Online Data Supplement

Supplemental Methods

RNA extraction and quantitative RT-PCR

Primer and probe sequences for these assays are presented in Data Supplement

Table I. Standard RNA was made for all assays by the T7 polymerase method

(Ambion, Austin, TX) with the use of total RNA isolated from mouse hearts. The

correlation between the C_t (the number of PCR cycles required for the fluorescent

signal to reach a detection threshold) and the amount of standard was linear over at

least a five-log range of RNA for all assays. β-actin was used as control.

Immunoblotting

Western blotting was performed using cardiac tissue lysates. 40 μg of total protein

extracts were resolved by SDS-PAGE and transferred to polyvinylidene fluoride

membranes. Overnight incubation (4°C) were performed with the primary antibodies

against acetyl-CoA carboxylase (ACC), phosphorylated ERK(p-ERK),

phosphorylated JNK(p-JNK), phosphorylated p38 MAPK(p-p38 MAPK), ERK, JNK,

p38 MAPK, β-Actin (Cell Signaling Technology), phosphorlamban (PLN, Upstate

USA Inc), phosphorylated phospholamban(p-PLN, Upstate USA Inc), sodium-

calcium exchanger protein 1 (NCX1, Abcam), and sarcoplasmic reticulum calcium

ATPase (SERCA 2a, kindly provided by Federica del Monte, Harvard Medical

School). Protein bands were visualized by chemiluminescent signal using ECL (Cell

Signaling Technology) and quantified by densitometry using ImageQuant 5.0

(Molecular Dynamics, Sunnyvale, CA).

Supplemental Table I

Primer and probe sequences used in real time quantitative RT-PCR

Gene	Primer/Probe	Sequence
ACC α	Forward	5′-GGCCAGTGCTATGCTGAGAT-3′
	Reverse	5′-AGGGTCAAGTGCTGCTCCA-3′
	Probe	5′-FAM-CGTTTGACATAATGGATGCAGCCAGATT-TAMRA-3′
ACC β	Forward	5′-ACTTTGACCTGACCGCTGTG-3′
	Reverse	5′-CTGAGTGCCGGATAATGGC-3′
	Probe	5′-FAM-TGCATCTTTACCTGGGAGCCGCTAAG-TAMRA-3′
CD36	Forward	5′-ATTGCGACATGATTAATGGCA-3′
	Reverse	5′-GATGGACCTGCAAATGTCAGA-3′
	Probe	5′-FAM-AGATGCAGCCTCCTTTCCACCTTTTG-TAMRA-3′
CPT1	Forward	5′-ATCATGTATCGCCGCAAACT-3′
	Reverse	5′-GGGATGCGTGTAGTGTTGAAC-3′
	Probe	5′-FAM-TCAAGCCGGTCATGGCACTGGG-TAMRA-3′
LCAD	Forward	5′-GGAGTAAGAACGAACGCCAA-3′
	Reverse	5′-GCCACGACGATCACGAGAT-3′
	Probe	5′-FAM-CCATTAGTGATGAACACCTTGCTTCCATTGA-TAMRA-3′
MCAD	Forward	5′-TGGCATATGGGTGTACAGGG-3′
	Reverse	5′-CCAAATACTTCTTCTTCTGTTGATCA-3′
	Probe	5′-FAM-AGGCATTTGCCCCAAAGAATTTGCTTC-TAMRA-3′
Mte1	Forward	5′-TGGGAACACCATCTCCTACAA-3′
	Reverse	5′-CCACGACATCCAAGAGACCA-3′
	Probe	5′-FAM-AGACTATACCCCCTGTGTCCCTTGTGAGAA-TAMRA-3′
PDK4	Forward	5′-TTCACACCTTCACCACATGC-3′
	Reverse	5′-AAAGGGCGGTTTTCTTGATG-3′
	Probe	5′-FAM-CGTGGCCCTCATGGCATTCTTG-TAMRA-3′
PPAR α	Forward	5′-CAAGGCCTCAGGGTACCACT-3′
	Reverse	5′-TTGCAGCTCCGATCACACTT-3′
	Probe	5′-FAM-AGGCTGTAAGGGCTTCTTTCGGC-TAMRA-3′
PPAR δ	Forward	5′- AGAGGAAGTGGCCATGGGT-3′
	Reverse	5′-CATCACAGCCCATCTGCAG-3′
	Probe	5′-FAM-CTGGGAGAGGTCTGCACAGCTGCT-TAMRA-3′
RXR α	Forward	5′-ACAGGAACAGCGCTCACAGT-3′
	Reverse	5′-CACGCATCTTCGACACCAG-3′
	Probe	5′-FAM-CGTTAGCACCCTGTCAAAGATGGCG-TAMRA-3′
SCOT	Forward	5′-AAGCCATCACGGGAGATTTT-3′
	Reverse	5′-CCACGGTAGTTCCTGCAGC-3′
	Probe	5′-FAM-CACATTGGCAAATTGAAGTTTCTTGCACTTTT-TAMRA-3′
UCP3	Forward	5′-TGCTGAGATGGTGACCTACGA-3′
	Reverse	5′-CCAAAGGCAGAGACAAAGTGA-3′
	Probe	5′-FAM-AAGTTGTCAGTAAACAGGTGAGACTCCAGCAA-TAMRA-3′

