Responses of GLUT4-Deficient Hearts to Ischemia Underscore the Importance of Glycolysis
Rong Tian, MD, PhD; E. Dale Abel, MBBS, DPhil

**Background**—The ischemic heart is dependent on glycolysis for ATP generation, and therapies that increase glucose utilization during ischemia improve survival. Myocardial ischemia results in the translocation of the glucose transporter proteins GLUT1 and GLUT4 to the sarcolemma. The increased glucose entry via these transporters contributes to enhanced glycolysis during ischemia.

**Methods and Results**—To determine the role of GLUT4 in mediating increased glycolytic flux during ischemia, hearts from mice with cardiac-selective GLUT4 deficiency (G4H−−/−) were subjected to global low-flow ischemia. During normal perfusion, hearts from fed G4H−−/− mice showed increased GLUT1-mediated glucose uptake, higher concentrations of glycogen and phosphocreatine, but delayed recovery after ischemia. When these compensatory changes were eliminated by a 20-hour fast, G4H−−/− hearts exhibited depressed glucose utilization during ischemia and developed profound and irreversible systolic and diastolic dysfunction associated with accelerated ATP depletion during ischemia and diminished regeneration of high-energy phosphate compounds on reperfusion.

**Conclusions**—GLUT4 is an important mediator of enhanced glycolysis during ischemia and represents an important protective mechanism against ischemic injury. *(Circulation. 2001;103:2961-2966.)*

**Key Words:** GLUT4 • ischemia • glucose • metabolism

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Ischemic heart disease is a major cause of morbidity and mortality worldwide. Recent clinical trials have suggested that therapeutic measures, such as glucose-insulin-potassium infusion, that augment glucose metabolism in the ischemic heart may have significant effects on survival. The mechanisms that govern this beneficial effect remain to be completely elucidated but may include enhanced glucose uptake and glycolysis by the ischemic myocardium. During ischemia, increased glycolysis becomes a major mechanism by which the heart maintains ATP concentrations in the face of impaired oxidative phosphorylation. Glucose enters cardiac myocytes via the facilitative glucose transporters GLUT1 and GLUT4, and glucose transport is a major determinant of glycolytic flux in muscle cells. GLUT4, the most abundant transporter, resides in intracellular vesicles under basal conditions, translocates to the plasma membrane in response to insulin, ischemia, and hypoxia, and most likely represents the major mechanism by which the heart increases glucose uptake under these circumstances. GLUT1 is less abundant, and a large proportion of GLUT1 resides in the sarcolemma and mediates basal cardiac glucose uptake. Stimuli that induce GLUT4 translocation, however, also stimulate GLUT1 translocation. To test the hypothesis that GLUT4-mediated glucose transport represents an essential protective mechanism against ischemic insults, we studied the responses of GLUT4-deficient hearts to ischemia. The hearts were obtained from mice with cardiac-selective ablation of the GLUT4 gene (G4H−−/−). The model is unique in that cardiac-selective ablation of GLUT4 is not associated with changes in systemic substrate metabolism, which may indirectly influence cardiac function.

**Methods**

**Animals**
Mice with cardiac-specific ablation of the GLUT4 gene (G4H−−/−) were generated by crossing mice (C57/BL6) bearing loxP sites flanking exon 10 of the GLUT4 gene with transgenic mice (FVB) with cardiac-specific expression of cre recombinase. All aspects of animal care and experimentation performed in this study were approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center and Harvard Medical School.

**Experimental Protocols**
Isolated hearts were obtained and perfused in the Langendorf mode as previously described. All hearts were perfused with phosphate-free Krebs-Henseleit buffer containing (in mmol/L) NaCl 118, NaHCO3 25, KCl 5.3, CaCl2 2.5, MgSO4 1.2, EDTA 0.5, glucose 10, and hexanoate 0.5 unless otherwise stated. 1P NMR spectra were collected simultaneously with the measurements of coronary flow and ventricular pressures of isolated heart preparations as previously described.
Three experimental protocols were performed in this study. For each protocol, half of the animals were fed and half were fasted for 20 hours.

