Cardiac-Specific Overexpression of GLUT1 Prevents the Development of Abnormal Ventricular Function in Diabetic Mice: an Investigation with MR Tagging and Spectroscopy

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Introduction

Diabetes mellitus is associated with increased cardiac morbidity and mortality. Cardiomyopathy occurs commonly in diabetics independent of known risk factors such as coronary artery disease or hypertension. Although there is currently no consensus as to the pathogenesis of diabetic cardiomyopathy, evidence is emerging that it may be related to derangements in myocardial energy metabolism. The heart metabolizes a variety of substrates to meet its energy requirements. Cardiac function is tightly linked to substrate uptake and utilization. GLUT1 is a major glucose transporter that mediates basal cardiac glucose uptake (1). Previously, it has been demonstrated that increasing myocardial glucose uptake through cardiac-specific GLUT1 overexpression (GLUT1-TG) protects against myocardial dysfunction in mice with chronic pressure overload (2). It also has been proposed that down regulation of GLUT1 expression partly contribute to myocardial dysfunction in diabetics (3). However, the functional significance of cardiac-specific overexpression of GLUT1 remains undefined in the diabetic heart.

In the current study, we aimed to test the hypothesis that normalized/enhanced glucose transport through GLUT1 overexpression will have the beneficial effects of increasing glycolysis and glucose oxidation in diabetic hearts. Such improvement will lead to normalized cardiac function in diabetic hearts. To test this hypothesis, we investigated cardiac functional changes in diabetic mouse hearts with MR tagging. Glucose uptake and utilization was examined with ^{13}C NMR spectroscopy.

Methods

Animal Model Type I diabetes was induced by streptozotocin (STZ, 50mg/kg body wt) injection in 4 month old GLUT1-TG mice (n=11) and their wildtype littermates (WT, n=11). Saline-injected, age-matched TG (n=11) and WT (n=11) mice were used as the controls. Blood glucose level was measured two weeks after STZ injection. Mice with a blood glucose level above 250 mg/dl were considered diabetic. The animals (n=44) had a mean age of 35.8±7.4 weeks and an STZ injection period of 16.5±2.7 weeks upon imaging.

MR Imaging and Image Analysis Animals (n=44) were scanned on a Bruker (Bruker Biospec, Germany) 7T scanner with a 2.5 cm volume coil. The body temperature was kept at 34.0±0.8°C during imaging. WT diabetic mice had a higher mean heart rate among the four groups (P<0.05). Tagged short-axis images were acquired with 1 mm slice thickness at base, mid-ventricle, and apex. ECG and respiratory gated tagged images (FLASH with SPAMM1331) were acquired with the following imaging parameters: TE, 2.5 ms; field of view, 4 cm × 4 cm; matrix size, 256×128. 15 frames were acquired per cardiac cycle. Cine images at same positions were acquired with the same parameters except a matrix size of 128×128.

Images were analyzed using a MATLAB-based Cardiovascular MR Image Analysis Tool (CVMRI). Epicardial and endocardial contours were traced interactively using B-spline interpolation with 8 control points on cine images. Intersecting tag points were tracked semi-automatically with HARP-based approach (4). Subsequently, circumferential and radial strains were calculated by 2D homogenous strain analysis.

^{13}C NMR spectroscopy *In vitro* high-resolution ^{13}C NMR spectra were acquired from perchloric acid extracts of isolated hearts perfused with ^{13}C-labeled substrates. The acquisition was performed on a 900MHz Bruker Spectrometer with 5 mm ^{13}C/^{1}H probe. Proton decoupled ^{13}C spectra were acquired with 1024 scans (90° pulse, 0.18s acquisition, 4s delay and 8000 data points). Two groups of perfusion experiments were performed to determine substrate utilization. In group 1, hearts were supplied with [U-^{13}C] palmitate, [3-^{13}C] lactate and unlabeled glucose and β-hydroxybutyrate, while in group 2, hearts were supplied with [U-^{13}C] glucose, [2,4-^{13}C] β-hydroxybutyrate, and unlabeled palmitate and lactate. These two complementary groups allowed the relative contribution of fatty acid, lactate, glucose, and ketone to oxidative metabolism to be determined from isotopmer analysis (5).

