Supplemental Material

Materials and Methods

The db/db mice model has been proven to be a suitable model to study the consequences of diabetes on the structure, energy metabolism and subcellular Ca\(^{2+}\) cycling of the heart. Here we studied the male diabetic (BKS.Cg-m +/+ Lepdb/Bom Tac) (20 exercised and 20 sedentary mice) and the sedentary (n=23) and exercise trained (n=6) non-diabetic healthy heterozygote (BKS.Cg-m +/+ Lepdb/+ lean); all age-matched (7 weeks at study start).

Exercise training and maximal oxygen uptake (\(V_{O2\text{max}}\))

To determine \(V_{O2\text{max}}\), mice ran until exhaustion on a customized treadmill in a metabolic chamber, and high intensity aerobic interval training was performed as uphill running, alternating between 4 min at 85%-90% of \(V_{O2\text{max}}\) and 2 min at 50% of \(V_{O2\text{max}}\) for 80 min/day, 5 days/week, for 13 weeks. We and others have previously demonstrated the efficacy and relevance of this exercise regime by both clinical trials and experimental studies (e.g.).

Cardiomyocyte isolation and Ca\(^{2+}\) measurements

Left ventricular myocytes were isolated as previously described. The Norwegian council for Animal Research approved the study, which was in accordance with Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996).

Cardiomyocyte shortening and Ca\(^{2+}\)-handling

Fura-2/AM-loaded (2 µM, Molecular Probes, Eugene, OR) cardiomyocytes were stimulated by bipolar electrical pulses for Ca\(^{2+}\) handling measurements including SR Ca\(^{2+}\) leak. CaMKII inhibitor and PKA inhibitor were used, with increasing frequencies (1-3 Hz) (HEPES-based solution 1.8 mM Ca\(^{2+}\), 37\(^\circ\)C). Contractility was recorded by video-based sarcomere spacing. We adapted an established protocol to determine diastolic SR Ca\(^{2+}\) leak. H-89 (3µM) to inhibit the effect of PKA or the membrane permeable autocalytide-2 related inhibitory peptide AIP (1 µM) to inhibit the effect of CaMKIIδ were added. Additional non-specific effects of the inhibitors were minimized by using lowest possible concentrations. Rate constants of Ca\(^{2+}\) -decline under three different conditions were used to quantify the contribution from: (i) SR Ca\(^{2+}\) ATPase (SERCA2a); (ii) NCX and (iii) mitochondrial uniporter and sarcolemmal Ca\(^{2+}\) ATPase as previously described.

Confocal imaging of Ca\(^{2+}\) waves, Ca\(^{2+}\) release synchrony and T-tubules

Cardiomyocytes loaded with Fluo-3/AM (10 µM, Molecular Probes) were used to count Ca2+ waves and determine Ca2+ release synchrony as previously described. Quiescent, non-perfused cardiomyocytes loaded with the membrane specific Di-8-ANEPPS dye (10 µM, Molecular Probes) were confocal Z-stack scanned. The relative density of T-tubules normalized to cell size was obtained from 5 images/cell captured from the middle of each cell. Images were analyzed with custom-made applications in IDL 6.0 (ITT Visual, Boulder, CO, USA), by counting pixels stained with the dye relative to the total number of pixels after removing pixels associated with the non-T-tubular sarcolemma.
Echocardiography
High-resolution echocardiography (Vevo 770, VisualSonics, Toronto, Canada), using a single-element mechanical transducer with a center frequency of 30 MHz, was performed on self-breathing mice under anesthesia (2% isoflurane and 98% oxygen).

Western blot analyses
100 µg of total lysate were loaded onto 4-12% Tris-Glycine precasted Novex Gel (Invitrogen, Carlsbad, CA) for protein detection. Proteins were transferred onto PVDF (BioRad, Hercules, CA) and membranes were blocked with PBS-T/milk for 1 hour at room temperature followed by overnight incubation with antibodies: total PLN and phospho-Thr-17-PLN antibodies (Badrilla, Leeds, UK), phospho-Ser-16-PLN antibody (Upstate, Charlottesville, VA), phospho-Thr-287-CaMKII, RyR2 (Affinity Bioreagents, Golden, CO), CaMKIIδ (Santa Cruz Biotechnology, California, USA), SERCA2a (ABR, Rockford, IL) and P-RyR2 S2808 and S2814 were kindly given by Dr. Xander Wehrens. Horseradish peroxidase-conjugate secondary antibodies and enhanced chemiluminescence (ECL) (Thermo Fisher Scientific Inc, Rockford, IL) were used for protein detection with GBOX/Chemi-HR16E (Synoptics, Cambridge, UK). All protein levels were normalized to total tubulin (Novus Biologicals, MI, US), GAPDH (Cell Signaling) or total PLN, RyR2 and quantified using ImageJ software (NIH, Bethesda, Maryland).

Real-time quantitative RT-PCR
Fresh samples from perfused heart were immersed in RNAlater (Qiagen, Hilden, Germany) and stored at 4°C until RNA extraction. Total RNA was extracted according to the RNeasy Fibrous Tissue Protocol kit (Qiagen Nordic-Norway). RNA concentration was measured spectrophotometrically (NanoDrop, Witec, Switzerland), and stored at -80°C before use. cDNAs were obtained from 1 µg total RNA according to iScript cDNA Synthesis Kit (BioRad, Sundbyberg, Sweden). Real-time PCR (qPCR) was performed using a 1:4 dilution of the cDNA and the TaqMan Fast Universal PCR master mix (ABI PRISM 7900 HT Fast, Applied Biosystems, Foster City, CA). The primer/probe sequences for the genes studied were obtained from Eurogentec Ltd (Seraing, Belgium) or Roche Diagnostics GmbH (Mannheim, Germany). Primers and TaqMan probes (2 µl of cDNA) were used in a 20 µl final volume. A negative control without cDNA template was included in every assay. The PCR efficiency for all genes was determined by performing a dilution series of a pool of all samples. The expression of Peroxisome proliferator activated receptor γ co-activator 1α (PGC-1α) mRNAs was normalised to the geometric mean of three housekeeping genes: cyclo (cyclophilin), sdha (succinate dehydrogenase complex subunit A) and hprt (hypoxanthine-guanine phosphoribosyl transferase).

Free FAs, triglycerides, insulin and blood glucose
Blood markers of energy metabolism were measured using standard procedures at St. Olavs University Hospital, Trondheim, Norway.


