Hypertrophied rat hearts are less responsive to the metabolic and functional effects of insulin

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Allard, Michael F., Richard B. Wambolt, Sarah L. Longnus, Mark Grist, Carmen P. Lydell, Hannah L. Parsons, Brian Rodrigues, Jennifer L. Hall, William C. Stanley, and Gregory P. Bondy. Hypertrophied rat hearts are less responsive to the metabolic and functional effects of insulin. Am J Physiol Endocrinol Metab 279: E487–E493, 2000.—We determined the effect of insulin on the fate of glucose and contractile function in isolated working hypertrophied hearts from rats with an aortic constriction (n = 27) and control hearts from sham-operated rats (n = 27). Insulin increased glycolysis and glycogen in control and hypertrophied hearts. The change in glycogen was brought about by increased glycogen synthesis and decreased glycolysis in both groups. However, the magnitude of change in glycolysis, glycogen synthesis, and glycogenolysis caused by insulin was lower in hypertrophied hearts than in control hearts. Insulin also increased glucose oxidation and contractile function in control hearts but not in hypertrophied hearts. Protein content of glucose transporters, protein kinase B, and phosphatidylinositol 3-kinase was not different between the two groups. Thus hypertrophied hearts are less responsive to the metabolic and functional effects of insulin. The reduced responsiveness involves multiple aspects of glucose metabolism, including glycolysis, glucose oxidation, and glycogen metabolism. The absence of changes in content of key regulatory molecules indicates that other sites, pathways, or factors regulating glucose utilization are responsible for these findings.

cardiac hypertrophy; insulin resistance; glycolysis; glucose oxidation; glycogen

GLUCOSE TAKEN UP by the myocardium is phosphorylated and is then either catabolized directly or incorporated into glycogen (12, 26). When catabolized directly, glucose passes through the glycolytic pathway to be oxidized to CO2 or nonoxidatively catabolized to lactate and alanine (26). Glucose can also be converted into glycogen via the action of glycogen synthase, a major regulatory enzyme of glycogen synthesis (26). Glucose utilization and its fate in the myocardium are controlled by many factors, including cardiac work, ischemia, or hypoxia, and a number of hormones, with insulin as one of the most important (16, 26).

Insulin has both direct and indirect effects on glucose and glycogen metabolism in the heart (see Ref. 6 for review). Insulin directly increases glucose transport (30, 34), glycolysis (19), and, possibly, glucose oxidation. Glycogen metabolism is also directly affected by way of insulin-mediated effects on the enzyme glycogen synthase (1, 26). Insulin indirectly affects myocardial glucose and glycogen metabolism by lowering circulating fatty acid concentrations in vivo (6).

Resistance to the effects of insulin on whole body and skeletal muscle glucose uptake has been described in both patients and experimental animals with hypertension (7, 27). A reduction in insulin-induced glucose uptake in the myocardium has also been observed in hypertrophied hearts from spontaneously hypertensive rats (23) and patients (24). In these latter studies, it was not determined whether the reduced responsiveness of the hypertrophied hearts to insulin was limited to glucose uptake or the major metabolic fates of glucose (glycolysis, glucose oxidation, glycogen) in the myocardium were also less responsive to insulin.

Hearts, including hypertrophied hearts, generate most of their energy from the catabolism of fatty acids (3). However, the fate of glucose and the extent of its use in the myocardium are known to significantly affect heart function (31, 32). As a consequence, there is increasing interest in approaches to therapeutically enhance glucose use to improve heart function in such settings as congestive heart failure and ischemic heart disease (31). Glucose-insulin-potassium (GIK) therapy
is one such approach that is receiving renewed interest as a means to improve ischemic and posts ischemic heart function (31, 32). It has very recently been proposed that patients with left ventricular hypertrophy may be a subgroup especially likely to derive clinical benefit from GIK therapy (4). To provide a physiologically relevant baseline for comparison before embarking on studies investigating GIK therapy in hypertrophied hearts during ischemia and reperfusion, characterization of the effect of insulin on all aspects of glucose metabolism in the nonischemic hypertrophied heart is required. Thus we determined whether the major fates of glucose (glycolysis, glucose oxidation, glycogen) in the nonischemic hypertrophied heart are less responsive to the effects of physiological levels of insulin.