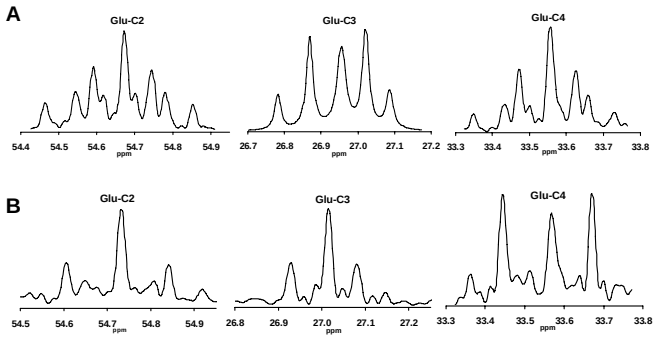

Figure 1. High-resolution ^{13}C NMR spectra of tissue extracts. Peak assignments: Glu-C2, 2-carbon of glutamate; Glu-C3, 3-carbon of glutamate; Glu-C4, 4-carbon of glutamate. ^{13}C-labeled substrates were A. [U-^{13}C] glucose and [2,4-^{13}C] β-hydroxybutyrate; B. [U-^{13}C] palmitate and [3-^{13}C] lactate.

Results

Figure 1 shows representative glutamate ^{13}C NMR spectra from group 1 and 2. Analysis of the multiplet structure of glutamate spectra revealed a 22% increase in fatty acid oxidation in WT diabetic mice. However, fatty acid oxidation in GLUT1-TG mice remained unchanged after STZ injection and was 28% lower than the WT diabetic mice.

The ejection fraction (EF) of WT diabetic mice decreased significantly when compared with controls (49.0±7.0% vs. 57.5±4.8%, P<0.05). However, EF of the GLUT1-TG mice with STZ injection showed no difference comparing with GLUT1-TG controls (51.3±8.3% vs. 51.5±5.2%, P=NS). Figure 2 shows peak systolic radial and circumferential strains at apex, mid-ventricle, and base. Decreased radial strain was observed in WT diabetic mice at apex and mid-ventricle comparing with WT controls (P<0.05), while no difference in radial strains was evident at all levels in GLUT1-TG groups (P=NS). WT diabetic mice also exhibited decreased circumferential strain at mid-ventricle and base (P<0.05). However, no changes in circumferential strain were observed in GLUT1-TG groups (P=NS).

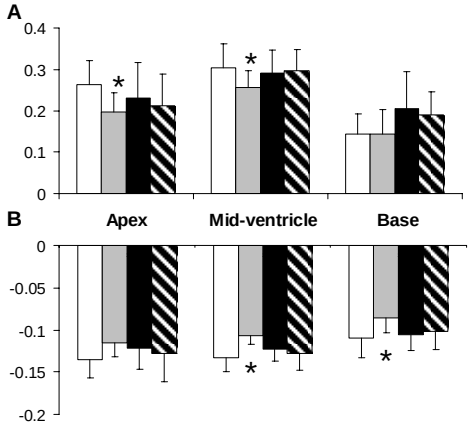

Figure 2. Radial (A) and circumferential (B) strains of WT control (open bars), STZ-treated WT (grey bars), GLUT1-TG (solid bars), and STZ-treated GLUT1-TG (hatched bars) at apex, mid-ventricle, and base. *: P<0.05 when compared with WT control.

Conclusion

In the current study, myocardial contractility was examined in STZ-treated WT and GLUT1-TG mice. Decreased EF, radial, and circumferential strains were observed in WT diabetic mice with a shift in substrate utilization towards enhanced fatty acid oxidation. However, diabetes-associated ventricular dysfunction was absent in STZ-treated GLUT1-TG mice showed with no alterations in fatty acid oxidation. Our results suggest that normalized/enhanced glucose transport can prevent functional deterioration in diabetic hearts.

References

1. Burcelin, R., et al. *Am. J. Physiol.* 265:E392–E401, 1993
2. Garvey,W.T., et al. *Am. J. Physiol.* 264:H837–H844,1993
3. Liao, R., et al. *Circulation.* 106: 2125-2131, 2002
4. Liu, W., et al. *Magn. Res. Med.* 52:1282-1290, 2004
5. Malloy C.R., et al. *Am J Physiol.* 259:H987–H995, 1990