Protocol 1
Protocol 1 determined contractile function, high-energy phosphate content, and intracellular pH (pH i) in hearts from G4H –/– and their wild-type (WT) littermates at baseline, during 28-minute low-flow ischemia, and during 40 minutes of reperfusion. Two baseline NMR spectra were collected simultaneously with measurement of left ventricular pressure after a 20-minute stabilization period. Global low-flow ischemia was then imposed by decreasing the coronary flow to 5% of the baseline value in hearts maintained at 37°C and surrounded by the perfusate. During ischemia and reperfusion, functional measurements and NMR spectra were collected every 4 minutes. Lactate content in the coronary effluent was measured, and the glycolytic activity during ischemia was assessed by the total lactate production during ischemia.

Protocol 2
Protocol 2 determined glycolyn and ATP contents. WT and G4H –/– hearts were perfused as in protocol 1. Hearts were freeze-clamped before or at the end of the 28-minute ischemic period for glycogen assays. Myocardial ATP content before ischemia was determined in WT and G4H –/– hearts by high-performance liquid chromatography (HPLC) and used to calibrate the NMR spectra (see below).

Protocol 3
Protocol 3 determined the rate of glucose transport in isolated perfused heart by 31P NMR spectroscopy using the glucose analogue 2-deoxyglucose (2-DG). After stabilization, the heart was switched to a glucose-free buffer in which 2 mmol/L 2-DG was added. 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 mU/mL) was added to the perfusate.14 During 2-DG perfusion, 1.2 mmol/L KH2PO4 was supplied to replenish the intracellular inorganic phosphate (Pi) pool.

Biochemical Assays
Lactate content in the coronary effluent was measured by a fluorometric assay.16 Total creatine (Cr) content in the heart was measured by a HPLC assay in this study, was 27.1 ± 4.6 nmol/mg protein for WT (n = 8) and 25.9 ± 4.0 nmol/mg protein for G4H –/– (n = 6) hearts at the end of the stabilization period. Using a value of 0.16 mg protein per mg blotted wet tissue and a value of 0.48 mL intracellular water per gram blotted wet tissue,17 [ATP] was calculated to be 9.03 ± 1.52 and 8.64 ± 1.33 mmol/L for the WT and G4H –/– groups, respectively (P = NS). Therefore, the ATP peak areas of the NMR spectra obtained at baseline were normalized to 9 mmol/L. Concentrations of phosphocreatine (PCr), Pi, and 2-DG-P were calculated by use of the ratios of their peak areas to ATP peak area. pH i was determined by comparing the chemical shift of Pi but not PCr changes with pH.

Statistical Analysis
Results are presented as mean ± SEM. Differences between the WT and G4H –/– hearts in either the fed or fasted condition were compared by 2-tailed Student’s t test or 1-way factorial ANOVA, and changes during ischemia and reperfusion were compared by repeated-measures ANOVA. Statistical analyses were performed with the Statview software program (Brainpower Inc), and a value of P < 0.05 was considered significant.

Results
Fasting Alters Glucose Utilization and Cardiac Function in GLUT4-Deficient Hearts
The characteristics of the animals studied are summarized in Table 1, and the baseline functions of the isolated perfused hearts are shown in Table 2. As previously reported, G4H –/– mice develop mild cardiac hypertrophy, and isovolumic contractile function was preserved in fed G4H –/– hearts.14 Fasting, however, resulted in a 20% decrease in left ventricular systolic pressure in G4H –/– mice (P < 0.05 versus WT or fed G4H –/–, Table 2). Heart rates and coronary flow rates were the same in all groups and were unaffected by fasting.

<table>
<thead>
<tr>
<th>TABLE 1. General Characteristics</th>
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<td>N (Sex)</td>
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<tr>
<td>WT</td>
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<td>G4H –/–</td>
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N indicates number of animals. *P < 0.05 vs WT.

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<th>TABLE 2. Baseline Function</th>
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<td>LVSP, mm Hg</td>
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<td>Fed</td>
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<td>G4H –/–</td>
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N indicates number of animals; LVSP, left ventricular systolic pressure; HR, heart rate; RPP, rate-pressure product; and CF, coronary flow.
*P < 0.05 vs WT; †P < 0.05 vs fed G4H –/–.
method and 31P NMR spectroscopy. All data are mean±SEM. Open symbols represent data obtained during insulin-free (basal) perfusion; solid symbols, data obtained during maximal insulin stimulation (2 mU/mL).

