Long-Term Effects of Increased Glucose Entry on Mouse Hearts During Normal Aging and Ischemic Stress

Ivan Luptak, MD, PhD*; Jie Yan, MD, PhD*; Lei Cui, MD; Mohit Jain, MD, PhD; Ronglih Liao, PhD; Rong Tian, MD, PhD

Background—A shift of substrate preference toward glucose in the heart is considered a reversion to fetal metabolic profile, but its role in the pathogenesis of cardiac diseases is incompletely understood.

Methods and Results—We performed a 2-year follow-up study in transgenic mice with sustained high glucose uptake and utilization in the heart by cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 (GLUT1-TG). Compared with wild-type litter mates, the GLUT1-TG mice showed a normal survival rate and unaltered contractile function of the heart monitored by serial echocardiography and by pressure–volume studies in isolated perfused hearts in the 2-year period. Furthermore, when hearts were subjected to ischemia-reperfusion, cardiac function of young and old GLUT1-TG recovered to the same level (86% and 83%, respectively) and exceeded that of both young and old wild-type hearts (52% and 35%, respectively; \( P < 0.05 \)). Nuclear magnetic resonance spectroscopic measurements with \(^{31}\)P showed delayed ATP depletion, reduced acidosis during ischemia, and improved recovery of high-energy phosphate content in old GLUT1-TG hearts (\( P < 0.05 \) versus old wild-type). During reperfusion, glucose oxidation was 3-fold higher and fatty acid oxidation was 45% lower in old GLUT1-TG hearts compared with old wild-type (\( P < 0.05 \)), which suggests that the deleterious effects of excessive fatty acid oxidation during reperfusion was prevented in old GLUT1-TG hearts.

Conclusions—We have demonstrated that a normal heart is able to adapt to long-term increases in basal glucose entry into cardiomyocytes without development of glucotoxicity. Furthermore, life-long increases in glucose uptake result in a favorable metabolic phenotype that affords protections against aging-associated increase of susceptibility to ischemic injury. (Circulation. 2007;116:901-909.)

Key Words: aging ■ glucose ■ ischemia ■ metabolism

The heart can utilize multiple classes of energy substrates such as carbohydrates (glucose and lactate), fat (free fatty acids and lipids), and ketones. Although fatty acids serve as the primary energy source in adult hearts, shifts in substrate preference have been observed in a variety of physiological and pathological conditions such as fasting, exercise, aging, diabetes mellitus, myocardial ischemia, and heart failure.1,2 For example, in cardiac hypertrophy and aging, fatty acid oxidation rate decreases in the heart, whereas the relative contribution of glucose increases.3–5 The functional significance of substrate switch under these conditions is poorly understood. We have previously shown that increased insulin-independent glucose transport and utilization in mouse hearts subjected to pressure overload blunted the progression to failure.6 These results suggest that increased reliance on glucose in the hypertrophied heart is adaptive and...
dial energetics and rendered the old hearts more resistant to ischemia-reperfusion injury. The present findings provide an important basis for our understanding of substrate metabolism in the heart as well as for pharmacological manipulation of energy metabolism in human diseases.

Methods

Animal Models

Transgenic mice that overexpress GLUT1 in the heart (GLUT1-TG) were generated on the FVB background as previously described.6 Age- and gender-matched wild-type (WT) litter mates were used as controls. All mice were kept on a 12-hour light/dark cycle with water and food ad libitum. All procedures related to the handling of the mice in the present study were approved by the Harvard Medical Area Standing Committee on Animals.

Transthoracic Echocardiography

Murine transthoracic echocardiography was conducted in conscious mice at 5- to 6-month intervals with an Acuson Sequoia C-256 echocardiograph machine (Siemens, Malvern, Pa) and a 15-MHz probe.6,12 Briefly, the heart was imaged in the 2-dimensional parasternal short-axis view and an M-mode echocardiogram of the mid-ventricle was recorded at the level of papillary muscles. All mice were imaged by a single operator. Heart rate, posterior and interventricular septum wall thicknesses, and the end-diastolic and end-systolic internal dimensions of the left ventricle (LV) were measured from the M-mode image. On the basis of our prior experience, these measurements were reproduced with an interreader variation <10%. LV wall thickness was assessed as the average of posterior wall and septum thickness. LV fractional shortening was defined as the end-diastolic diameter minus the end-systolic diameter normalized for the end-diastolic diameter; this was used as an index of cardiac contractile function.

