SUPPLEMENTAL MATERIAL

Methods

Metabolic determinations (S1, S2)

The plasma glucose levels were measured using YSI Glucose Analyzer (YSI, Pittsburgh, PA). The measurements of non-esterified fatty acids (NEFA) in the plasma were carried out using the NEFA C test kit purchased from Wako Diagnostics (Richmond, VA). The measurements of plasma insulin and glucagon levels were carried out using specific RIA kits from Linco Research, Inc. (St. Charles, MO). The measurements of total plasma NO levels were carried out using a kit purchased from R&D Systems, Inc. (Minneapolis, MN). Blood was drawn from the coronary sinus and aorta simultaneously and collected in tubes containing EDTA as an anticoagulant. Since most of the NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), the concentrations of these anions are used as a quantitative measurement of NO production. After the enzymatic conversion of nitrate to nitrite by nitrate reductase, the spectrophotometric measurement at 540 nm of NO_2^- is accomplished by using the Greiss Reaction (**S2**). A standard curve of known nitrite levels was used and total NO values of experimental samples were determined using a linear curve-fit analysis.

GLP-1 preparation (S3)

Synthetic GLP-1 was mixed in 2.8 ml of normal saline and 0.2 ml of fresh plasma prepared from each animal. GLP-1 (7-36) amide was synthesized in the protein/peptide core facility of the Endocrine Unit of the Massachusetts General Hospital. The peptide content was 99% pure and gave a single peak on high performance liquid chromatography. The peptide was lyophilized in vials under sterile conditions for single

use and was certified to be both pyrogen-free and sterile. Net peptide content was used for all calculations. The peptide used in this protocol was from a single lot.

Hyperinsulinemic-Euglycemic Clamps (S1, S3)

Myocardial insulin sensitivity was assessed in the a) baseline state (n=18), b) following the development of DCM (day 28, n=9), and c) after a 48-hour infusion of GLP-1 (day 30, n=9) or vehicle (day 30, n=6) using the hyperinsulinemic-euglycemic clamp technique (12-14). In the fasting state, a primed constant infusion of insulin (480 $pmol \bullet m^{-2} \bullet min^{-1}$) was administered for 120 minutes to create a steady state concentration of plasma insulin (~1100 pmol/L). Arterial glucose concentrations were measured every 5 minutes and glucose was infused to maintain plasma glucose concentrations at 5 mmol/L ±10%. Myocardial glucose balance and CBF were sampled every 15 minutes to determine myocardial glucose uptake.

References

S1. Nikolaidis LA, Stuzu A, Stolarrki C, Alahi D, Shen YT and Shannon RP. The development of myocardial insulin resistance in conscious dogs with advanced dilated c!rdiomyopathy. Cardiovasc. Res. 2004; 61:297-306.

S2. Miles AM, Wink DA, Cook JC, Grisham MB. Determination of nitric oxide using fluorescence spectroscopy. Methods in Enzymology. 1996; 268:105-20.

S3. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, Stolarski C, Shen YT, Shannon RP. Recombinant GLP-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy.

Supplemental Figure Legend

Supplemental Figure 1:

Expression of GLUT-1 in whole cell homogenates, cytosolic fractions, Intracellular vesicles and purified sarcolemmal membrane preparations from central (n=6), dogs with dilated cardiomyopathy (n=6), dogs with DCM treated with vehicle for 3 days (n=4) and dogs treated with DCM treated GLP-1 for 3 days.

Supplemental Figure 1