Cardiac-Specific Overexpression of GLUT1 Prevents the Development of Heart Failure Attributable to Pressure Overload in Mice

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Background—Increased rates of glucose uptake and glycolysis have been repeatedly observed in cardiac hypertrophy and failure. Although these changes have been considered part of the fetal gene reactivation program, the functional significance of increased glucose utilization in hypertrophied and failing myocardium is poorly understood.

Methods and Results—We generated transgenic (TG) mice with cardiac-specific overexpression of insulin-independent glucose transporter GLUT1 to recapitulate the increases in basal glucose uptake rate observed in hypertrophied hearts. Isolated perfused TG hearts showed a greater rate of basal glucose uptake and glycolysis than hearts isolated from wild-type littermates, which persisted after pressure overload by ascending aortic constriction (AAC). The in vivo cardiac function in TG mice, assessed by echocardiography, was unaltered. When subjected to AAC, wild-type mice exhibited a progressive decline in left ventricular (LV) fractional shortening accompanied by ventricular dilation and decreased phosphocreatine to ATP ratio and reached a mortality rate of 40% at 8 weeks. In contrast, TG-AAC mice maintained LV function and phosphocreatine to ATP ratio and had <10% mortality.

Conclusions—We found that increasing insulin-independent glucose uptake and glycolysis in adult hearts does not compromise cardiac function. Furthermore, we demonstrate that increasing glucose utilization in hypertrophied hearts protects against contractile dysfunction and LV dilation after chronic pressure overload. (Circulation. 2002;106:2125-2131.)

Key Words: glucose ■ heart failure ■ metabolism

Despite significant advances in the treatment of cardiovascular disease, the incidence of congestive heart failure continues to rise, and heart failure remains the leading cause of mortality in the United States. Heart failure represents a common final pathway for heart diseases of numerous etiologies in which the compensatory mechanisms of the heart eventually fail to maintain an appropriate cardiac output. For example, in hearts with chronic hemodynamic overload, such as that occurring in patients with hypertension, valvular disease, or postmyocardial infarction, the overload initially results in cardiomyocyte hypertrophy and augmented force generation. This apparent compensatory response, however, is followed by a process of deleterious remodeling in which the hypertrophied heart dilates and fails to contract effectively.¹

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The process of transition from cardiac hypertrophy to heart failure has long been recognized, but its underlying mechanisms remain poorly understood despite intensive studies. In hypertrophied and failing hearts, increased glucose uptake and utilization have been observed in both animal models and patients.²⁻⁷ We and others have shown that increased glucose uptake in hypertrophied hearts is insulin independent and is associated with increased expression of basal glucose transporter GLUT1 and decreased expression of insulin-regulated glucose transporter GLUT4.⁸⁻¹¹ However, the functional significance of increased basal glucose uptake and utilization in hypertrophied hearts has not been defined. In recent clinical studies, pharmacological interventions that decrease fatty acid oxidation and promote glucose utilization have been found to improve cardiac function and increase exercise capacity in patients with chronic ischemic heart disease.¹²⁻¹⁴ Thus, we hypothesize that increased glucose utilization represents an adaptive response that renders hypertrophied hearts more tolerant to chronic hemodynamic overload. To test this hypothesis, we generated transgenic (TG) mice with cardiac-specific overexpression of the basal glucose transporter...
GLUT1 to enhance basal glucose transport in the heart and subjected them to chronic pressure overload by ascending aortic constriction (AAC). In this study, we demonstrate that increasing myocardial glucose uptake protects against the progression to heart failure and improves survival in mice with chronic pressure overload.

Methods

Experimental Animals
TG mice were generated by injecting a recombinant DNA construct containing a cDNA fragment encoding the human GLUT1 (kind gift of Dr Mike Mueckler), regulated by a mouse α-myosin heavy chain promoter (Figure 1a), into the pronuclei of fertilized oocytes obtained from female FVB mice. The transgenic mouse colony was established by breeding the founder mice with wild-type (WT) FVB mice purchased from Charles River Laboratories (Wilmington, Mass), and all of the control mice used in this study were WT littermates. All of the procedures related to the handling of the mice in this study were approved by Harvard Medical Area Standing Committee on Animals.

