Supplemental Data

METHODS

Materials

Ad-GFP-DN-SREBP-1 and Ad-GFP-SREBP-1c (ADD-1) were gifts from Dr. Bruce Spiegelman, Dana Farber Cancer Institute and Harvard Medical School, Boston. Ad-GFP-βgal was a kind gift from Dr. Anthony Rosenzweig, Beth Israel Deaconess Hospital and Harvard Medical School, Boston. The heterozygous male Akita *Ins2*$^{\mathbf{Cys96Tyr}}$ mice and the littermate wild type mice were from the Jackson Laboratories. The heterozygous Akita Ins2$^{\mathbf{Cys96Tyr}}$ mice were selected by restriction digestion analysis of a PCR product of the *Ins2* gene using genomic DNA of Akita mice as described.[1] To monitor the progression of disease, measurements of urine glucose, protein and ketones were made with Keto-Diastix Reagent Strips for Urinalysis (Bayer). Serum glucose was determined by the hexokinase method using a Hitachi 747; hemoglobin-A1c by HPLC; blood gases and electrolytes measured in the clinical lab. Osmolarity was measured by freezing point depression (Idexx Laboratories, Grafton, MA). Phloridzin (0.4 g/kg, subcutaneously 3 times a day) was from Sigma-Aldrich. All vertebrate animal-related procedures described here were approved by the Institutional Animal Care Committee at Tufts Medical Center.

Cell culture

Embryonic chick atrial myocytes were cultured as described.[2] For electrophysiological studies dissociated atrial myocytes from mouse atria were prepared by a retrograde Langendorf perfusion method as described.[3]

ECG monitoring in conscious mice

An implantable wireless radiofrequency transmitter was inserted and the ECG signal was recorded with the use of a telemetry receiver and an analog to digital acquisition system (Data Sciences International) as described.[4] In one set of experiments, age-matched male WT and Akita mice (DM), 4-6 months old were studied, and in a second set of experiments, age-matched male Akita mice before and 1 week after implantation of slow-release insulin pellets (LinShin, Inc, Canada, 2U of insulin/day) were studied. Treatment regimens for propranolol and carbamylcholine are outlined in the text. The recording was analyzed using custom built software: Beat-to-beat heart rate data were obtained from the ECG signal by R-wave peak detection. Artifacts and non-sinus rhythms were detected and removed after manual review. Heart rate measurements for statistical analysis were obtained from moving average beat data. Baseline heart rate immediately prior to carbamylcholine injection and the lowest heart rate following carbamylcholine injection were used to compute the heart rate response and the 80% recovery point. The elapsed time from the carbamylcholine induced bradycardia until the initiation of recovery was defined as the plateau time of bradycardia. The recovery phase was characterized by the time elapsed until achieving 80% recovery of the baseline heart rate.

Western blot analysis

To determine GIRK1 and SREBP-1 levels, Western blot analysis has been done as described previously.[2, 3] A GIRK1 specific antibody from Alomone Labs (Jerusalem, Israel) and SREBP-1 antibodies from Santa Cruz were used. A rabbit polyclonal Gαs and a rabbit polyclonal Gβ antibodies were from Santa Cruz.

Cellular Electrophysiology

Membrane currents were measured by the patch-clamp technique in whole-cell mode using an LM-EPC7 amplifier as described.[3] Briefly, immediately prior to seal formation, the

extracellular solution was replaced with a high K^+ solution without Ca^{2+} to suppress contraction: 100 mM NaCl, 50 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 5.5 mM D-glucose, adjusted to pH 7.4. Patch electrodes were pulled from 1.5 mm diameter Fischer brand glass capillaries and demonstrated a 2 MΩ resistance when filled with pipette solution: 140 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM EGTA, 5 mM Mg_2ATP, 0.1 mM GTP, and pH adjusted to 7.2 with KOH. In order to obtain and maintain good seal formation required for membrane current recording, we found it necessary to suppress contractions with high external K^+, which leads to persistent membrane depolarization and inactivation of voltage-activated Na^+ channels, and 0 external $Ca2^+$. Both of these conditions have been shown to have no effect I_{KACh}.[5] It was necessary to suppress contraction, because of difficulty in maintaining seals on spontaneously beating cells. Data were acquired by an Axon Instrument Digitizer (Digidata 1322B) with pClamp 9 software. Whole cell currents were elicited at room temperature in the presence and absence of 20 μM carbamylcholine introduced by focal perfusion over 10-15 seconds followed by washout as described.[3, 6] Currents returned to baseline within 10-15 seconds of washout. In order to correct for differences in cell size currents were normalized to the cell capacitance determined via capacitance compensation and data presented as current density in pA/pF. Current-voltage (I-V) plots were constructed from a series of data points obtained from the carbamylcholine current responses at given voltages.