Supplemental Table II

General Characteristics of the Mice

	LFWT (n=7)	HFWT (n=9)	LFTG (n=9)	HFTG (n=8)
HW/BW, mg/g	4.07±0.15	3.85±0.26	4.29±0.15	3.96±0.08
HW/TL, mg/mm	7.53±0.54	7.95±0.26	8.67±0.60	8.73±0.45
Lung, wet/dry	4.04±0.12	4.07±0.08	4.11±0.07	4.13±0.07
Liver, wet/dry	3.00±0.04	2.92±0.07	2.91±0.03	2.92±0.04

Values are expressed as mean ± SEM. HW/BW, heart weight normalized to body weight; HW/TL, heart weight normalized to tibia length.

Supplemental figures

Figure I
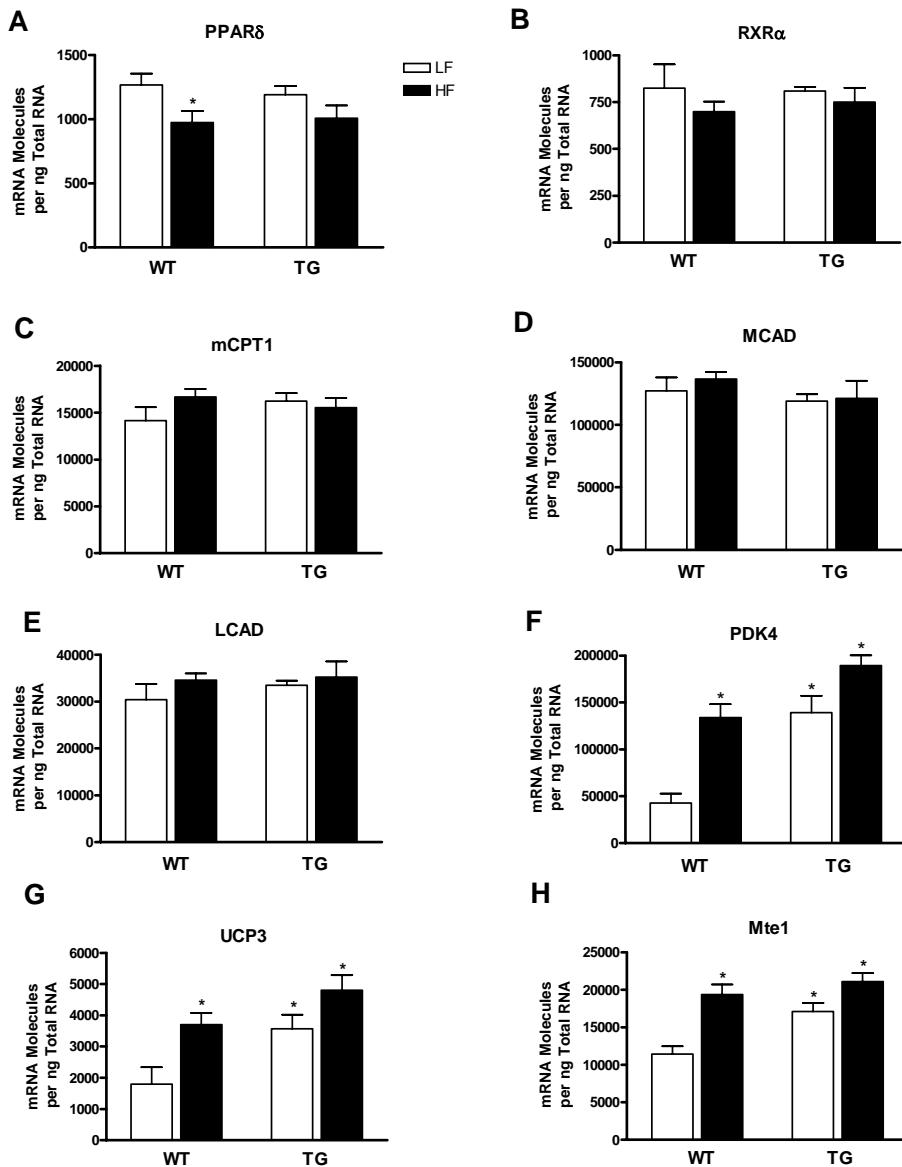
Figure I. Expression of PPAR δ, RXRα and PPAR α target genes determined by real-time PCR. (A and B) Expression of PPAR δ and RXRα, (C-H) Expression of mCPT1, MCAD, LCAD, PDK4, UCP3 and Mte1 in the hearts of WT and TG mice fed a HF or LF diet for 20 weeks. Data are mean ± SEM (n=6 per group). *p < 0.05 vs LFWT.

