SUPPLEMENTAL MATERIAL

Supplemental Methods

Fractional shortening and Ca^{2+} transient measurements in isolated cardiomyocytes

Explanted hearts were placed in ice-cold Ca^{2+} free Krebs-Henseleit buffer (KHB) containing (in mmol/L) 12.5 glucose, 5.4 KCl, 1 lactic acid, 1.2 $MgSO_4$, 130 NaCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 2 Na-pyruvate (pH 7.4 with NaOH). A small catheter was placed into the lumen of the LAD coronary artery that supplied the free-wall region of the left ventricle. The perfused myocardial region was cut out from the heart and rinsed for 15 minutes with non-recirculating KHB with 10 mM taurine and the perfusion maintained for 30 -45 minutes with recirculating KHB containing 180 U/mL collagenase (Worthington, type 2), 20mM 2,3 butanedione monoxime (BDM), 20mM taurine, and 0.05mM $CaCl_2$. The softened myocardial tissue was then removed from the cannula and minced to dissociate myocytes from the mid-myocardial layer. The resulting cell suspension was filtered and the myocytes were allowed to settle by gravity. The supernatant was removed and the cells were resuspended in KHB containing 1% weight/volume bovine serum albumin (BSA), 10 mM taurine, and 0.25 mM $CaCl_2$ and then oxygenated for 2-3 hours with 95% O_2 + 5% CO_2. The temperature of all solutions was kept at 37°C throughout the isolation procedure and all solutions were equilibriated with 95% O_2 and 5% CO_2. All experiments were conducted within 3 hours from cell isolation.

Fractional shortening and Ca^{2+} transients were measured as previously described (29-30). Myocytes were loaded with 5-10 µM Fluo-4 AM (Molecular Probes) and placed in a heated chamber (35°C) on the stage of an inverted microscope and perfused with a normal

physiological Tyrode's solution containing (in mM): 150 NaCl, 5.4 KCl, 1.2 $MgCl_2$, 10 glucose, 2 Na-pyruvate, 1 $CaCl_2$ and 5 HEPES, pH 7.4. They were paced at 0.2 Hz and fractional shortening data was collected using edge detection (Crescent Electronics).

Real-time PCR

RNA Primer pairs were designed with Primer-3 software and all had an optimal annealing temperature at 61 °C. The housekeeping gene 18S rRNA was used as an internal standard for gene expression screening. RT-PCR primers were synthesized by Sigma-Aldrich (Milan, Italy).

Supplemental Figures

Figure 1

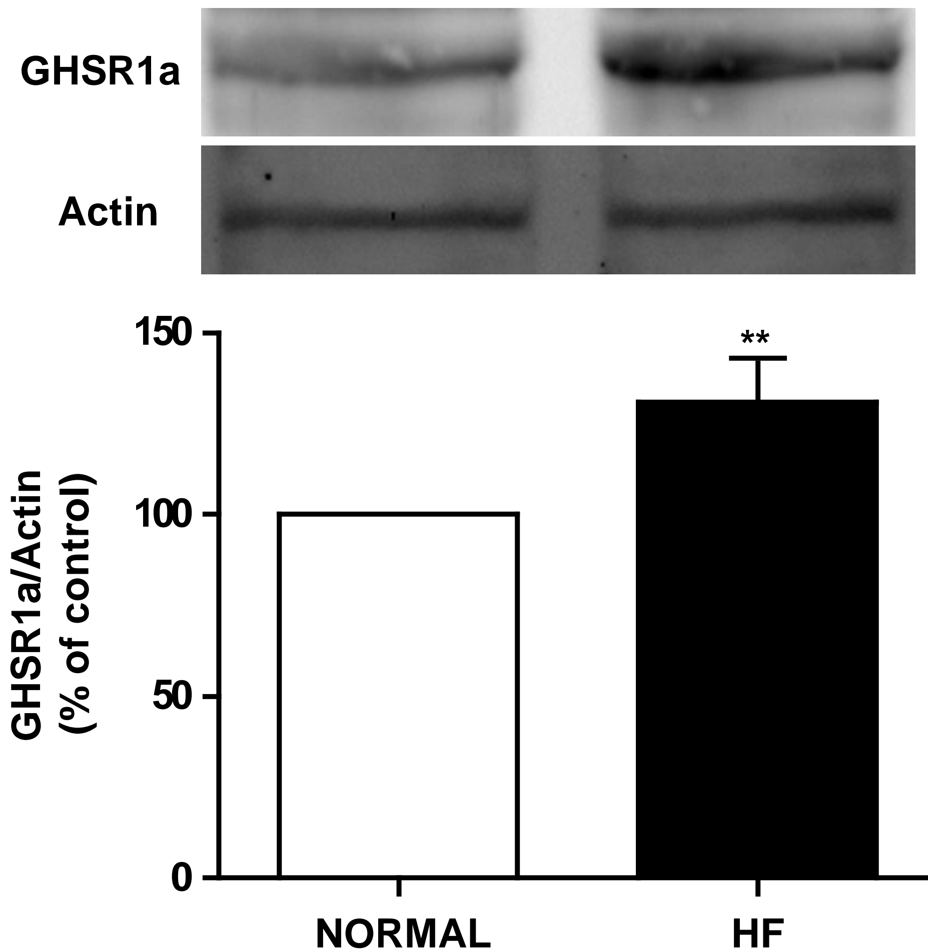

Figure Legends

Figure 1. Ghrelin receptor (GHSR-1a) protein expression determined by Western blot in normal and HF heart samples. The bar graph represents the densitometric quantification of GHSR-1a\ normalized to total proteins and reported as percent of control (n=3 per group, P<0.05 vs. normal).