Echocardiography

Echocardiographic studies were performed as previously described.[7] Briefly, a commercially available echocardiography system (Sonos 7500, Phillips Medical Systems) was utilized with a dynamically focused linear array transducer (15-6L Intraoperative Linear Array, Phillips Medical Systems) using a depth setting of 0.5–1.0 cm. Anesthesia was induced with inhaled 2.5%

isoflurane in oxygen and maintained with inhaled 2.0% isoflurane in oxygen. Animals were placed on a warming pad to maintain body temperature at 36.5 to 37.5^{o}C. Two-dimensional images and M-mode tracings (sweep speed 50–100 mm/s) were then recorded from the short-axis view at the papillary muscle level. Using M-mode tracings, LV end-diastolic diameter (EDD) and end-systolic diameter (ESD) were measured to the nearest 0.1 mm, averaging three cardiac cycles. Fractional shortening (FS) was calculated using the standard equation: FS (%) = (EDD – ESD)/EDD x 100. Ejection fraction (EF) was calculated using the standard equation: EF (%) = (LVEDV - LVESV)*100/ LVEDV; LVEDV (LV end diastolic volume) = (7 * LVEDd3)/(2.4 + LVEDd); LVESV (LV end systolic volume) = (7 * LVESd3)/(2.4 + LVESd); LVEDd, LV end diastolic diameter; LVESd, LV end systolic diameter.

Blood gases

For blood gases mice were anesthetized with 2.5% isoflurane in oxygen, intubated and body temperature maintained using a rectal probe and a feedback heating pad. The heart was exposed via a midline sternotomy and blood obtained via a percutaneous intraluminal left ventricular stick using a 25 gauge needle and a 1cc heparinized syringe.

Statistical analysis

All values are expressed as mean ±SEM. Statistical differences between mean values were calculated by ANOVA, followed by Bonferroni's test for unpaired comparisons where appropriate. For comparison of WT and DM mice, Student's t-test was applied. The effect of insulin treatment on DM mice was assessed using a paired t-test. A 2-tailed p value ≤0.05 was considered significant.

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Online Table S1. Blood parameters of WT and Akita mice

	Akita DM	WT
[1]**Blood glucose**	585 ± 18	149 ± 8
[2]**Hemoglobin A1c**	9.41 ± 0.4	4.37 ± 0.09
[3]**pH**	7.35 ± 0.03	7.37 ± 0.03
[3]**pCO_2**	38.93 ± 3.7	35.98 ± 2.5
[3]**HCO_3^-**	19.45 ± 0.7	19.95 ± 1.0
[3]**Na^+**	144 ± 1.5	144 ± 1.8
[3]**K^+**	4.1 ± 0.09	4.4 ± 0.16
[3]**Cl^-**	111 ± 1.1	111 ± 0.7
[4]**Anion Gap**	17.5 ± 1.5	16.3 ± 1.5

1. Blood glucose (mg/dL), 33 Akita DM and 27 WT ($P < 0.001$); 2. Hemoglobin A1c (%), 9 Akita DM and 7 WT ($P < 0.001$); 3. Blood gases (mm Hg) and electrolytes (mmol/L), *ns*.; 4. Anion gap (mmol/L) = ($[Na^+] + [K^+]$) - ($[Cl^-] + [HCO3^-]$), *ns*. Both WT and Akita mice did demonstrate a mild metabolic acidosis, which was probably secondary to the effects of general anesthesia

Online Table S2. Echocardiographic analysis of LV structure and function of WT and Akita mice

	Akita DM	WT
EDD	2.78±0.08	2.92±0.04
ESD	1.46±0.07	1.52±0.06
Post wall	1.18±0.04	1.21±0.03
Ant wall	1.17±0.03	1.13±0.03
HR	400±10	414±6
FS	47.3±2.36	48.0±1.45
EF	79.8±2.24	80.6±1.47

Akita DM (n=9) and WT mice (n=10). EDD, end-diastolic dimension (mm); ESD, end-systolic dimension (mm); Post wall, posterior wall thickness (mm); Ant wall, anterior wall thickness (mm); HR, heart rate (beats/min); FS, fractional shortening (%); EF, ejection fraction (%). No statistically significant difference was found in these parameters.

Online Figure S1. Streptozotocin treatment decreases SREBP-1 levels in the heart.

Twelve to sixteen weeks old male C57BL/6 mice were treated intraperitoneally (i.p.) with a single dose of streptozotocin (STZ) in citrate buffer (pH4.5), 180 mg/kg body weight, as described previously.[8] Control mice were injected with an equal volume of citrate buffer. A subset of STZ treated mice received insulin (Regular Iletin II porcine insulin, Eli Lilly). Blood glucose in control mice, STZ treated mice and STZ+insulin treated mice were 130±4 mg/dL (n=12), 457±12 mg/dL (n=10), 140±14 mg/dL (n=11), respectively. Expression of SREBP-1 from atria of control, STZ and STZ+insulin treated mice were determined by Western blot analysis as described in Methods. These data are typical of 3 similar experiments.

Online Supplemental data Fig. S1