METHODS

Animal Model

As previously described (3), a mild pressure-overload left ventricular hypertrophy was produced in 3-wk-old male Sprague-Dawley rats by constriction of the suprarenal abdominal aorta with a metallic clip (0.4 mm diameter). In control rats, the aorta was isolated but not constricted. Experiments were performed on hearts excised from animals 8 wk after surgery. Food and water were available ad libitum. These experiments were approved by the University of British Columbia Committee on Animal Care in accordance with guidelines set out by the Canadian Council on Animal Care.

Isolated Heart Preparation and Perfusion Protocol

As described (2, 3), the aorta and left atrium were cannulated in hearts removed from halothane (3–4%)-anesthetized rats. Hearts were then perfused with modified Krebs-Henseleit (KH) solution as isolated working preparations for 30 min at a left atrial preload of 11.5 mmHg and an aortic afterload of 80 mmHg. The KH solution contained 1.2 mM palmitate prebound in albumin, 5.5 mM [5-3H]glucose/[U-14C]glucose, 0.5 mM lactate, and 2.5 mM calcium. Palmitate and lactate were included in the perfusate because others have shown that alternative substrates are required to demonstrate insulin responsiveness of glucose uptake in isolated working hearts (29). High concentrations of palmitate were used to minimize differences in rates of fatty acid oxidation between control and hypertrophied hearts. Low rates of palmitate oxidation, as seen in hypertrophied hearts perfused with low palmitate concentrations (0.4 mM; see Ref. 3), lead to a stimulation of glucose use that may mask any response to insulin. Because insulin-mediated effects on glucose and glycogen metabolism were the focus of this investigation, fatty oxidation was not measured. During the perfusion, the KH solution was oxygenated with 95% O2 and 5% CO2, and was maintained at 37°C, and contained either 0, 40, or 100 mU/l insulin. These concentrations of insulin were chosen because they span the physiological range in the rat (14).

Heart function was measured at 10-min intervals during the working heart perfusions. Heart rate and peak systolic pressure were recorded with a pressure transducer (Viggo-spectramed, Oxnard, CA) in the aortic afterload line. Cardiac output and aortic flow were measured by means of flow probes (Transonic Systems, Ithaca, NY) in the preload and aortic outflow lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. The rate pressure product (heart rate multiplied by peak systolic pressure) and hydraulic power (the product of cardiac output and peak systolic pressure) were calculated to determine the work performed by the heart (3, 10, 20).

A separate series of experiments was also performed to determine whether differences in coronary flow were responsible for any difference in glucose metabolism observed between control and hypertrophied hearts. This series was identical to the first except that all perfusate contained 100 mU/l insulin, and hearts were exposed to different aortic afterloads (control, 60 mmHg, n = 6; hypertrophy, 80 mmHg, n = 6) such that rates of coronary flow per unit mass of myocardium were comparable between the two groups.

At the end of the perfusion period, hearts were frozen quickly by clamping with tongs cooled to the temperature of liquid nitrogen. Selected hearts were also frozen at the end of the initial 10-min Langendorff perfusion. The frozen tissue was weighed to determine ventricular weight, powdered, and then stored at −70°C. Any remaining atrial tissue and a portion of ventricular tissue were dried, weighed, and used in the calculation of total heart weight.

Measurement of Glucose Oxidation and Glycolysis

Glucose oxidation rates were measured by quantitatively determining the rate of 14CO2 production from [U-14C]glucose (liberated at the pyruvate dehydrogenase reaction and in the tricarboxylic acid cycle) released as a gas and dissolved in the perfusate as [14C]bicarbonate (2, 3). Rates of glycolysis were determined by measuring the rate of 3H2O production from [5-3H]glucose (2, 3). Samples for analysis were taken every 10 min throughout the perfusion. Samples were ultimately placed in vials containing scintillation cocktail and were counted by standard techniques. In these experiments, glycolysis and glucose oxidation refer to glycolysis and oxidation of exogenous glucose, because the contribution of glycogen was not measured.

