Preservation of glucose metabolism in hypertrophic GLUT4-null hearts

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Departments of 1Biochemistry, 2Medicine, 4Physiology and Biophysics, and 5Pathology, Albert Einstein College of Medicine, Bronx, New York 10461-1602; 2Division of NMR Research, Department of Radiology, and 6Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2195; and 7Division of Nephrology, Department of Medicine, University of Medicine and Dentistry at New Jersey, Newark, New Jersey 07103

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Stenbit, Antine E., Ellen B. Katz, John C. Chatham, David L. Geenen, Stephen M. Factor, Robert G. Weiss, Tsu-Shuein Tsao, Ashwani Malhotra, V. P. Chacko, Christopher Ocampo, Linda A. Jelicks, and Maureen J. Charron, Preservation of glucose metabolism in hypertrophic GLUT4-null hearts. Am J Physiol Heart Circ Physiol 279: H313–H318, 2000.—GLUT4-null mice lacking the insulin-sensitive glucose transporter are not diabetic but do exhibit abnormalities in glucose and lipid metabolism. The most striking morphological consequence of ablating GLUT4 is cardiac hypertrophy. GLUT4-null hearts display characteristics of hypertrophy caused by hypertension. However, GLUT4-null mice have normal blood pressure and maintain a normal cardiac contractile protein profile. Unexpectedly, although they lack the predominant glucose transporter in the heart, GLUT4-null hearts transport glucose and synthesize glycogen at normal levels, but gene expression of rate-limiting enzymes involved in fatty acid oxidation is decreased. The GLUT4-null heart represents a unique model of hypertrophy that may be used to study the consequences of altered substrate utilization in normal and pathophysiologically conditions.

To determine its role in whole body glucose homeostasis, GLUT4, the insulin-sensitive glucose transporter, has been disrupted in the mouse by use of embryonic stem cell technology (12). GLUT4, the predominant glucose transporter in the heart, skeletal muscle, and adipose tissue, translocates from an intracellular compartment to the cell membrane in response to an insulin signal and is the rate-limiting step in glucose utilization (11, 25). Surprisingly, GLUT4 null mice are not diabetic but do exhibit abnormalities in glucose and lipid metabolism (12). One of the most prominent morphological changes caused by ablation of GLUT4 is the visible cardiac hypertrophy. The adaptive response of cardiac hypertrophy can be brought about by many stimuli, including hormones, mechanical load, and hypertension (1, 4, 6, 7, 11, 18, 23, 26). The molecular changes that occur with the development of these hypertrophies include the increase in cell size and the upregulation of fetal genes (11). We determined whether the GLUT4-null mice possess the morphological and molecular characteristics of the cardiac hypertrophies associated with the factors mentioned above. The results of these histological, biochemical, and functional studies reveal the unique nature of the GLUT4-null cardiac hypertrophy.

METHODS

Histopathology. Hearts from 3- to 5-mo-old GLUT4-null and control mice (n = 6–8/genotype) were perfused with PBS followed by Trump’s fixative (1% glutaraldehyde-4% paraformaldehyde). The fixed tissues were embedded in paraffin and sectioned at 5 μm. Sections were stained with hematoxylin and eosin or trichrome.

Magnetic resonance imaging. Magnetic resonance images (MRIs) were obtained as described previously (22). Briefly, nine GLUT4-null (26.82 ± 0.64 g) and five control (32.47 ± 1.31 g) male mice 8–12 wk of age were anesthetized with pentobarbital sodium (15 mg/kg). Once the mice were anesthetized, a standard set of electrocardiogram (ECG) leads was attached to the limbs, and an ECG signal was fed to a Gould ECG amplifier associated with a Gould 2200S recorder. The anesthetized mouse was wrapped in a small blanket and was then placed in a plastic animal holder designed to position the mouse within the 40-mm imaging coil. The probe temperature was maintained at 30°C by a water-cooling system of gradients. The Gould recorder was used to trigger the GE Omega 400 WB spectrometer to acquire images during diastole and systole. The rising phase...
arterial and ventricular pressures were obtained. Isoproterenol was infused at a constant flow of 3 ml/min with a Krebs-Henseleit bicarbonate buffer containing 3% BSA (fatty acid free), with 5 mM glucose, 0.4 mM sodium palmitate, 0.2 mM D-β-hydroxybutyrate, and 50 mM U-14C-insulin (9). Hearts were inserted into a 10-mm NMR tube and placed in a commercial broad-band 10-mm NMR probe in a Bruker 500MSL spectrometer equipped with an 11.85-T magnet. Baseline 31P- and 13C-NMR spectra were recorded, and perfusate was then switched to that containing [1-13C]glucose, [U-13C]palmitate, and 2-deoxyglucose (0.3 mM). Substrate concentrations were kept the same, and D-β-hydroxybutyrate and insulin were also unchanged. Four 3-min [1-13C]glucose NMR spectra and one 31P-NMR spectrum were collected. This sequence was repeated four times for a total of ~75 min.