Assessment of Glucose Uptake, Glycolysis, and High-Energy Phosphate Content in Isolated Perfused Mouse Hearts
We determined the rate of glucose transport in perfused hearts isolated from TG mice and their WT littermates by a nontracer method using 31P NMR spectroscopy and the glucose analogue 2-deoxyglucose (2-DG), as previously described. The transport rate of 2-DG, assessed by the time-dependent accumulation of 2-DG-phosphate (2-DG-P), was measured both before and after insulin (2 U/L) was added to the perfusate. To determine whether increased glucose uptake in TG hearts persisted after chronic pressure overload, 2-DG uptake experiments were also performed in hearts 3 to 4 weeks after AAC. All hearts were perfused with a constant pressure system with the perfusion pressure of 75 mm Hg for normal hearts and 100 mm Hg for pressure-overloaded hearts. Using this approach, we were able to achieve comparable myocardial flow per unit heart weight for the hypertrophied hearts and the control hearts. The rate of anaerobic glycolysis was assessed by net lactate production, measured by the difference of lactate concentration in coronary effluent and coronary perfusate. 15

High-energy phosphate contents were measured using 31P NMR spectroscopy in isolated perfused hearts 4 to 5 weeks after AAC or sham operation, and the ratio of phosphocreatine to ATP (PCr/ATP) was used as an index for the energetic status of these hearts.

Ascending Aortic Constriction and Measurement of Pressure Gradient
AAC was performed in anesthetized (pentobarbital 15 mg/kg, IP) and ventilated mice. The thorax was opened by an anterolateral thoracotomy, and aortic constriction was induced by ligating the ascending aorta around a 27-gauge needle using 7-0 silk suture. Sham-operated animals underwent a similar procedure without banding the aorta. For the assessment of trans-constriction pressure gradients, the right carotid artery was isolated and a partial incision was made to introduce a calibrated 1.4F Millar pressure transducer into the artery lumen. Mice then underwent a left thoracotomy to expose the apex of the heart. A sterile 22-gauge needle was used to puncture the apex, and a second 1.4F Millar pressure transducer was introduced into the left ventricle. Aortic and ventricular pressures were simultaneously determined, and the pressure difference represented trans-constriction pressure gradient.

Trans-Thoracic Echocardiography
Murine trans-thoracic echocardiography was conducted in conscious mice as previously described using an Acuson Sequoia C-256 echocardiograph machine and a 15-MHz probe. Briefly, the heart was imaged in the two-dimensional parasternal short-axis view, and an M-mode echocardiogram of the midventricle was recorded at the level of papillary muscles. Heart rate, posterior wall thickness, and end-diastolic and end-systolic internal dimensions of the left ventricle were measured from the M-mode image. LV fractional shortening was defined as the end-diastolic dimension minus the end-systolic dimension normalized for the end-diastolic dimension and was used as an index of cardiac contractile function.

Organ Weight and Histology
Body weight, tibia length, and heart weight were determined in mice 8 to 10 weeks after surgery. Lung and liver samples were removed, and their wet and dry weight was recorded; their wet to dry ratios were calculated and used as an indirect assessment of pulmonary or hepatic congestion, respectively. Hearts were arrested in diastole by KCl (30 mmol/L), followed by perfusion fixation with 10% buffered formalin. Longitudinal sections of hearts were stained with Trichrome for histological assessment.
Immunoblotting, Glycogen Assay, and Measurement of Blood Glucose Level

The protein levels of GLUT1 and GLUT4 were assessed by immunoblotting on postnuclear membranes prepared from cardiac tissue. Myocardial glycogen content was determined as previously described. The blood glucose level was measured by ONE TOUCH Glucose Monitor (Lifescan Inc) using blood samples obtained by tail bleeding.