Isolated Perfused Heart Experiments and 31P Nuclear Magnetic Resonance Spectroscopy

Mice were heparinized (100 U, intraperitoneally) and anesthetized by sodium pentobarbital (150 mg/kg, intraperitoneally). The heart was excised and perfused at a constant pressure of 80 mm Hg at 37°C as previously described.13 The perfusate contained the following (in mmol/L): NaCl (118), NaHCO3 (25), KCl (5.3), CaCl2 (2), MgSO4 (1.2), EDTA (0.5), glucose (5.5), mixed long-chain fatty acids (0.4, bound to 1% albumin), DL-β-hydroxybutyrate (0.38), lactate (1.0), and insulin (50 μU/mL); the perfusate was equilibrated with 95% O2 and 5% CO2 in a water-jacketed chamber. The perfusate pH was determined with a glass electrode and a water-jacketed pH probe.6,12 Briefly, the heart was imaged in the 2-dimensional parasternal short-axis view and an M-mode echocardiogram of the mid-ventricle was recorded at the level of papillary muscles. All mice were imaged by a single operator. Heart rate, posterior and interventricular septum wall thicknesses, and the end-diastolic and end-systolic internal dimensions of the left ventricle (LV) were measured from the M-mode image. On the basis of our prior experience, these measurements were reproduced with an interreader variation <10%. LV wall thickness was assessed as the average of posterior wall and septum thickness. LV fractional shortening was defined as the end-diastolic diameter minus the end-systolic diameter normalized for the end-diastolic diameter; this was used as an index of cardiac contractile function.

13C NMR Spectroscopy

Perchloric acid extracts were prepared from freeze-clamped heart tissue, neutralized by KOH, lyophilized, and subsequently dissolved in 300 μL D2O. Proton-decoupled 13C NMR spectra of tissue extracts were obtained with a 3-mm NMR probe on the Varian Inova 400 spectrometer. The contributions of each labeled substrate and the total of the unlabeled exogenous and endogenous substrates to the oxidative metabolism were determined from the peak areas of 13C isotopomers at C3 and C4 of glutamate by modeling of the TCA cycle fluxes as previously described.14–16

Biochemical Measurements and Calibration of 31P NMR Spectra

Cardiac glycogen content was determined by use of an alkaline extraction procedure to separate glycogen and exogenous glucose in the tissue.17 Glucose released from glycogen and lactate content in the coronary effluent were measured with Sigma assay kits (Sigma-Aldrich, St Louis, Mo). Freeze-clamped tissues obtained during baseline perfusion were used for determination of myocardial content of ATP by high-performance liquid chromatography as reported previously.13 Myocardial ATP content measured by high-performance liquid chromatography was converted to ATP with an assumption of an intracellular water content of 0.48 mL/g and a protein content of 0.15 g/g of blotted wet tissue. These values were used to calibrate ATP peak areas in baseline 31P NMR spectra for the respective groups. The average of β- and γ-ATP peak areas obtained under baseline conditions was set to 100% and used as the reference value for all peaks in all 31P NMR spectra. Intracellular pH was determined by comparison of the chemical shift of inorganic phosphorus and phosphocholine (PCr) in each spectrum with a standard curve because the chemical shift of inorganic phosphorous but not PCr changes with pH.13

Statistical Analyses

All data are presented as the mean±SEM. One-way ANOVA with Bonferroni post test was performed, and the unpaired t test was used for 2-group comparisons. Two-way repeated measure ANOVA was used to compare the P–V relationships and the responses to ischemia-reperfusion. Survival data were assessed by Kaplan-Meier survival analysis. Analyses were performed with GraphPad Prism (GraphPad Software, San Diego, Calif), and a value of P<0.05 was considered to be significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