RESULTS

Histological and morphometric characteristics of GLUT4-null cardiac hypertrophy. The heart weight-to-body weight ratio was used to measure the extent of hypertrophy in GLUT4-null hearts (12). GLUT4-null mice exhibit a 2.5-fold increase in this ratio compared with age-matched controls (12). Histological examination of GLUT4-null hearts revealed myocyte hypertrophy in all four chambers, vascular sclerosis, and interstitial fibrosis (Fig. 1A). The interstitial fibrosis was noted throughout the ventricles. Although the extent of fibrosis was not specifically quantitated, pathology was present in ~1–5% of the ventricular area. Control hearts did not exhibit any of the above pathologies (data not shown). Additional morphometric characterization of the extent of the hypertrophy was carried out using cardiac-gated MRI (Fig. 1B). MRI studies indicate that the thickness of the left ventricular free wall of the GLUT4-null hearts increased by 1.5-fold, whereas the other walls increased by 1.3-fold compared with control hearts (Table 1, Fig. 1B). The left ventricular internal diameter in GLUT4-null hearts was not significantly different from that in control hearts, implying an increase in wall-to-volume ratio. However, the average EF was the same in GLUT4-null and control hearts (62.7 ± 7.2 and 63.3 ± 4.7%, respectively), demonstrating that the cardiac function was similar under anesthesia.

Blood pressure measurements. The histological and morphometric results suggest that the GLUT4-null cardiac hypertrophy is characterized by concentric hypertrophy and is similar to that seen in hypertrophy associated with hypertension (1, 6, 18, 23, 26). However, unlike most rodent models of hypertrophy, GLUT4-null mice do not exhibit an increase in blood pressure compared with controls. Simultaneous arterial and left ventricular pressure tracings in anesthetized mice reveal normal blood pressure; however, the GLUT4-null hearts failed to respond to isoproterenol (Fig. 2A). Open-chest left ventricular pressure was 75 ± 8 and 80 ± 9 mmHg (P > 0.05) in the control and GLUT4-null hearts at baseline. With isoproterenol in-
myosin (V1) to the fetal form (V3) with coincident hypertrophy, there is a switch from the adult isoform of tropomyosin (TnT) and troponin I (TnI) to the fetal isoforms (V1, V3, TnT, TnI). In most animal models of cardiac hypertrophy, there are qualitative and quantitative changes in the profile of normal adult contractile proteins, including a decrease in myosin ATPase activity and the abundance of TnI and TnT.

Pathological cardiac hypertrophy is associated with diminished contractile function accompanied by a decrease in ATPase activity in pathological hypertrophy (10, 26). However, pyrophosphate gel electrophoresis and immunoblot analysis, respectively, show that GLUT4-null and normal age-matched control hearts contain the same amount of V1 myosin (V1 = 100%; Fig. 2B). Although they display histological and morphological characteristics of hypertrophy associated with hypertension, GLUT4-null hearts maintain a normal myosin protein profile.