Figure II
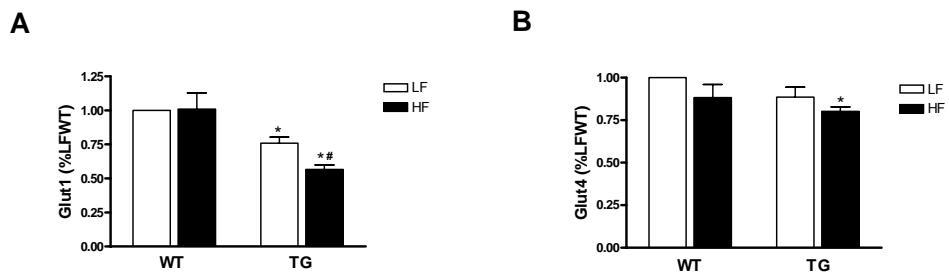
Figure II. mRNA expression levels of Glut1and Glut4 in the hearts of WT and TG mice fed a HF or LF diet for 20 weeks. Data are mean ± SEM (n=3 per group). *p < 0.05 vs LFWT; [#]p < 0.05 vs LFTG.

Figure III
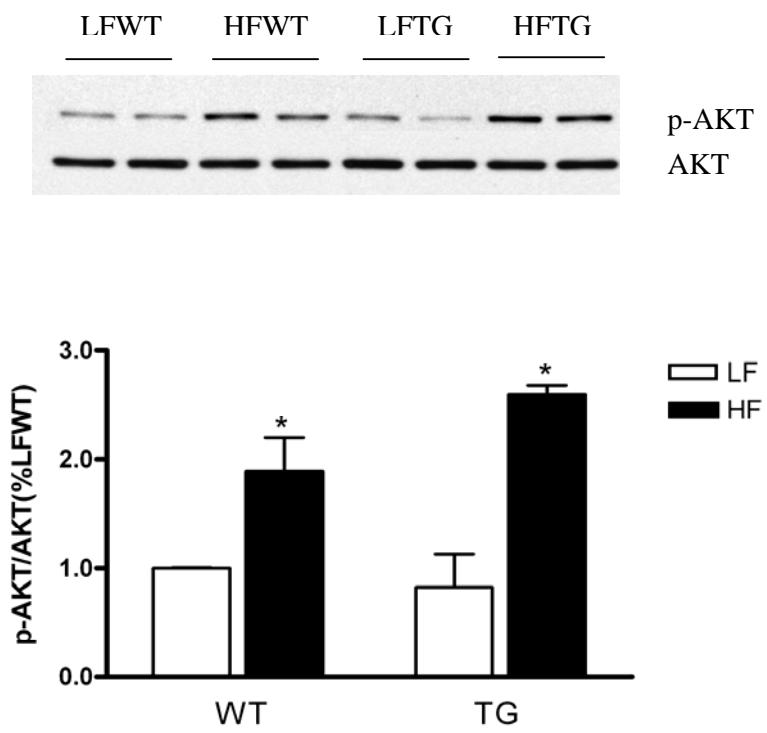
Figure III. Protein levels of phosphorylated AKT and total AKT in the hearts of WT and TG fed a HF or LF diet for 20 weeks. Data are expressed as mean ± SEM from 4-5 hearts for each group. *p < 0.05 vs LFWT.