Statistical Analysis

Results are presented as mean ± SEM. Differences between the WT and TG hearts were compared by 2-tailed Student’s t test or 1-way factorial ANOVA for all in vitro measurements. The growth curve and the serial echocardiographic measurements of WT and TG mice were first compared by 2-way repeated-measures ANOVA. Once \( P < 0.05 \) was found, comparisons among the groups at the same time point were performed by 1-way factorial ANOVA. Survival data were assessed by Kaplan-Meier survival analysis. A value of \( P < 0.05 \) was considered significant.

Results

Characterization of Mice With Cardiac Specific Overexpression of GLUT1

Three founder mice (Nos. 54, 53, and 39) were identified by Southern blot analysis, all of which had passed the transgene to their offspring. Overexpression of GLUT1 resulted in substantial increases in GLUT1 protein with no changes in the total amount of GLUT4 protein in the heart (Figure 1b). Mice from all 3 transgenic lines were followed for 15 months, and no early death or altered growth pattern was observed (Figure 1c). Results reported here were obtained from the No. 54 line, unless otherwise stated.

Overexpressing GLUT1 in Mouse Hearts Results in Increased Glucose Utilization

In isolated perfused heart, we found that the uptake rate of 2DG, a glucose analogue, increased markedly (≈40-fold) in TG hearts during insulin-free perfusion and remained more than 2-fold higher than WT hearts during insulin stimulation (Figures 2a and 2b). The greater rate of glucose uptake persisted in TG hearts after AAC and remained more than 10-fold higher than WT-AAC, even though the rate of glucose uptake in WT hearts also increased after AAC (Figure 2b). Furthermore, changes in glucose uptake rate are consistent with the amount of GLUT1 protein in these hearts (2.7 ± 0.9, 8.5 ± 1.2, and 55 ± 5 AU for WT-sham, WT-AAC, and TG-AAC, respectively, \( n = 3 \) each). These results confirm the plasma membrane localization and the functionality of overexpressed GLUT1 glucose transporters. They also show that increased amount of GLUT1 in the heart results in a significant higher rate for basal glucose transport in both normal hearts and hypertrophied hearts with pressure overload.

Increasing glucose transport in the heart, however, did not alter systemic glucose homeostasis. Blood glucose levels were similar in WT and TG mice either at fed state (6.9 ± 0.3 versus 6.7 ± 0.2 mmol/L, \( P = \text{NS} \)) or after overnight fasting (3.9 ± 0.2 versus 4.1 ± 0.4 mmol/L, \( P = \text{NS} \)). Augmented glucose uptake resulted in an increase in cardiac glycogen content in TG No. 39 and TG No. 54 of 3- and 5-fold, respectively, compared with WT hearts (Figure 2c). The increase in glycogen content in TG No. 39 and TG No. 54 hearts was parallel with the amount of GLUT1 expressed in these transgenic lines (Figure 1b), suggesting a dose effect of transgene expression.

Lactate production, an indirect assessment of glycolytic activity, was also significantly elevated in isolated perfused TG hearts at baseline and increased additionally at peak work induced by increasing LV volume (Starling mechanism) (Figure 2d). These results suggest a marked increase in glycolytic flux in TG hearts, assuming unaltered glucose oxidation rate between TG and WT hearts. Furthermore, increased glycolysis at baseline did not impair the ability of the TG heart to additionally increase its glycolytic flux in response to high workload.
Overexpressing GLUT1 in Mouse Hearts Attenuates the Development of Contractile Dysfunction and Depletion of High-Energy Phosphate and Improves the Long-Term Survival After Ascending Aortic Constriction

Before AAC, the baseline in vivo cardiac function in TG mice was not different from WT mice, as assessed by trans-thoracic echocardiography in conscious mice (Figures 3a through 3f). Immediately after AAC, similar pressure gradients were found in WT (n=4) and TG (n=4) mice (63±3 versus 66±3 mm Hg, P=NS). At 1 week after AAC, similar increases in the LV posterior wall thickness were found in WT and TG mice (Figure 3c). With time, however, WT-AAC mice exhibited a progressive decrease of the LV wall thickness (Figure 3c) and increases in LV end-diastolic (Figure 3d) and end-systolic (Figure 3e) dimensions, indicative of LV dysfunction.
Body Weight and Organ Weight
decreased significantly in WT-AAC (NS versus Sham), whereas PCr/ATP was preserved PCr/ATP status was also improved in TG-AAC, as indicated by a similar degree of pressure overload. Myocardial energetic (Figure 3f). Thus, no evidence of the transition to failure was ing was not impaired in TG-AAC mice at 8 weeks after AAC evidence of LV dilation. Furthermore, the fractional shorteneections demonstrate that the tolerance to pressure overload was together with the echocardiographic evidence, these observa-
Table)