General Characteristics of the Mice

To determine the long-term effects of increased glucose uptake in the heart, we followed a cohort of WT (n=42, 48% male) and GLUT1-TG (n=72, 45% male) mice housed in a standard pathogen-free animal facility for up to 2 years. The survival rates of the mice at 22 months were 76% for WT and 72% for GLUT1-TG (Figure 1; P=0.863). Mice 22 to 24 months old were used for the aging studies reported here unless otherwise specified. In young adult mice (5 months),

Figure 1. Long-term survival curve. The percentage survival is presented for GLUT1-TG (n=72, 45% male) and WT littermates (n=42, 48% male).
no differences existed in body weight, heart weight, and wet/dry weight ratios for the lung and the liver (Table 1). In old mice (22 to 24 months old), heart weight normalized to tibia length increased by 20% in both WT and GLUT1-TG group. The wet/dry ratio of the lung was slightly but significantly higher in old WT compared with young WT mice ($P<0.05$), and this difference was not found in GLUT1-TG mice (Table 1).

### Cardiac Function of Old GLUT1-TG Mice

We tracked cardiac function of the GLUT1-TG mice by echocardiography in conscious mice every 5 to 6 months during the 2-year period. As shown in Figure 2, an age-dependent increase in LV wall thickness and LV internal diameter existed for both WT and GLUT1-TG hearts, consistent with the increase in heart weight shown above. The LV fractional shortening was sustained up to 18 months and declined modestly at 2 years for both genotypes. No difference was observed between WT and GLUT1-TG mice at any age point.

To determine myocardial contractile function independent of neurohormonal regulation, we assessed LV P–V relationships in isolated perfused hearts. Compared with young hearts (4 months old), the peak LV developed pressure in old hearts (22 months old) was decreased in both genotypes (Figure 3; $P<0.05$), which suggests an age-associated decline in systolic function. When WT and GLUT1-TG hearts were compared at each age point, the P–V relationships as well as the peak LV developed pressures were similar at 4 months (110±9 (WT) versus 120±7 mm Hg (GLUT1-TG); $P=0.333$) as well as at 22 months (72±5 (WT) versus 86±5 mm Hg (GLUT1-TG); $P=0.09$). The P–V relationship for end-diastolic pressure was indistinguishable for WT and GLUT1-TG hearts at 4 months of age (Figure 3A). At 22 months, the relationship shifted upward and leftward in WT compared with GLUT1-TG (Figure 3, B and C; $P=0.019\times2$-way repeated measure ANOVA), which suggests an impaired diastolic function in old WT but not in old GLUT1-TG hearts. This finding was consistent with the modest increase of water content found only in the lung of old WT mice.

### Responses of Old GLUT1-TG Hearts to Ischemia

To determine the long-term effects of increased glucose uptake on responses to ischemic injury in old hearts, we assessed cardiac function in young and old GLUT1-TG hearts subjected to 25 minutes of low-flow ischemia (3% of baseline daily workload).

### Table 1. General Characteristics of the Mice

<table>
<thead>
<tr>
<th>Age</th>
<th>Body weight, g</th>
<th>Heart weight, mg</th>
<th>HW/T, mg/mm</th>
<th>Lung, wet/dry</th>
<th>Liver, wet/dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Months</td>
<td>WT (n=8)</td>
<td>TG (n=14)</td>
<td>$P$</td>
<td>WT (n=8)</td>
<td>TG (n=14)</td>
</tr>
<tr>
<td>Old</td>
<td>WT (n=8)</td>
<td>TG (n=14)</td>
<td>$P$</td>
<td>WT (n=8)</td>
<td>TG (n=14)</td>
</tr>
<tr>
<td>5 Months</td>
<td>27±1</td>
<td>26±1</td>
<td>0.464</td>
<td>32±2</td>
<td>32±1</td>
</tr>
<tr>
<td>22 to 24</td>
<td>124±7</td>
<td>136±5</td>
<td>0.849</td>
<td>158±10</td>
<td>170±10</td>
</tr>
<tr>
<td>Months</td>
<td>7.25±0.29</td>
<td>7.90±0.23</td>
<td>0.095</td>
<td>8.70±0.49</td>
<td>9.57±0.5</td>
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<td></td>
<td>4.43±0.05</td>
<td>4.44±0.04</td>
<td>0.474</td>
<td>4.67±0.07</td>
<td>4.40±0.04</td>
</tr>
<tr>
<td>22 to 24</td>
<td>3.25±0.05</td>
<td>3.21±0.03</td>
<td>0.562</td>
<td>3.26±0.06</td>
<td>3.30±0.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. HW indicates heart weight; TL, tibia length; and TG, GLUT1-transgenic mice.

