^{2+} levels were assessed as the 360 nm/380 nm excitation ratio of the fura-2 emission at 510 nm at room temperature (22-25 °C), using the InCa++ Ca^{2+} Imaging System (Intracellular Imaging, Cincinnati, OH) as previously described (1;2;4-10). Basal Ca^{2+} levels were measured in physiologic salt solution (PSS) composed of the following (in mM): 2 $CaCl_2$, 138 NaCl, 1 $MgCl_2$, 5 KCl, 10 HEPES, 10 glucose; pH 7.4). Voltage-gated calcium channels were activated by depolarization with high (80 mM) K^+ solution (2 $CaCl_2$, 63 NaCl, 1 MgCl, 80 KCl, 10 HEPES, 10 glucose; pH 7.4). Sarcoplasmic reticulum (SR) Ca^{2+} stores were released with 5 mM caffeine in Ca^{2+}-free solution (138 NaCl, 1 $MgCl_2$, 5 KCl, 10 HEPES, 10^{-5} M K^+-EGTA, 10 glucose; pH 7.4).

Intravenous glucose tolerance testing.

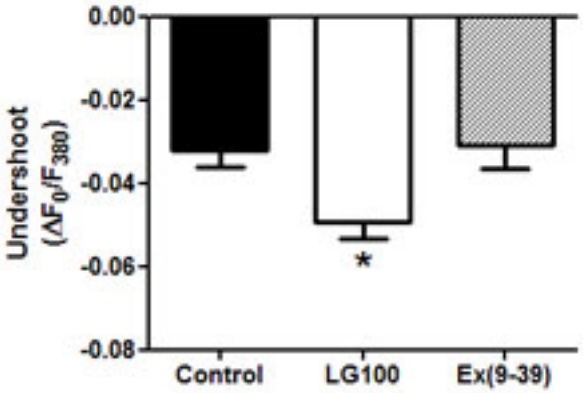
Briefly, swine were pre-acclimated to sling-restraint. Following an overnight fast, swine were restrained in the low stress sling and baseline blood samples and tail-cuff blood pressures were obtained. Next, glucose (1 g/kg body weight) was administered intravenously as a bolus and timed blood samples were collected. Blood glucose was measured immediately (YSI 2300 STAT Plus Glucose analyzer, YSI Life Sciences, Yellow Springs, OH). Plasma insulin assays were performed offsite (Millipore, Inc., St Charles, MO).

Intravascular ultrasound.

After 12 months on diet, following an overnight fast, swine received 2.2 mg/kg xylazine and 5.5 mg/kg telazol, similar to previous reports (1-3;5;11;12). Swine were intubated and anesthesia was maintained at 2-4% isoflurane in 100% O_2 as a carrier gas. A 7 F introducer sheath was inserted into the right femoral artery and heparin (200 U/kg) was administered. A 7 F guiding catheter (Amplatz L, sizes 0.75-2.0; Corndis, Bridgewater, NJ) was advanced to engage either the right or left coronary ostium. A 3.2 F, 40 MHz IVUS catheter (Boston Scientific, Natick, MA) was advanced over a guide wire and positioned in the coronary artery. Automated IVUS pullbacks were performed at 0.5 mm/sec. Angiography was performed throughout the procedure to assist in catheter placement.

SUPPLEMENTARY DATA

Supplementary Figure 1. Liraglutide increases SERCA activity in CSM from lean, healthy Ossabaw Swine, and this effect is prevented in the presence of GLP-1 receptor antagonist, Exendin (9-39).



SUPPLEMENTARY DATA

Supplementary Table S1. Phenotypic Characteristics of Ossabaw Swine Groups.

Parameter	Lean	Placebo			AC3174			Significance, * (p < 0.05)
Week on Treatment		**0**	**12**	**24**	**0**	**12**	**24**	
Body Weight	61 ± 6	75 ± 3	100 ± 2*	116 ± 2*	73 ± 4	87 ± 5	100 ± 6*	Lean < AC3174 < Placebo
Blood Pressure								
Systolic (mmHg)	143 ± 8	149 ± 8	151 ± 4	170 ± 7	132 ± 5	149 ± 6	152 ± 6	None
Diastolic (mmHg)	76 ± 5	79 ± 5	84 ± 3	89 ± 5	74 ± 4	82 ± 5	85 ± 4	None
Mean Arterial Pressure (mmHg)	98 ± 5	102 ± 5	106 ± 2	116 ± 5	93 ± 4	104 ± 5	107 ± 4	None
Carbohydrate Metabolism								
Fasting plasma glucose (mg/dL)	77 ± 5	74 ± 2	78 ± 2	81 ± 2	72 ± 3	73 ± 3	68 ± 2	None
Peak plasma insulin (µU/dL)	88 ± 33	47 ± 17	47 ± 6	67 ± 12	46 ± 10	139 ± 29*	122 ± 25*	Placebo < AC3174
Lipids								
Total cholesterol (mg/dL)	66 ± 10	430 ± 95	420 ± 68	240 ± 20	460 ± 78	369 ± 65	354 ± 61	Lean < Placebo, AC3174
Triglycerides (mg/dL	29 ± 5	67 ± 16	46 ± 6	41 ± 4*	60 ± 6	64 ± 13	74 ± 7*	Lean < Placebo < AC3174
Kidney Function								
BUN (mg/dL)	13 ± 1.0	15 ± 1.4	13 ± 0.9	13 ± 1.2	16 ± 1.2	14 ± 1.4	15 ± 1.2	None
Creatinine (mg/dL)	1.2 ± 0.09	1 ± 0.04	0.9 ± 0.05	1 ± 0.04	1.1 ± 0.09	1 ± 0.07	1 ± 0.04	None
Plasma Proteins								
Total Protein (g/dL)	7.1 ± 0.2	6.2 ± 0.4	6.7 ± 0.2	6.8 ± 0.2	6.4 ± 0.4	6.8 ± 0.7	6.6 ± 0.2	None
Albumin (g/dL)	4 ± 0.2	3.6 ± 0.1	3.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	None
Globulin (g/DL)	3.3 ± 0.2	2.9 ± 0.1	3.1 ± 0.2	3.1 ± 0.1	2.8 ± 0.2	2.9 ± 0.1	2.8 ± 0.1	None
Electrolytes								
Phosphorus (mg/dL)	5.8 ± 0.2	7 ± 0.1*	6.7 ± 0.2	6.2 ± 0.2	6.7 ± 0.2*	6.1 ± 0.2*	6 ± 0.2	Lean < Placebo > AC3174
Calcium (mg/dL)	10.3 ± 0.1	9.7 ± 0.2	9.4 ± 0.2	9.5 ± 0.3	9.7 ± 0.3	9.8 ± 0.3	9.5 ± 0.2	None
Magnesium (mEq/L)	1.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	None
Sodium(mEq/L)	140 ± 1	140 ± 1	137 ± 2	139 ± 1	141 ± 1	137 ± 2	138 ± 2	None
Potassium (mEq/L)	4.3 ± 0.3	4.2 ± 0.1	4.3 ± 0.1	4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	None
Chloride (mEq/L)	102 ± 1	99 ± 1	97 ± 1	106 ± 8	100 ± 1	97 ± 1	98 ± 1	None
Other								
Creatine Phosphokinase	537 ± 225	422 ± 40	502 ± 56	448 ± 54	364 ± 54	410 ± 72	645 ± 321	None

Lean control; Placebo = MetS + Placebo treatment; AC3174 = MetS + AC3174 treatment.

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