Biochemical Analysis

Myocardial glycogen and net incorporation of radiolabeled glucose into glycogen were determined as previously described (28). Homogenate samples containing 40–50 μg total protein were solubilized by boiling in sample buffer (50 mM Tris-HCl, 2% SDS, 10% glycerol, and 0.01% bromphenol blue, pH 6.8), separated by electrophoresis on 10% SDS-polyacrylamide gels (200 V, 45 min), and transferred by electroblotting to a nitrocellulose membrane (110 V, 1 h). Equivalence of protein loading was confirmed by Ponceau S staining and/or detection of glyceraldehyde-3-phosphate dehydrogenase. Blots were blocked against nonspecific protein binding by incubation in blocking buffer (PBS, 5% nonfat milk protein, and 0.1% Tween 20) at room temperature for at least 1 h. They were then incubated overnight with the following primary antibodies: rabbit polyclonal GLUT-1 (1:1,000 dilution) and GLUT-3 (1:2,500 dilution; Alpha Diagnostics, San Antonio, TX), mouse monoclonal GLUT-4 (IF-8 clone, 1:3,000 dilution; Biogenesis, Bournemouth, UK), sheep polyclonal protein kinase B (PKB)-a (1:2,000; Upstate Biotechnology, Lake Placid, NY), or mouse monoclonal phosphatidylinositol 3-kinase (PI3K) p85 (UB93–3 clone, 1:400;
Table 1. Mechanical function of control and hypertrophied working rat hearts exposed to different insulin concentrations

<table>
<thead>
<tr>
<th>Insulin, mU/l</th>
<th>Control</th>
<th>Hypertrophy</th>
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<tr>
<td></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>263 ± 8</td>
<td>285 ± 7a</td>
</tr>
<tr>
<td>Peak systolic pressure, mmHg</td>
<td>129 ± 5</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>59 ± 3</td>
<td>65 ± 5</td>
</tr>
<tr>
<td>Rate pressure product, mmHg·beats·min⁻¹·10⁻⁸</td>
<td>33 ± 1</td>
<td>36 ± 1a</td>
</tr>
<tr>
<td>Hydraulic power, mW</td>
<td>17.8 ± 1.4</td>
<td>20.6 ± 1.3</td>
</tr>
<tr>
<td>Coronary flow, ml·min⁻¹·g wet wt⁻¹</td>
<td>18 ± 1</td>
<td>17 ± 2</td>
</tr>
</tbody>
</table>

Values, measured at the end of perfusion, are means ± SE; n = 5/group. *Different from control, P < 0.05. †Different from 0 mU/l insulin hypertrophy, P < 0.05. ‡Different from 0 mU/l insulin control, P < 0.05. §Different from 100 mU/l insulin control, P < 0.05.

Upstate Biotechnology). Unbound primary antibody was removed by three consecutive 15-min washes in PBS. Blots were then incubated at room temperature for 1 h with anti-rabbit secondary antibody (Bio-Rad, Hercules, CA) for GLUT-1 (1:5,000 dilution) and GLUT-3 (1:2,500 dilution), anti-mouse secondary antibody (Bio-Rad) for GLUT-4 (1:2,000 dilution) and PI3K (1:4,000 dilution), or anti-sheep secondary antibody (Upstate Biotechnology) for PKB (1:4,000 dilution). Detection of the secondary antibody was performed after three consecutive 15-min washes in PBS using the enhanced chemiluminescence-based detection system (Renaissance; NEN Life Science Products, Boston, MA) by exposure to Kodak X-Omat blue film. Bands were quantified by densitometry.

**Statistical Analysis**

Weight, glucose transporter, PKB, PI3K, and glycogen data were analyzed using a one- or two-way ANOVA. Left ventricular function, glucose oxidation, and glycolysis were examined using a repeated-measures two-way ANOVA. The sequential rejective Bonferroni procedure was used to correct for multiple comparisons and tests (13). Values are expressed as means ± SE. A corrected P value of >0.05 was considered to be nonsignificant (NS).