*P<0.05 versus young WT mice and old GLUT1-TG mice.

Figure 2. Echocardiographic assessments of GLUT1-TG and WT mice at 4 ages during 2 years of follow-up (n=5 to 10 for each group). All measurements were obtained in conscious mice at heart rates of 600 to 700 bpm (no differences in heart rates between 2 genotypes at any age point; data not shown). Results are presented as (top) LV wall thickness (average of posterior and interventricular septum wall), (middle) LV internal diameter at end diastole, and (bottom) LV fractional shortening. Data are shown as mean±SEM. FS indicates fractional shortening.
coronary flow) and 45 minutes reperfusion. Compared with age-matched WT hearts, the GLUT1-TG hearts showed less diastolic contracture during ischemia and a greater recovery of LV end-diastolic pressure during reperfusion (Figure 4A). In WT group, impairment of diastolic function was worsened in old hearts consistent with the notion that aging hearts are more susceptible to ischemic insults. However, age-associated diastolic dysfunction was attenuated in GLUT1-TG hearts during reperfusion, the diastolic pressure returned to pre-ischemic level in both young and old hearts after reperfusion (Figure 4). Similarly, the recovery of LV developed pressure at the end of reperfusion reached 86% and 83% of the pre-ischemic level in young and old GLUT1-TG hearts, respectively (P was nonsignificant) compared with that of 52% and 35% in young and old WT hearts, respectively (Figure 4B, P<0.05). The coronary flow rate was similar during ischemia for WT and GLUT1-TG hearts (≈0.07 mL/min), and the recovery at the end of reperfusion was not different among the groups (Figure 4C). Together, these results suggested that the tolerance to ischemia-reperfusion injury was markedly improved in old GLUT1-TG hearts, and age-associated deterioration was reduced in GLUT1-TG.

To determine whether the functional improvement in old GLUT1-TG hearts can be attributed to preserved myocardial energetic status, we measured dynamic changes of high-energy phosphate content in the heart. Before ischemia, ATP was similar in all groups. The concentration of PCr, the energy reserve compound, was lower in old WT hearts but was sustained in old GLUT1-TG hearts (Figure 5A, P<0.05). Although PCr decreased rapidly during ischemia, the rate of ATP depletion was significantly blunted in GLUT1-TG hearts (Figure 5B). ATP was ≈2-fold higher in GLUT1-TG compared with age-matched WT at the end of ischemia. By

Figure 3. Left ventricular pressure–volume relationships in isolated perfused hearts in (A) 4-month-old and (B) 22-month-old mice (n=6 to 8 for each group). C, Comparison of age-related changes in pressure–volume relationship is shown by normalization of the LV volume to heart weight. Data are shown as mean±SEM. *P<0.05 TG versus WT; †P<0.05 old versus young. DevP indicates developed pressure; LVEDP, LV end-diastolic pressure; YWT, young WT; OWT, old WT; YTG, young GLUT1-TG; and OTG, old GLUT1-TG.
the end of reperfusion, ATP in old GLUT1-TG was 3 times higher than old WT hearts and exceeded the level of young WT hearts. Of note, a smaller decline of intracellular pH occurred in old GLUT1-TG hearts during ischemia (Figure 5C, *P < 0.05) despite the assumption that increased glycolysis in these hearts would aggravate acidosis. Thus, these results suggested that improved energy supply in old GLUT1-TG hearts during ischemia was associated with preserved myocardial energetics and fewer disturbances of ion homeostasis.