Figure 1 shows glucose uptake rates in hearts as assessed by time-dependent accumulation of 2-DG-P. Hearts of fed G4H−/− mice revealed a compensatory 3-fold increase in basal glucose uptake compared with WT [0.26 versus 0.88 (mmol/L)/min], and this corresponds to a 3-fold upregulation of GLUT1 protein.14 The compensatory increase in basal glucose uptake was completely abolished after 20 hours of fasting, although there was no change in GLUT1 content (compared with fed G4H−/−, data not shown). Thus, fasted G4H−/− had rates of basal glucose uptake identical to those of the WT (Figure 1). As expected, neither fed nor fasted G4H−/− hearts increased glucose uptake rates after insulin administration (Figure 1).

Differences in preischemic cardiac glycogen content were also noted between fed and fasted groups (Table 3). Glycogen content was 54% higher in fed G4H−/− than in fed WT (P<0.05). Fasting caused a marked decrease in glycogen content in G4H−/− hearts, possibly due to decreased glucose uptake rates during the 20-minute stabilization period. As a result, there was no difference in myocardial glycogen in fasted G4H−/− and fasted WT mice.

Intolerance to Ischemia in GLUT4-Deficient Hearts Is Associated With Impaired Glycolysis

Figure 2 shows changes in left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDevP) in WT and G4H−/− hearts during ischemia and after reperfusion. Fasted G4H−/− mice developed early and profound diastolic dysfunction (ischemic contracture), as evidenced by the marked rise in LVEDP during ischemia. On reperfusion, LVEDP remained elevated at 47±8 mm Hg in fasted G4H−/− hearts. In contrast, the degree of ischemic contracture was less severe in WT, and LVEDP was lower after reperfusion (23±11 mm Hg, P<0.05 versus G4H−/−) (Figure 2A). Furthermore, LVDevP recovered to only 40% of baseline in fasted G4H−/− at the end of reperfusion, compared with 71% in WT hearts (Figure 2B). In fed mice, changes in LV pressure during ischemia were not different in G4H−/− and WT hearts. Although there was a tendency toward higher LVDevP in fed G4H−/− mice, the difference between fed WT and G4H−/− was not statistically significant (Figure 2C). During reperfusion, the recovery of LVDevP was delayed in fed G4H−/− (P=0.048 by repeated-measures ANOVA). Ventricular function of fed G4H−/− was improved, however, compared with fasted G4H−/− (P<0.05) and recovered to an extent similar to that of WT by the end of reperfusion (84±12% versus 96±9%, P=NS).

To estimate myocardial glucose utilization (glycolytic flux) during ischemia, glycogen content at the end of ischemia and total lactate production during the ischemic period were measured (Table 3). Lactate production during ischemia in fed WT hearts was 28% higher than in fed G4H−/−

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<th>TABLE 3. Glycogen Content and Lactate Production</th>
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<td>Preischemic Glycogen, µmol Glucose/g wet wt</td>
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*P<0.05 vs corresponding WT; †P<0.05 vs corresponding fed group.
hearts. This difference became greater in fasted mice, with lactate production in WT being 75% higher than in G4H−/−. Furthermore, lactate production not accounted for by glycogen breakdown was decreased by >50% in fed and fasted G4H−/− versus their respective controls, consistent with impaired glucose transport in G4H−/− hearts during ischemia (Table 3). Thus, the ability to generate ATP by glycolysis during ischemia was markedly impaired in G4H−/− hearts from fasted mice, which was partially compensated for by increased GLUT1-mediated glucose uptake (calculated) and glycogen stores in fed G4H−/− hearts. The inability to utilize glucose during ischemia, as evidenced by lower lactate production, is therefore closely associated with functional deterioration in G4H−/− hearts.