**RESULTS**

**Animal Data**

Heart weight of aortic-banded rats (2.02 ± 0.05 g, n = 27) was increased compared with the heart weight of sham-operated control rats (1.57 ± 0.03 g, n = 27, P < 0.05). Body weight of aortic-banded rats (433 ± 7 g) was not significantly different from sham-operated control rats (428 ± 9 g, P = NS). There were no differences in heart weight or body weight among different insulin groups in either control or aortic-banded rats (data not shown). Plasma insulin was lower in aortic-banded rats (44.6 ± 4.6 mU/l, n = 3) than in control rats (61.8 ± 6.2 mU/l, n = 3, P < 0.05).

**Mechanical Function of Control and Hypertrophied Hearts**

Mechanical function of control and hypertrophied hearts exposed to different insulin concentrations is summarized in Table 1. All parameters measured were stable throughout the perfusion period in all groups (data not shown). In control hearts, 40 mU/l insulin produced a small, but significant, increase in heart rate and rate pressure product. However, all indexes of function, except coronary flow, were significantly increased above basal in control hearts perfused with 100 mU/l insulin. There were no significant differences between control hearts perfused with 40 mU/l and those perfused with 100 mU/l insulin. Insulin failed to increase any parameter of function in hypertrophied hearts with the exception of heart rate, which was increased in hypertrophied hearts perfused with 100 mU/l insulin.

Under basal (insulin-free) conditions, cardiac output and hydraulic power were significantly lower in hypertrophied hearts than in control hearts. In the presence of insulin, all parameters of function were lower in hypertrophied hearts than in control hearts, with the exception of peak systolic pressure at 40 mU/l insulin and heart rate at 100 mU/l insulin. Coronary flow was lower in hypertrophied hearts than in control hearts under all perfusion conditions.

Function of control hearts exposed to 60 mmHg and hypertrophied hearts exposed to 80 mmHg is summarized in Table 2. Peak systolic pressure, rate pressure product, and hydraulic power were greater in hypertrophied hearts exposed to 80 mmHg than in control hearts exposed to 60 mmHg. There were no differences in coronary flow. Coronary resistance was higher in hypertrophied hearts than in control hearts in both

Table 2. Mechanical function of control and hypertrophied working rat hearts exposed to different aortic afterloads

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypertrophy</th>
</tr>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>223 ± 7</td>
<td>227 ± 6</td>
</tr>
<tr>
<td>Peak systolic pressure, mmHg</td>
<td>104 ± 5</td>
<td>132 ± 3*</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>44 ± 1</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Rate pressure product, mmHg·beats·min⁻¹·10⁻⁸</td>
<td>23 ± 1</td>
<td>30 ± 1*</td>
</tr>
<tr>
<td>Hydraulic power, mW</td>
<td>10.2 ± 0.4</td>
<td>14.3 ± 0.6a</td>
</tr>
<tr>
<td>Coronary flow, ml·min⁻¹·g wet wt⁻¹</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>

Values, measured at the end of perfusion, are means ± SE; n = 6/group. *Different from control, P < 0.05.
series and was not changed appreciably by insulin or perfusion conditions (data not shown).

**Myocardial Glucose Metabolism**

**Glucose oxidation.** Rates of glucose oxidation were increased by insulin in control hearts (Fig. 1; 31 ± 13 and 87 ± 8% above basal by 40 and 100 mU/l insulin, respectively). Unlike control hearts, insulin did not significantly augment glucose oxidation in hypertrophied hearts (Fig. 1). Indeed, in the presence of 100 mU/l insulin, glucose oxidation was lower in hypertrophied hearts than in control hearts.

Rates of glucose oxidation were not significantly different between hypertrophied hearts exposed to an 80-mmHg aortic afterload (289 ± 24 nmol·min⁻¹·g dry wt⁻¹) and control hearts exposed to a 60-mmHg aortic afterload (232 ± 18 nmol·min⁻¹·g dry wt⁻¹, P = NS).