To determine the role of substrate metabolism in the postischemic recovery of myocardial energetics and contractile function, we assessed net changes in glycogen content, glycolytic activity, and substrate oxidation in hearts during ischemia and reperfusion. The lactate output from old GLUT1-TG hearts during normal perfusion was 5-fold higher than age-matched WT hearts (Figure 6A), consistent with our prior observations in young GLUT1-TG hearts. During the low-flow ischemia, lactate output was low in all groups, whereas a large amount of lactate was detected in the coronary effluent in the first 5 minutes of reperfusion (Figure 6A). These results suggested that the low flow rate during ischemia was not sufficient to wash out lactate from the heart in either group. Thus, lactate washout during the first 5 minutes of reperfusion was included in the estimation of total amount of lactate produced during ischemia. Cardiac glycogen content at baseline, at the end of ischemia, and at the end of reperfusion was shown in Table 2, and the net glycogen depletion during ischemia was compared with the total lactate output during ischemia. Glycogen content was higher in GLUT1-TG hearts before ischemia, but the net glycogen depletion during ischemia was similar in WT and GLUT1-TG hearts. Even if 100% of net glycogenolysis contributed to anaerobic glycolysis, it would account for 39% and 35% of total lactate output in WT and GLUT1-TG hearts. Therefore, increased glucose uptake rather than higher glycogen content likely accounted for increased glycolysis in GLUT1-TG hearts.

The substrate oxidation profile was determined during reperfusion period with 13C NMR isotopomer analysis (Figure 6B). Similar to previous observations in adult hearts, the contribution of fatty acids to oxidative metabolism predominates during reperfusion. However, the contribution of glucose was increased by 2- and 3-fold in young and old GLUT1-TG hearts compared with age-matched WT. Moreover, the relative contribution of fatty acid oxidation was markedly reduced in old GLUT1-TG hearts (decreased by 45% versus old WT). Thus, glucose and fatty acids contribute to the similar extents in old GLUT1-TG hearts during reperfusion. Interestingly, enhanced oxidation of glucose over fatty acids was associated with marked improvement of energetic and functional recovery in the old transgenic hearts even though the rate of lactate output remained higher in the old GLUT1-TG hearts during reperfusion compared with old WT hearts; thus a significant fraction of glycolytic flux remained uncoupled from oxidation.

**Discussion**

The present study demonstrates that life-long increases of continuous basal glucose uptake (insulin-independent) in the mouse heart do not adversely alter cardiac morphology, contractile function, or survival during the 2-year period. Instead, we have found evidence of improved diastolic function in mouse hearts with long-term increases in glucose uptake during normal aging. Furthermore, long-term increases in glucose uptake and utilization have preserved myocardial energetics and attenuated aging-caused suscepti-
bility to ischemia-reperfusion in mouse hearts. These observations strongly suggest that the heart is able to adapt to sustained high rates of glucose transport into cardiac myocytes and that long-term increases in intracellular glucose by themselves cause no long-term adverse consequences.

It is generally recognized that the adult heart utilizes fatty acids as the primary energy substrate, whereas the fetal heart mainly relies on carbohydrates (glucose and lactate). Recent studies of energy metabolism in cardiac hypertrophy and failure suggest that the substrate preference in failing hearts is shifted toward glucose, manifested as increased glucose uptake and glycolysis compared with normal hearts when supplied with identical compositions of carbohydrates and fatty acids. Such a change is noted as a reversion to fetal metabolic profile by many, and it remains unclear whether it is adaptive or maladaptive. Prior studies of cardiac myocytes exposed to high levels of glucose have demonstrated contractile dysfunction and cell death that are dependent on the increases in intracellular glucose, which suggests that increased glucose entry causes glucotoxicity. In the present study, we use a transgenic mouse model in which myocardial glucose uptake rates are ∼40-fold higher in the absence of insulin and 2-fold higher than maximum insulin-stimulated glucose uptake in WT. Despite the substantially

Figure 5. Measurements made by 31P nuclear magnetic resonance spectroscopy in GLUT1-TG and WT hearts (n=4 to 7 each group) before and during ischemia-reperfusion. Data are shown as mean±SEM for (A) PCr, (B) ATP, and (C) intracellular pH in old (left panel) and young hearts (right panel). *P<0.05 TG versus WT.
Elevated glucose entry, the cardiac morphology and function of normal WT mice during the 2-year period. We have also shown that these hearts adapt to increased glucose influx by alteration of the expressions of proteins involved in glucose and fatty acids metabolism, which results in increased glycolysis and glucose oxidation and decreased contribution of noncarbohydrates such as fatty acids and ketones to oxidative metabolism. Thus, although increased myocardial glucose uptake and a greater reliance on glucose present as reappearance of fetal metabolic profile, the normal heart is able to adapt to such a metabolic pattern for life. Moreover, increased glucose uptake and utilization has been found to be beneficial for hypertrophied and failing hearts and in hearts with impaired fatty acid oxidation.