No qualitative changes in expression of the adult isoforms of TnI or TnT were detected. Interestingly, the abundance of TnI and TnT is increased in GLUT4-null hearts compared with controls (Fig. 2B). Scanning laser densitometric quantitation (expressed in arbitrary optical density units) demonstrated that the abundance of TnI was increased 52–62% in GLUT4-null hearts compared with controls. This increase was statistically significant in females (513 ± 40.0 and 337.9 ± 38.0 for GLUT4-null and control hearts, respectively, P < 0.02, n = 4) but not in males (373.1 ± 76.8 and 230 ± 6.6 for GLUT4-null and control hearts, respectively, P < 0.11, n = 4). Similarly, expression of TnT was increased in GLUT4-null hearts. A significant 78% increase in TnT expression was measured in female GLUT4-null hearts compared with controls (1,050.7 ± 60.8 and 571.0 ± 57.2 for GLUT4-null and control hearts, respectively, P < 0.001, n = 4). A modest 31% increase in TnT expression was measured in male GLUT4-null hearts compared with controls, which did not achieve statistical significance (831.4 ± 131.2 and 636.1 ± 86.7 for GLUT4-null and control hearts, respectively, P < 0.27, n = 4).

Glucose uptake and glycogen synthesis in GLUT4-null heart. In addition to changes in contractile proteins in most models of cardiac hypertrophy, there are also alterations in substrate metabolism (13, 22). Rodent models of cardiac hypertrophy induced by overload are accompanied by a return to a fetal type of metabolism, with glucose representing the major energy source (13, 22). The lack of the predominant glucose transporter would suggest that the hypertrophy seen in the GLUT4-null hearts would not be accompanied by a shift from fatty acid to glucose metabolism. Consequently, we determined the combined rates of glucose transport and phosphorylation by using 31P-NMR spectroscopy to measure 2-deoxyglucose-6-phosphate accumulation after perfusion with 2-deoxyglucose (Fig. 3A). Unexpectedly, GLUT4-null hearts have the same rate of 2-deoxyglucose-6-phosphate accumulation compared with controls, which did not achieve statistical significance.

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**Table 1. Left ventricular wall diastolic thickness of control and GLUT4-null hearts**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Left Free Wall</th>
<th>Septum</th>
<th>Anterior Free Wall</th>
<th>Posterior Free Wall</th>
<th>A-P Internal Diameter</th>
<th>L-M Internal Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1.30 ± 0.16</td>
<td>1.17 ± 0.06</td>
<td>1.10 ± 0.10</td>
<td>1.20 ± 0.18</td>
<td>3.07 ± 0.27</td>
<td>3.54 ± 0.28</td>
</tr>
<tr>
<td>GLUT4-null</td>
<td>9</td>
<td>1.95 ± 0.15a</td>
<td>1.51 ± 0.06a</td>
<td>1.42 ± 0.09a</td>
<td>1.56 ± 0.07a</td>
<td>2.36 ± 0.26</td>
<td>3.50 ± 0.32</td>
</tr>
<tr>
<td>Magnitude increase</td>
<td>1.5</td>
<td>1.3</td>
<td></td>
<td>1.3</td>
<td>1.3</td>
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</tr>
</tbody>
</table>

Values are means ± SE in mm; n, no. of mice. Gated magnetic resonance images were used to determine the wall thickness of control and GLUT4-null hearts. Gating to 75% of the R-R interval was considered diastole. A-P, anterior-posterior; L-M, lateral-medial. *P < 0.05, by Student’s t-test.

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![Histological images of GLUT4-null hearts](image_url)
**H316 GLUCOSE METABOLISM IN HYPERTROPHIC GLUT4-NULL HEARTS**

**DISCUSSION**

GLUT4-null mice exhibit a unique cardiac hypertrophy. Histological and morphometric analyses suggest that GLUT4-null hearts have characteristics seen in pathological hypertrophy. The vascular sclerosis, extensive interstitial fibrosis, and concentric hypertrophy in the GLUT4-null heart are similar to those seen in hypertrophy associated with hypertension (1, 6, 18, 23, 26). However, blood pressure is not increased in the GLUT4-null mice. Additionally, the failure of the GLUT4-null hearts to respond to isoproterenol is in contrast to that in models of hypertrophy associated with hypertension. In these models, the relationship between the plasma membrane Ca\(^{2+}\) current and the Ca\(^{2+}\) release from the sarcoplasmic reticulum has been shown to be reduced (8). Stimulation with isoproterenol overcomes this difference to increase the contractility of these hearts (8). This lack of hemodynamic change with the inotrope in the GLUT4-null heart suggests alterations in the β-adrenergic receptor number and/or affinity, alterations of the adenylate cyclase pathway, or changes in Ca\(^{2+}\) handling within the cell (8). Isoproterenol is known to have chronotropic as well as inotropic effects on the heart. A significant increase in heart rates was not observed in GLUT4-null hearts compared with controls in response to isoproterenol. Thus it is unlikely that increases in heart rate could account for the absence of a pressure rise in GLUT4-null hearts.