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<th>Group</th>
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<th>Body Weight, g</th>
<th>Tibia Length, mm</th>
<th>Heart Weight, mg</th>
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*P<0.01 vs WT-Sham; †P<0.01 vs TG-Sham.

Figure 4. PCr/ATP in WT-sham (n=3), WT-AAC (n=4), TG-sham (n=4), or TG-AAC (n=3) hearts.

Discussion
This study demonstrates that metabolic interventions have the potential to alter the natural history of heart failure. Increasing myocardial glucose uptake in transgenic mice by cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 prevented the development of heart failure and improved survival of mice subjected to chronic pressure overload for the period of this study.

In this study, we assessed in vivo cardiac function in conscious mice using echocardiography. This approach has the advantage of repeatedly measuring cardiac function without the influence of anesthesia on the heart. Using this approach, we found impaired cardiac function and a transition from concentric hypertrophy to LV dilatation in WT mice after 4 to 8 weeks of pressure overload. This is consistent with previous studies showing evidence of contractile dysfunction in isolated cardiac myocytes at 7 weeks of pressure overload in the same model.19,20 In contrast, in vivo contractile function was maintained in TG hearts with 8 weeks of pressure overload. It is unlikely that these findings resulted from less severe pressure overload in TG mice for several reasons. First, the pressure gradient induced by constricting the ascending aorta is highly reproducible. This is in contrast to the model of transverse aortic constriction, in which variable blood flow via brachiocephalic artery (located proximal to the constriction) resulted in a wide range of pressure gradients.21 We measured the pressure gradient after AAC in a subgroup of mice in this study and confirmed the reproducibility of this model. Importantly, we found no difference in pressure gradient generated by this procedure in WT and TG mice. Finally, our conclusion that WT and TG mice were dilated. At 4 and 8 weeks after AAC, WT-AAC mice exhibited reduced fractional shortening, consistent with overt contractile dysfunction. In contrast, TG-AAC mice maintained increased LV wall thickness and did not show any evidence of LV dilation. Furthermore, the fractional shortening was not impaired in TG-AAC mice at 8 weeks after AAC (Figure 3f). Thus, no evidence of the transition to failure was observed in TG-AAC mice for the period of this study despite similar degree of pressure overload. Myocardial energetic status was also improved in TG-AAC, as indicated by a preserved PCr/ATP (P=NS versus Sham), whereas PCr/ATP decreased significantly in WT-AAC (P<0.05 versus Sham) (Figure 4).

The acute mortality rate of AAC or sham operation was not different for WT and TG mice. In the first 72 hours, 21% of WT (4 of 19) and 25% of TG (4 of 16) mice subjected to AAC died (P=NS), and 13% of WT (2 of 15) and 18% of TG (2 of 11) mice subjected to sham operation died (P=NS). In mice that survived the acute perioperative period, 40% of WT-AAC mice died in the following 8 weeks, whereas <10% of TG-AAC mice died during the same period (P<0.05, Figure 5). Although similar increases in heart weight were found in both groups 8 to 10 weeks after AAC (Table), greater dilatation of the LV and enlargement of the left atrium were observed in histological sections of WT-AAC hearts (Figure 6). Furthermore, 8 weeks after AAC, WT-AAC but not TG-AAC mice exhibited increased lung and liver wet to dry ratios, indicating the development of pulmonary and hepatic congestion, respectively. Taken together with the echocardiographic evidence, these observations demonstrate that the tolerance to pressure overload was improved and the onset of heart failure delayed in the hearts of TG-AAC mice.