It has been suggested that exposure of cardiac myocytes to high-glucose media resulted in impaired E-C coupling and prolonged relaxation that was dependent on increased glucose entry and metabolism. In addition, we have previously shown that impaired cell relengthening in senescent mouse cardiac myocytes was a mechanism for diastolic dysfunction in aging hearts. Thus, we investigated whether long-term increases in glucose uptake had a negative impact on diastolic function during aging in GLUT1-TG hearts. Interestingly, we have shown that the diastolic P−V relationship is preserved in old GLUT1-TG hearts compared with age-matched WT hearts. We speculate that the differences in the present observations made in mouse hearts compared with the previous findings in cardiac myocytes cultured in high-glucose media are the result of the nonbeating nature of cultured myocytes, which renders the metabolic rate markedly lower than a beating heart, and hence we caution the interpretation of metabolic studies that use quiescent cardiac myocytes.

Previous studies by others and us have shown that glucose supply plays a critical role in cardiac responses to ischemia and increased glucose delivery during acute ischemia is cardioprotective. Results from the present study suggest that long-term increases in glucose delivery to the heart do not alter function of normal hearts but improve tolerance to ischemic stress, and these benefits are independent of changes in signaling mechanisms involved in cardioprotection such as Akt and AMPK (online-only Data Supplement). Importantly, we show that long-term increases in glucose uptake reduce aging-associated increases of susceptibility to ischemia-reperfusion insult. Old GLUT1-TG hearts are not only protected from ischemia-reperfusion injury compared with age-matched WT hearts, but old GLUT1-TG hearts also recovered to the similar level as young GLUT1-TG hearts during reperfusion. This is consistent with previous reports that showed that aged hearts develop increased dependence on glucose and glycogen and yet have reduced ability to upregulate glucose utilization during stresses.

Several differences in energy metabolism observed in the present study likely contribute to the improved tolerance to ischemia-reperfusion injury in old transgenic hearts. First, a greater level of the energy reserve compound PCr is present in old transgenic hearts. It has been shown that aging is associated with a decline of PCr content in the heart. Consistently, we found that PCr was lower in old WT hearts but was maintained at normal adult levels in old transgenic hearts. PCr serves as an energy reserve with a rapid transfer of

### TABLE 2. Estimation of Glycolysis and Glycogenolysis During Ischemia

<table>
<thead>
<tr>
<th>Glycogen Content (μmol glucose/g)</th>
<th>Glycogen Use During Ischemia, μmol glucose/g</th>
<th>Lactate Output During Ischemia, μmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>25.1±1.9</td>
<td>11.8</td>
</tr>
<tr>
<td>TG</td>
<td>53.9±5.2</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>End-Ischemia</strong></td>
<td>13.3±0.4</td>
<td>60.6±7.8</td>
</tr>
<tr>
<td><strong>End-Reperfusion</strong></td>
<td>27.5±6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.3±2.8</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Each group comprised 2 to 4 mice.

*P<0.05 for WT mouse versus GLUT1-TG mice.
its high-energy phosphoryl group to ATP at the onset of ischemia to temporarily offset the depletion of ATP. Sustained energy reserve in old transgenic hearts results in a significant, albeit brief, delay in the depletion of ATP during the first 4 minutes of ischemia. The favorable energy status is maintained by a robust glycolytic ATP production in the old transgenic hearts fueled by a greater rate of glucose uptake. Although it has been repeatedly shown that increased glycolytic ATP synthesis is protective during ischemia, concerns have been raised that excessive anaerobic glycolysis may exacerbate intracellular acidosis and hence worsen ischemic injury. It has been speculated that the benefit of enhanced glycolysis during low-flow ischemia found in perfused hearts ex vivo could be biased as a result of the washout of lactate by a relatively high coronary flow rate. In the present study, at a flow rate of \(0.07\) mL/min during ischemia, lactate washout was incomplete in both WT and transgenic hearts. Despite the fact that a smaller fraction of lactate was washed out in old transgenic hearts, less acidosis was present in these hearts versus WT hearts. These results suggest that proton homeostasis is better maintained in old transgenic hearts. Because we observed no active contractile activity in any of the hearts during ischemia, we speculate that improved ATP supply in the old transgenic hearts is most likely used for the maintenance of ion pump function and hence minimizes myocardial injury.