Myocardial High-Energy Phosphate Content

Figure 3 shows representative 31P NMR spectra of isolated perfused hearts from a WT and a fed and a fasted G4H−/− mouse. From left to right, the peaks are for Pi, PCr, and α-, γ-, and β-phosphates of ATP. The area under each peak represents the amount of each compound in the heart. In the WT heart shown, 28-minute ischemia resulted in complete depletion of PCr, partial depletion of ATP, and a substantial increase in Pi. The chemical shift of Pi to the right indicates intracellular acidosis during ischemia. During reperfusion, PCr of the WT heart recovered completely. ATP recovered to 50% of the preischemia level, and the chemical shift of Pi, returned to the preischemia position, indicating a full recovery of pH. In contrast, both PCr and ATP were undetectable at the end of ischemia in the fasted G4H−/− heart, and their recovery during reperfusion was markedly depressed relative to the WT heart. Note that the baseline PCr peak in the fed G4H−/− was higher than in the WT heart and was completely depleted by the end of ischemia. Changes in ATP during ischemia and reperfusion in the fed G4H−/− heart were similar to those in the WT heart.

Figure 4 summarizes the mean values of the NMR measurements from multiple experiments. In fasted mice, there were no differences in basal [PCr], but there was a 50% increase in [PCr] in the hearts of fed G4H−/−. PCr was depleted by ischemia in all hearts. In fasted G4H−/−, PCr recovery on reperfusion was blunted, returning to 41±10% of baseline concentrations, in contrast to 75±10% in fasted WT (P<0.05). In fed mice, PCr recovery was more complete, returning to 82±9% and 95±3% of baseline concentrations in G4H−/− and WT hearts, respectively. Basal [ATP] was similar in all hearts. Ischemia resulted in accelerated depletion of ATP in fasted G4H−/− hearts. On reperfusion, ATP levels recovered to only 16±4% of baseline values in fasted G4H−/−, which contrasts with 35±13% of baseline values in fasted WT and ~60% of baseline in fed WT and G4H−/−. The [Pi] during ischemia was higher in both fed and fasted G4H−/−. In fasted G4H−/−, this was due to the greater hydrolysis of ATP. In fed G4H−/−, the higher [Pi] during ischemia represents loss of phosphate from the significantly larger PCr pool. pH declined to similar degrees in the fasted G4H−/− and WT hearts during ischemia, whereas pH was lower in the hearts of fed G4H−/−. In all groups, intracellular pH rapidly returned to baseline on reperfusion.

To further investigate the mechanisms governing the increased [PCr] in fed G4H−/−, myocardial total Cr content was determined. Total Cr content was 93±3 and 109±8 nmol/mg protein in the hearts of fed and fasted G4H−/−, respectively. These concentrations were significantly higher than those of fed and fasted WT (70±6 and 79±7 nmol/mg
protein, respectively, $P<0.05$). In fed mice, the proportion of total Cr that was phosphorylated (PCr/Cr) was similar for G4H−/− (91±12%) and WT (89±9%) hearts, resulting in higher [PCr] in fed G4H−/− than in WT. In contrast, there was a significant reduction in PCr/Cr in the hearts of fasted G4H−/− (67±5%) compared with fasted WT (81±9%). Because of this, [PCr] in the hearts of fasted G4H−/− mice was similar to that of WT mice despite the higher total Cr content.

**Discussion**

The studies described here suggest that GLUT4-mediated glucose transport represents a major mechanism by which the heart increases glucose uptake during ischemia. This mechanism is critical for myocardial protection during ischemia and for subsequent recovery of the heart after ischemic insults. Furthermore, our study also supports the hypothesis that glucose availability plays a key role in regulating glycolysis during ischemia and that enhanced glycolysis is critical to myocardial recovery. These findings offer mechanistic insight into clinical observations, suggesting that therapeutic maneuvers that enhance glucose delivery during ischemia may reduce ischemic injury and enhance survival.1–4

The more severe changes in fasted G4H−/− mice occurred as a result of loss of compensatory mechanisms established in the hearts of fed G4H−/− mice. Upregulation of GLUT1 (by 3-fold) in G4H−/− hearts is associated with a marked increase in basal glucose uptake and glycogen content in fed mice. This finding is similar to the observation of increased basal glucose utilization and glycogen content in skeletal muscle of mice overexpressing GLUT1.18 Results from this study show that these adaptations may partially compensate for the loss of GLUT4-mediated glucose transport during ischemia, as evidenced by higher glycolytic activity (calculated) and less severe functional impairment in fed versus fasted G4H−/− hearts during low-flow ischemia. The compensation in fed G4H−/− hearts was only partial, however, as evidenced by slower functional recovery during reperfusion. Furthermore, once the compensatory increase in glycogen content was abrogated in fasted G4H−/− hearts, a greater functional impairment was observed during ischemia and reperfusion.