**Glycolysis.** Compared with basal, rates of glycolysis were increased 29 ± 7% by 40 mU/l insulin and 92 ± 4% by 100 mU/l insulin in control hearts (Fig. 2). Rates of glycolysis in hypertrophied hearts were significantly greater than those in corresponding control hearts under all perfusion conditions (Fig. 2). Insulin also increased rates of glycolysis in hypertrophied hearts (Fig. 2). However, the magnitude of the insulin-induced increase in rates of glycolysis in hypertrophied hearts was substantially less than that observed in control hearts (16 ± 1 and 27 ± 3% at 40 and 100 mU/l insulin, respectively).

Rates of glycolysis were significantly greater in hypertrophied hearts exposed to an 80-mmHg aortic afterload (3,250 ± 110 nmol·min⁻¹·g dry wt⁻¹) compared with control hearts exposed to a 60-mmHg aortic afterload (2,263 ± 108 nmol·min⁻¹·g dry wt⁻¹, P < 0.05).

**Myocardial Glycogen**

Myocardial glycogen content of control and hypertrophied hearts is summarized in Table 3. Glycogen content was higher in hypertrophied hearts compared with control hearts, but only in the insulin-free group. Hearts in both groups synthesized (as reflected by net incorporation of radiolabeled glucose) and degraded (as reflected by the change in unlabeled glycogen) glycogen during all of the working heart perfusions. In the absence of insulin, net degradation of glycogen exceeded glycogen synthesis in both groups. Insulin increased glycogen synthesis and decreased glycogen degradation in both control and hypertrophied hearts. However, the extent to which insulin increased synthesis and decreased degradation of glycogen was lower in hypertrophied hearts than in control hearts. Glycogen degradation in control hearts was significantly decreased by 40 and further decreased by 100 mU/l insulin in control hearts. In hypertrophied hearts, on the other hand, glycogen degradation was only significantly decreased by 100 mU/l insulin. At this level of insulin, glycogen degradation was reduced 20-fold below basal in control hearts but only 5-fold below basal in hypertrophied hearts.

**Protein Content of Glucose Transporters, PKB, and PI3K**

Figure 3 shows immunobots of GLUT-1 (Fig. 3A), GLUT-4 (Fig. 3B), PKB (Fig. 3C), and PI3K (Fig. 3D) proteins in control and hypertrophied hearts. GLUT-3
protein was undetectable in both groups (data not shown). Densitometric analysis revealed no significant differences between control and hypertrophied hearts (GLUT-1, control 52.1 ± 6.10.9 vs. hypertrophy 61.6 ± 5.1 densitometric units, P < NS; GLUT-4, control 32.4 ± 6.3.5 vs. hypertrophy 32.4 ± 4.6 densitometric units, P = NS; PKB, control 72.1 ± 6.2 vs. hypertrophy 77.4 ± 2.0 densitometric units, P = NS; PI3K, control 57.4 ± 6.11 vs. hypertrophy 59.1 ± 6.10 densitometric units, P = NS).

DISCUSSION

In these experiments, we confirm and broaden the concept that hypertrophied hearts are less responsive to the metabolic actions of relevant concentrations of insulin. Of major importance, we show that the reduced responsiveness of the hypertrophied heart to insulin affects glucose metabolism widely, including glycolysis, glucose oxidation, and glycogen, and occurs in the absence of changes in glucose transporter, PKB, and PI3K protein content. An additional and unexpected finding is the observation that hypertrophied hearts are also less responsive to the effects of insulin on myocardial contractile function.