It has been well recognized that excessive fatty acid oxidation during reperfusion plays an important role in myocardial damage. Increased fatty acid oxidation during reperfusion leads to impaired glucose oxidation and uncouples glycolytic flux from oxidative metabolism, which thus increases proton load and decreases cardiac efficiency. Thus, an increase in glycolytic flux under such conditions would be undesirable. Interestingly, we found improved functional recovery in young GLUT1-TG was associated with high rates of fatty acid oxidation even though glucose oxidation also doubled. It is possible that the greater functional recovery primarily reflects improved ischemic tolerance in this group. Nevertheless, we found that old transgenic hearts were able to sustain enhanced glucose oxidation over fatty acid oxidation during reperfusion and demonstrated an improved postsischemic recovery in both energetics and function. Taken together, these results suggest that a normal heart is not only able to adapt to long-term increases of intracellular glucose but also develops a metabolic phenotype that is more tolerant to ischemic insult in old age.

Finally, it is important to recognize the limitations of studies that use transgenic mouse models such as the present model. Increased glucose uptake is initiated in the present mouse model at birth. It is possible that adaptations developed during maturation contribute to the phenotype of uncompromised cardiac function despite life-long increases in intracellular glucose. The nature of these adaptations that developed and importantly whether adult-onset of increased glucose supply also triggers similar responses are not addressed by the present study but should be pursued in future studies that use a modified model such as conditional transgenic mice. Moreover, the present proof-of-concept study is performed in otherwise healthy mice. Although such an approach allows us to dissect the functional consequence of a single metabolic alteration, additional studies are clearly necessary to integrate these results into complex disease conditions. We have previously shown that increased glucose uptake restores contractile reserve in hearts with impaired fatty acid oxidation, and that GLUT1-TG hearts are protected from the development of heart failure by chronic pressure overload, another condition of reduced capacity for fatty acid oxidation. The consequence of increased myocardial glucose uptake in hearts with high rates of fatty acid oxidation such as diabetes mellitus and obesity remains to be tested.

In summary, we have demonstrated that a normal heart is able to adapt to long-term increases in insulin-independent glucose entry into cardiomyocytes and that life-long increases in glucose uptake result in a favorable metabolic phenotype that affords protections against increased sensitivity to ischemic injury in old hearts. Observations made in the present study provide an important basis for future studies to address the role of altered energy substrate preference in complex disease conditions as well as to identify novel mechanisms for metabolic therapy of heart disease.

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Disclosures

None.

References

It is increasingly recognized that abnormal cardiac metabolism is integral to the pathogenesis of heart disease. Alterations in cardiac glucose and fatty acid metabolism have been reported in heart failure, ischemia, aging, obesity, and diabetes mellitus, but the mechanistic link between altered metabolism and cardiac dysfunction remains elusive. The present study provides proof-of-concept evidence that a normal heart is metabolically flexible to substrate availability and is able to maintain normal cardiac function for life by adaptation to long-term increases in glucose supply. Furthermore, the metabolic adaptations developed for long-term elevations of glucose uptake and utilization protect the heart from aging-related susceptibility to ischemic injury. These observations indicate that glucose per se does not constitute cardiac toxicity, even though increased glucose supply and/or uptake are often observed under conditions associated with cardiac dysfunction. Although the present study addresses the functional consequence of a single metabolic perturbation in a healthy heart, it provides an important basis for future studies to define the role of altered substrate metabolism in complex disease conditions as well as to identify novel mechanisms for metabolic therapy for heart disease.
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