SUPLEMENTAL MATERIALS, METHODS AND FIGURE LEGENDS

Western Blotting.

Non-infarcted areas of the LV from sham (n=5), saline (n=5), leptin (n=8) and MTII (n=8) treated rats were dissected and homogenized in lysis buffer (KPO_4, pH 7.4) and cleared by centrifugation (1,000 g, 5 min at 4ºC). After determination of supernatant protein concentration by the Bradford method (Bio-Rad, Hercules, CA), 50 μg of protein was separated in a 4-15% precast linear gradient polyacrymide gel. After transfer to nitrocellulose membrane, blots were rinsed in PBS and blocked in Odyssey blocking buffer (LI-COR, Linclon, NE) for 1 h at room temperature, and incubated with rabbit polyclonal anti-Phospho-AMPKα (p-AMPKα, Thr172) or AMPKα antibody (1:1,000; Cell Signaling, Danvers, MA), rabbit polyclonal anti-Phospho-MTOR (p-mTOR) or MTOR antibody (1:1,000; overnight at 4ºC). The membrane was probed for mouse anti-GAPDH (1:3,000; Abcam) as loading control. The membrane was then incubated with IR700-conjugated donkey anti-rabbit IgG and IR800-conjugated donkey and anti-rabbit antibodies (1:2,000, Rockland Immunologicals, Gilbertsville, PA). Antibody labeling was visualized using the Odyssey infrared scanner (LI-COR) for simultaneous detection of two probes. Fluorescence intensity analysis was performed using Odyssey software (LI-COR). p-AMPKα, AMPK, p-mTOR and mTOR levels were normalized to GAPDH after subtraction of background.

RT-PCR.

Hearts from saline (n=7), leptin (n=8) and MTII-treated rats (n=8) were quickly removed and cleaned on an ice-cold platform and healthy portions of the left ventricle away from the infarct region were used. Tissue samples were immediately frozen by immersion

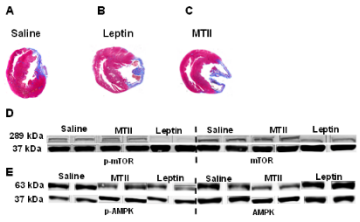
in liquid nitrogen and stored at -80ºC. Total RNA was extracted from approximately 50 mg tissue, cleaned and quantified. RNA (2.5 μg) was reverse transcribed by SuperScript VILO kit (Thermo Fisher Scientific, Waltham, MA) and treated with DNase for genomic DNA removal. qRT-PCR was performed using 4 μl cDNA with the StepOne Plus qRT-PCR system with PowerUP SYBR green master mix (Thermo Fisher Scientific). The following primer pairs were used: PPARα (peroxisome proliferator-activated receptor α) : 5'-CCTGTGAACTGACATTTGTC-3' (forward) and 5'-TCATCTGCTTCAAGTGGG -3' (reverse), PGC1α (PPARγ coactivator 1 α): 5'-ACCAAACCCACAGACAAC-3' (forward) and 5'-GGGTCAGAGGAAGAGATAAAG-3' (reverse), CPT1b: (carnitine palmitoyltransferase 1b) 5'-CAAAGCAGTACCCCAATC-3' (forward) and 5'-GCGATCATGTAGGAAACCC-3' (reverse), CD36 (fatty acid translocase): 5'-GGGAAAGTTATTGCGACATG-3' (forward) and 5'-CAGATCCAAACACAGCATAG-3' (reverse), FATP1 (fatty acid transport protein 1): 5'-GGACCCTAACTCAATGTACC -3' (forward) and 5'-TCTGGATCTTGAAGGTGC-3' (reverse); HIF1α (hypoxia-inducible factor 1α): 5'-CGATCATATCACTGGACTTCG -3' (forward) and 5'-TTCAGAGGCAGGTAATGG -3' (reverse); GLUT4 (glucose transporter 4): 5'-GTAACTTCATCGTTGGCATG-3' (forward) and 5'-GGTTTCAGGCACTCTTAGG-3' (reverse); VEGFα (vascular endotelial growth factor α): 5'-ACGTCACTATGCAGATCATG-3' (forward) and 5'-CCTTTCCCTTTCCTCGAAC-3' (reverse); IL1β (interleukin 1β): 5'-CACCTTCTTTTCCTTCATCTTTG-3' (forward) and 5'-GTCGTTGCTTGTCTCTCCTTGTA-3' (reverse); PDK4 (pyruvate dehydrogenase kinase 4): 5'-CCCGCTGTCCATGAAGCAGC-3' (forward) and 5'-