Although G4H−/− hearts developed mild hypertrophy, it is unlikely that cardiac hypertrophy is responsible for the poor tolerance to ischemia in these hearts. Fed G4H−/− hearts showed significantly less susceptibility to ischemia than fasted G4H−/− hearts despite similar degrees of cardiac hypertrophy. Furthermore, hypertrophied hearts are not always more susceptible to ischemia. For example, mouse hearts with a moderate increase in protein kinase C expression develop cardiac hypertrophy and yet show improved tolerance to ischemia.19

A surprising change in G4H−/− hearts is the increase in cardiac Cr content. Cr plays an important role in myocardial energetics. The phosphorylated form of Cr, PCr, serves as the energy reserve for the heart by rapidly rephosphorylating ADP via the creatine kinase reaction so that ATP concentrations can be maintained in the heart. Impairment of this reserve mechanism jeopardizes contractile function of the heart.20 Thus, increased [PCr] consequent to the increased total Cr pool also represents an important compensatory mechanism that protected the fed G4H−/− from ischemic injury.

Interestingly, increased Cr content was found in G4H−/− hearts despite the presence of cardiac hypertrophy. This contrasts with other models of cardiac hypertrophy and/or heart failure in which total Cr content in the heart is reduced.21 The underlying mechanisms for the increased cardiac Cr content in G4H−/− have not been elucidated in the present study, but our observations raise the possibility that glucose transport is linked with Cr content in cardiac muscle. Elucidation of the molecular basis for this observation may lead to novel strategies for increasing cardiac Cr content, which will be of great clinical significance for the treatment of heart failure.

There are certain limitations of this study. The substrates used in this study, glucose and hexanoate, do not completely mimic physiological substrates in vivo. This may have increased the relative dependence on glucose of the hearts, primarily during normal perfusion. The lack of long-chain fatty acids in the perfusate could also contribute to decreased systolic function in fasted G4H−/− hearts during baseline perfusion. Myocardial glycogen content measured after 20 minutes of perfusion may not necessarily reflect the in vivo glycogen levels. Nevertheless, because glycogen content in fasted G4H−/− hearts is not different from that in fasted WT hearts, the functional differences observed between these 2 groups allow direct assessment of the significance of GLUT4-mediated glucose transport during ischemia and reperfusion. Glucose uptake and glycolysis during ischemia were not directly measured in this study. Instead, we measured lactate production and glycogen breakdown during ischemia. Results obtained from these measurements showed lower glycolytic activity (decreased total lactate production) and decreased exogenous glucose utilization, supporting the hypothesis of impaired glucose delivery in G4H−/− hearts during ischemia.

In summary, our results show that in the absence of the compensatory changes seen in fed G4H−/− mice, GLUT4 deficiency predisposes the heart to profound ischemic injury. The impairment in glucose utilization, as evidenced by the decreased lactate production during ischemia, resulted in dramatic depletion of ATP during ischemia and minimal recovery of ATP and PCr during reperfusion. This was associated with marked deterioration in systolic and diastolic function. Downregulation of cardiac GLUT4 has been reported in a number of pathological states, including diabetes and cardiac hypertrophy.22–25 These conditions are associated with poor tolerance to myocardial ischemia.2,26,27 Thus, strategies that enhance glucose transport represent a rational approach to the treatment of cardiac ischemia, particularly in these patients. It is of interest that the compensatory changes found in fed G4H−/− hearts offer partial protection from ischemic injury. Future efforts will be needed to elucidate the regulatory mechanisms underlying the compensatory changes. A greater understanding of these mechanisms may lead to novel approaches to myocardial protection in clinical practice.
Acknowledgments
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