As we (2, 3) and others (8) have previously observed in normoxic nonischemic hypertrophied hearts, rates of glycolysis are increased in hypertrophied hearts compared with those in control hearts under all perfusion conditions (Fig. 2). This observation is consistent with the increased accumulation of 2-deoxyglucose 6-phosphate observed in hypertrophied dog (36) and rat (15) hearts in vivo. In these studies, the authors concluded that the increased uptake of 2-deoxyglucose could not be explained by increased left ventricular pressure and energy expenditure or by differences in insulin concentration. Our own data indicate that hypertrophied hearts from aortic-banded rats are not exposed to greater insulin stimulation in vivo, because plasma insulin is not increased in these animals. The accelerated rates of glycolysis are also in keeping with findings that the activity of a number of glycolytic enzymes determined in vitro is greater in hearts exposed to a pressure overload than in normal hearts (33) and that isoenzymes of lactate dehydrogenase (5) and enolase

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**Table 3. Glycogen content in control and hypertrophied working rat hearts exposed to different insulin concentrations**

<table>
<thead>
<tr>
<th>Insulin, mU/l</th>
<th>Total glycogen</th>
<th>Net incorporation of labeled glucose</th>
<th>Unlabeled glycogen</th>
<th>Change in unlabeled glycogen</th>
<th>Net incorporation of labeled glucose</th>
<th>Unlabeled glycogen</th>
<th>Change in unlabeled glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypertrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>98.2 ± 2.7</td>
<td>+13.0 ± 1.2</td>
<td>85.2 ± 3.4</td>
<td>-29.7 ± 3.4</td>
<td>112.9 ± 2.2</td>
<td>+16.2 ± 0.6</td>
<td>-25.4 ± 2.3</td>
</tr>
<tr>
<td>40</td>
<td>120.7 ± 3.0</td>
<td>+16.9 ± 0.8</td>
<td>103.8 ± 2.8</td>
<td>-11.1 ± 2.8</td>
<td>125.1 ± 2.2</td>
<td>+22.8 ± 0.7</td>
<td>102.3 ± 2.1</td>
</tr>
<tr>
<td>100</td>
<td>133.2 ± 2.2</td>
<td>+19.7 ± 1.1</td>
<td>113.5 ± 2.6</td>
<td>-1.4 ± 2.6</td>
<td>137.7 ± 2.6</td>
<td>+20.5 ± 0.6</td>
<td>117.3 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7/group. Units are μmol/g dry wt. Change in unlabelled glycogen content was determined by comparison with values of glycogen at the end of the Langendorff perfusion (control, 114.9 ± 4.3 μmol/g dry wt, n = 8; hypertrophy, 122.1 ± 3.9 μmol/g dry wt, n = 8). *Different from 0 mU/l insulin control, P < 0.05. †Different from 0 mU/l insulin hypertrophy, P < 0.05. ‡Different from 40 mU/l insulin control, P < 0.05. §Different from 40 mU/l insulin hypertrophy, P < 0.05.
Insulin increases rates of glycolysis in both control and hypertrophied hearts compared with rates in control and hypertrophied hearts perfused in the absence of insulin (Fig. 2). This observation is entirely consistent with the well-described actions of insulin on myocardial glucose uptake/phosphorylation (16, 22, 26, 30, 34) and glycolysis (19). Our finding that glycolysis is less responsive to insulin in hypertrophied hearts than in control hearts expands upon the results of Paternostro et al. (23, 24). Our data show that responsiveness to insulin in the hypertrophied heart is reduced but not absent and involves not only glucose uptake but also glycolysis, an important insulin-sensitive fate of glucose in the myocardium.

Glucose oxidation is stimulated by insulin in control hearts (Fig. 1), a finding in keeping with that of others (21). Activation of pyruvate dehydrogenase phosphatase is believed to be the mechanism by which insulin directly activates the pyruvate dehydrogenase complex (25). However, enhanced production of pyruvate, brought about by the insulin-induced acceleration of glycolysis, may indirectly stimulate glucose oxidation by inhibiting pyruvate dehydrogenase kinase and may lead to a greater proportion of pyruvate dehydrogenase in the dephosphorylated active form (25). Thus, on the basis of the data obtained in this study, it is not possible to definitively state whether insulin stimulates glucose oxidation in control hearts by direct or indirect means.