CCAATGTGGCTTGGGTTTCC-3' (reverse); SERCA2 (sarco/endoplasmic reticulum Ca^{+2}-ATPase 2): 5'-GTGAAATGCCATCAGTATGACGG-3' (forward) and 5'-GTGAGAGCAGTCTCTGTGGCTT-3' (reverse); and TNF-α (tumor necrosis factor α): 5'-ACTGAACTTCGGGGTGATTG -3' (forward) and 5'-GCTTGGTGGTTTGCTACGAC-3' (reverse). Rat 18S rRNA was used as an internal control. Samples were incubated at 50°C for 2 min, then at 95°C for 2 min before cycling 40 times at 95/60/72°C for 15/30/60 sec. A melting curve was conducted to ensure a single product was amplified.

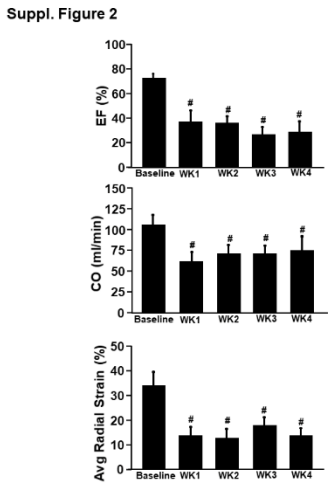
Supplemental Figures

Supplemental Figure S1 - Representative hearts from vehicle, leptin or MTII infused rats showing similar infarct size in all groups, and western blot analyses for mTOR and AMPK. Heart sections from (**A)** Vehicle, (**B**) leptin, and (**C**) MTII-infused rats. (**D**) Representative western blot analyses for p-mTOR and mTOR and (**E**) p-AMPK and AMPK performed using heart samples vehicle, leptin or MTII-treated rats.

Suppl. Figure 1

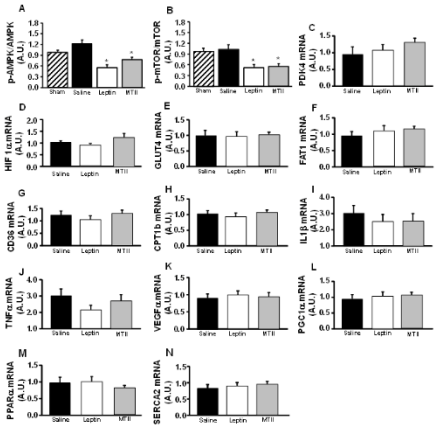


Supplemental Figure S2 – Reduction in food intake does not attenuate cardiac dysfunction after MI. (**A**) Ejection fraction (EF), (**B**) cardiac output (CO) and (**C**) average radial strain in pair-fed rats (n=5).



Supplemental Figure S3 - Chronic ICV leptin or MTII infusion for 4 weeks in rats with MI does not alter transcription of genes involved in cardiac tissue transcription regulation, remodeling, inflammation and neovascularization, while reducing p-AMPK and p-mTOR levels. (**A**) p-AMPK/APMK ratio, (**B**) p-mTOR/mTOR ratio, (**C**) PDK4 mRNA, (**D**) HIF-1α mRNA, (**E**) GLUT4 mRNA, (**F**) FAT1 mRNA, (**G**) CD36 mRNA, (**H**) CPT1b mRNA, (**I**) IL 1β mRNA, (**J**) TNFα mRNA, (**K**) VEGFα mRNA, (**L**) PGC1α mRNA, (**M**) PPARα mRNA,, and (**N**) SERCA2 mRNA levels in Sprague-Dawley rats with MI and infused ICV with vehicle (n=6), leptin (n=8) or MTII (n=8) for 4 weeks post-MI, and in sham control rats (n=5). *p<0.05 compared to vehicle-treated group.

Suppl. Figure 3



Supplemental Table S1. Cardiac function parameters in perfused hearts from ICV leptin-treated, ICV MTII-treated and ICV vehicle-treated rats 14 days after MI.

	Cardiac Output (mL/min/gdw)	**Coronary Blood Flow (mL/min/gdw)**	**Cardiac Power (mW/gdw)**	**O_2 Consumption (μmol/min/gdw)**
Vehicle	122.3±1.4	56.6±0.4	26.7±.0.2	41.8±0.1
Leptin	120.7±2.4	61.3±1.5	26.6±0.3	46.3±0.6*
MTII	171.0±3.8*#	60.6±1.1	34.1±0.6*#	46.6±0.2*

Values represent mean ± SEM obtained on day 14 of chronic ICV infusion. *$p<0.05$ compared to vehicle; # $p<0.05$ compared to ICV leptin treatment; (n = 5-6 per group). gdw, gram of dry heart weight.