Other indicators of hypertrophy were also studied. Analysis of contractile proteins, which are altered in hypertrophy associated with hypertension, show that these proteins remain unchanged in GLUT4-null hearts as age-matched controls under conditions in which the perfusate contains 5 mM glucose, which is similar to circulating glucose levels in vivo (12). Additionally, \(^{13}C\)-NMR spectra were collected from hearts perfused with \(^{13}C\)glucose for 75 min to assess the rate of glycogen synthesis. At 30 min, glycogen synthesis rates were three times higher than in age-matched controls (Fig. 3B). These studies show that GLUT4-null hearts take up normal amounts of glucose sufficient to maintain a high level of glucose metabolism.

Measurement of rate-limiting enzymes of fatty acid oxidation. In most rodent models of hypertrophy, as glucose metabolism increases, fatty acid oxidation decreases (22). The mRNA levels of medium- and long-chain acyl-CoA dehydrogenase (MCAD and LCAD, respectively) are directly related to the extent of fatty acid oxidation in myocytes (13, 22). LCAD, which catalyzes the first step in long-chain fatty acid oxidation, and MCAD, which is the rate-limiting step in medium-chain fatty acid oxidation, have been shown to be downregulated in cardiac hypertrophy (22). It was determined by Northern blot analysis that MCAD and LCAD mRNA expression are significantly decreased in GLUT4-null hearts compared with controls (Fig. 3C). The level of carnitine palmitoyl transferase (CPT1) mRNA, an enzyme responsible for the transport of long-chain fatty acid into the mitochondria, was not changed (data not shown). One of the factors leading to the decreased expression of LCAD and MCAD in GLUT4-null hearts could be the significantly decreased availability of fatty acids in the fed state in GLUT4-null mice, in which adipose tissue is severely diminished (12).
hearts. In the present study, no changes were observed in expression of the different isoforms of the cardiac regulatory proteins TnI and TnT. Interestingly, TnI and TnT protein levels were somewhat increased in GLUT4-null hearts. The elevated expression of cardiac regulatory proteins may represent a compensatory mechanism to improve contractility of the severely hypertrophied hearts of GLUT4-null mice (17). In an earlier study it was demonstrated that TnI was reduced in diabetic hearts, and this could be responsible for the loss in Ca$^{2+}$ sensitivity in the streptozotocin-induced model of diabetic cardiomyopathy (15, 16). Additionally, increased TnI phosphorylation was noted after constitutive overexpression of insulin-like growth factor I; this could provide the molecular basis for reduction in myofilament Ca$^{2+}$ sensitivity of tension in myocytes (19). It is possible that phosphorylation of TnI and/or TnT may be modulated in GLUT4-null hearts, inasmuch as expression of no other isoform was observed. The increase in regulatory protein content in GLUT4-null hearts could be one of several changes that permit more economical force generation by the heart (17). Additional studies that focus specifically on phosphorylation of cardiac regulatory proteins and Ca$^{2+}$ sensitivity of the contractile apparatus will reveal the effects of the altered expression of TnI and TnT noted in this unique model of hypertrophy.

Finally, although they lack the major glucose transporter, GLUT4-null hearts take up normal amounts of glucose and synthesize glycogen at normal-to-higher...
rates. These unexpected results are accompanied by a decrease in mRNA levels of enzymes of fatty acid oxidation, suggesting a decrease in use of fatty acids as an energy source. This could be due in part to the decreased availability of fatty acids in the fed state in GLUT4-null mice, in which adipose tissue is severely diminished (12). Cardiac hypertrophy occurs when the heart is forced to rely on glucose metabolism for energy by treating the animal with an inhibitor of carnitine palmitoyl transferase, such as etomoxir, to decrease fatty acid oxidation (21). The above results suggest that the hypertrophy of the GLUT4-null heart is more like that seen after treatment with fatty acid oxidation inhibitors or after endurance training. The GLUT4-null heart represents a novel model of hypertrophy that may be used to study molecular changes in substrate utilization that affect cardiac function under normal and pathological conditions.

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