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Figure 5. Kaplan-Meier survival plots for WT and TG mice subjected AAC or sham-operation (Sham) after 72-hour perioperative period.
been repeatedly observed. Although these changes have long been considered part of the fetal gene reactivation program in cardiac hypertrophy, their functional significance is poorly understood. In this study, we generated transgenic mice with cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 and used it as a tool to define the functional consequence of altered substrate utilization in the heart. Our results demonstrate that chronic increases in basal glucose uptake and glycolysis in adult hearts do not alter in vivo cardiac function, systemic glucose homeostasis, or survival in normal mice. When subjected to AAC, however, TG mice show greater tolerance to chronic pressure overload during chronic pressure overload and protects against contractile failure independent of the development of hypertrophy.

Fatty acids are the predominant fuel for energy production in adult hearts, whereas glucose and lactate are the major carbon sources for fetal and neonatal hearts. In hypertrophied hearts, increased glucose uptake and glycolysis have been repeatedly observed. Previous studies have shown that the increase in glucose uptake in hypertrophied hearts is insulin-independent. Although these changes have long been considered part of the fetal gene reactivation program in cardiac hypertrophy, their functional significance is poorly understood. In this study, we generated transgenic mice with cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 and used it as a tool to define the functional consequence of altered substrate utilization in the heart. Our results demonstrate that chronic increases in basal glucose uptake and glycolysis in adult hearts do not alter in vivo cardiac function, systemic glucose homeostasis, or survival in normal mice. When subjected to AAC, however, TG mice show greater tolerance to chronic pressure overload and delayed progression to heart failure. These results support the hypothesis that substantial increase in glucose utilization enables the hypertrophied hearts to maintain high-contractile performance during mechanic overload.

Although glucose utilization is increased in hypertrophied hearts of nontransgenic animals, our results suggest that this intrinsic mechanism is insufficient to support the overloaded hearts. This is because the increase in basal glucose uptake and glycolysis in the hypertrophied heart is modest, and it is associated with inability to additionally increase glucose utilization in response to high workload. In contrast, overexpressing GLUT1 in the heart renders marked increases in glucose uptake and glycolysis that exceeds the level that can be achieved in WT adult hearts. Despite increased basal glucose utilization, TG hearts were able to additionally increase glycolysis at high workload. Because fatty acid utilization is impaired in hypertrophied hearts and glucose contributes significantly to the ATP synthesis at high workload, enhancing glucose utilization may promote a favorable mode of energy supply that enables these hearts to sustain normal contractile performance at high workload during chronic pressure overload. This notion is supported by our observation that TG-AAC hearts show less depletion of energy reserve compound PCr. Furthermore, increasing the contribution of glucose as the energy substrate reduces myocardial oxygen consumption for the same workload, which is highly desirable for failing hearts.

The results of our study are of clinical importance. Recent clinical studies show that improvement of cardiac function in heart failure patients treated with β-adrenergic receptor blockers is associated with a shift in cardiac substrate utilization toward glucose. Our finding provides experimental basis for the benefit of increasing glucose utilization in hypertrophied and failing hearts. In support of this notion, recent studies show that acutely supplying pyruvate, a glycolytic product, to the failing human myocardium improves energetics, Ca2+ handling, and contractile function. In addition, our results also suggest that the benefit of metabolic intervention is not limited to failing hearts. Increasing glucose utilization at early stage alters the natural course for the development of heart failure in pressure-overloaded hearts. Together, these findings may contribute to the development of novel strategies for the prevention and treatment of heart failure.

**Acknowledgments**

This study was supported in part by National Institutes of Health grants HL-03377 (to Dr Liao), HL-59246, HL-67970, and AG-00837 (to Dr Tian) and by grants from Milton Fund and Harvard Medical School’s Center of Excellence in Women’s Health (to Dr Tian). The authors thank Drs Carl S. Apstein and Joanne S. Ingwall for critical review of the manuscript.

**References**


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*Circulation*. 2002;106:2125-2131
doi: 10.1161/01.CIR.0000034049.61181.F3

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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