In keeping with previous observations (2, 3, 8), coupling between glycolysis and glucose oxidation is lower in hypertrophied hearts than in control hearts regardless of insulin concentration (see Figs. 1 and 2). The mechanisms responsible for the reduced coupling between glycolysis and glucose oxidation in hypertrophied hearts are not yet known. Importantly, the fact that reduced coupling is present in hypertrophied hearts in the absence of insulin suggests that alterations in insulin signaling are not primarily responsible. Additionally, the finding that coupling is reduced in hypertrophied hearts even when coronary flow is similar to control indicates that ischemia and hypoxia are not responsible.

Insulin increases glycogen synthesis and decreases glycogenolysis in both control and hypertrophied hearts (Table 3). These findings are not surprising because insulin is known to activate glycogen synthase (1, 6, 26), the major enzyme regulating glycogen synthesis in the heart and other tissues (1), and to decrease glycogenolysis in isolated normal rat hearts (10). Higher rates of glucose uptake likely also contribute to the insulin-induced change in myocardial glycogen metabolism, presumably by increasing glucose 6-phosphate and UDP-glucose. Of great interest is the novel observation that the overall extent of change in synthesis and degradation of glycogen caused by insulin is lower in hypertrophied hearts than in control hearts (Table 3), with the difference particularly evident for glycogen degradation. Additionally, the insulin concentration required to significantly suppress glycogen degradation is higher in hypertrophied hearts than in control hearts. In other words, glycogen metabolism in hypertrophied hearts is not only less responsive to insulin but is also less sensitive to insulin.

Insulin significantly improves contractile function of control hearts (Table 1). Positive effects of insulin on myocardial function are well known, having been described in hearts in vivo and in vitro as well as in isolated muscle and myocyte preparations (9, 18, 35). The improvement in function with increasing insulin concentration cannot be attributed to increases in coronary flow, because rates of coronary flow are not different among the different insulin groups (Table 1). As with glucose and glycogen metabolism, contractile function of the hypertrophied hearts is less responsive to the effects of insulin. To our knowledge, reduced responsiveness of hypertrophied heart function to insulin has not been described previously.

From a mechanistic perspective, reduced responsiveness of hypertrophied hearts to insulin may be due to decreased glucose transporter content, as proposed by some (23, 24). However, our data (Fig. 3) indicate that alterations in glucose transporter expression are not responsible for the reduced responsiveness to insulin in this model of cardiac hypertrophy. Nevertheless, it is conceivable that changes occur in glucose transporter activity and/or distribution that contribute to the reduced responsiveness of hypertrophied hearts to insulin. Investigation of these possibilities, which was beyond the scope of the present study, is currently underway. An acceleration of glycolysis to near maximal values may also have contributed to the reduced responsiveness to insulin in hypertrophied hearts in this study. However, this change does not adequately account for the decreased responsiveness of glucose oxidation, glycogen metabolism, or contractile function to insulin. Alternatively, but not necessarily exclusively, alterations in insulin signaling, involving factors other than the content of PKB and PI3K, may be responsible for these findings. The intracellular signaling pathway responsible for insulin-induced changes in glucose uptake, glycolysis, and glycogen metabolism is separate from that for glucose oxidation (6). Because responsiveness to insulin is reduced in both pathways in hypertrophied hearts, only changes in either proximal (e.g., insulin receptor) and/or multiple distal sites in the insulin signaling cascade could account for these findings. Which, if any, of these possibilities is responsible remains to be determined.

The results of our study also have potentially important clinical implications, because they raise the possibility that GIK therapy may be ineffective in directly altering cardiac function and metabolism of glucose and glycogen in hypertrophied hearts during ischemia and reperfusion. Because our data are not completely applicable to the setting of ischemia and reperfusion, further studies using ischemic and postischemic hypertrophied hearts exposed to high concentrations of in-
insulin and glucose are required to determine whether this is the case.

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REFERENCES


21. Lopaschuk GD, Wambolt RB, and Barr RL. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. J Pharmacol Exp Ther 264: 135–144, 1993.


