Influence of Glucose and Insulin on the Exaggerated Diastolic and Systolic Dysfunction of Hypertrophied Rat Hearts During Hypoxia

Michael J. Cunningham, Carl S. Apstein, Ellen O. Weinberg, W. Mark Vogel, and Beverly H. Lorell

Myocardial hypertrophy can result in increased sensitivity toward the development of mechanical dysfunction during hypoxia. Alterations in glycolytic metabolism may contribute to this. We studied the response to 15 minutes of hypoxia in hypertrophied (deoxycorticosterone-salt hypertension model) and nonhypertrophied rat hearts and examined the influence of a high glucose (27.5 mM) and insulin (100 mU/ml) concentration. In response to hypoxia in the presence of a normal glucose concentration (5.5 mM), left ventricular end-diastolic pressure was higher in hypertrophied than in nonhypertrophied hearts (65±6 vs. 44±4 mm Hg; p<0.05). Perfusion with high glucose and insulin blunted the rise in left ventricular end-diastolic pressure in both hypertrophied and nonhypertrophied hearts and abolished the difference in diastolic dysfunction between groups during hypoxia (26±2 vs. 32±4 mm Hg, respectively; p=NS). At end hypoxia in the presence of a normal glucose concentration, developed pressure was more depressed in hypertrophied than in nonhypertrophied hearts (11±1 vs. 18±1% of baseline, respectively; p<0.05). Perfusion with high glucose and insulin resulted in improved function in both groups during hypoxia such that a greater impairment of developed pressure was no longer present in the hypertrophied versus nonhypertrophied hearts (21±1 vs. 24±2% of baseline, respectively; p=NS). At the end of hypoxic perfusion in the presence of a normal glucose concentration, hypertrophied hearts were producing 38% less lactate than nonhypertrophied hearts. Perfusion with high glucose and insulin increased lactate production in both groups and equalized lactate production between groups. Thus, the greater deterioration in hemodynamic function in hypertrophied hearts compared with nonhypertrophied hearts during hypoxia is associated with lower lactate production. Both the exaggerated hemodynamic dysfunction and deficient lactate production can be ameliorated by perfusion with a high glucose concentration and insulin. (Circulation Research 1990;66:406–415)

Pressure-overload cardiac hypertrophy serves as a beneficial adaptation by normalizing wall stress.1 Hypertrophy can be disadvantageous, however, in that it can result in an impairment of myocardial relaxation that is particularly pronounced during myocardial hypoxia.2–8 Previous work in this laboratory7,8 has shown that hypertrophied hearts from hypertensive rats develop greater diastolic dysfunction during hypoxia compared with controls. Other studies9–11 showing the importance of glycolytic metabolism in preserving diastolic function during ischemia and hypoxia in nonhypertrophied hearts raise the possibility that alterations in glycolytic metabolism could contribute to this greater impairment in diastolic function in hypertrophied hearts during hypoxia.
We studied the role that alterations in glucose and insulin availability play in modulating hypoxia in hypertrophied rat hearts with particular regard to the relation between glycolytic metabolism and diastolic dysfunction. In the presence of a normal glucose concentration, we found that diastolic and systolic function deteriorated to a greater extent during hypoxia in hypertrophied compared with nonhypertrophied rat hearts and was associated with lower lactate production in the hypertrophied hearts. High glucose and insulin abolished the more pronounced hemodynamic dysfunction and deficient lactate production in hypertrophied hearts so that their behavior was similar to nonhypertrophied hearts during hypoxia. These findings implicate alterations in glycolytic metabolism as an important contributor to the increased sensitivity of hypertrophied hearts to hypoxia.

Materials and Methods

We induced left ventricular hypertrophy in rats using the deoxycorticosterone-salt method previously used in our laboratory. Briefly, male Wistar-Kyoto rats from the Okamoto-Aoki strain (Charles River Breeding Laboratories, Kingston, New York) were uninephrectomized at 10 weeks of age and randomized to control or hypertensive/hypertrophy groups. The hypertensive/hypertrophy groups received deoxycorticosterone pivalate (Percorten, CIBA, Summit, New Jersey) subcutaneously (1.5 mg/100 g body wt twice weekly) for 7 weeks and had 1% saline substituted for drinking water. Control rats received no deoxycorticosterone and received regular drinking water. All animals were fed normal chow (Purina). Blood pressures were measured during the sixth week of therapy by a standard tail-cuff technique.

Perfusion Technique

An isolated isovolumic working heart preparation was used as previously described. Briefly, rats were injected intraperitoneally with pentobarbital (50 mg/ml). The thorax was opened, and the heart was excised. Coronary perfusion was initiated through a short cannula in the aortic root. Nonrecirculating modified Krebs-Henseleit buffer was used and had the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.0, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, Na₂EDTA 0.4, glucose 5.5, and lactate 1.0. The buffer for groups receiving high glucose and insulin was similar except that the glucose concentration was raised to 27.5 mM and insulin (100 mU/ml) was added. Buffer was equilibrated with 5% CO₂-95% O₂, which maintained the pH at 7.35–7.40 and the PO₂ at 580–620 mm Hg. For hypoxic perfusion, the buffer was equilibrated with 5% CO₂-95% N₂. The left ventricle was vented of Thebesian drainage with an apical cannula. A catheter was placed to empty the right ventricle and to allow collection of coronary sinus flow for lactate determination. A pacing electrode attached to a stimulator (model SS, Grass Instrument, Quincy, Massachusetts) and a temperature probe were placed in the right ventricle. A fluid-filled latex balloon connected to a Statham P23Db pressure transducer (Statham Instruments, Hato Rey, Puerto Rico) by a short length of stiff polyethylene tubing was then placed into the left atrium through an incision in the left atrium. Coronary perfusion pressure was measured via a Statham P23Db pressure transducer connected to the aortic cannula.

Hemodynamic Monitoring

Coronary perfusion pressure and left ventricular pressure were recorded on a multichannel recorder (Gould, Cleveland, Ohio). Left ventricular balloon volume was adjusted under oxygenated conditions to achieve an initial left ventricular end-diastolic pressure (LVEDP) of 10 mm Hg in all hearts. Balloon volume was then held constant throughout the experiment so that a rise in LVEDP represented decreased diastolic chamber distensibility. Coronary vascular resistance (CVR) was calculated by using the formula: CVR = (CPP – LVEDP)/CF/g LV, where CPP is coronary perfusion pressure, CF is coronary flow, and LV is left ventricle. Measurement of LVEDP in the absence of coronary turgor, to assess the myofiber component of diastolic pressure, was performed by abruptly reducing coronary flow to zero for a brief period as previously described.

Experimental Protocol

Four groups were studied. The first group (CTL) consisted of 14 nonhypertrophied hearts perfused with buffer containing a 5.5 mM glucose concentration. The second group (CTL/G+I) comprised seven nonhypertrophied hearts perfused with buffer containing 27.5 mM glucose and 100 mU/ml insulin. In both groups, coronary flow was adjusted during oxygenated perfusion to achieve a physiological perfusion pressure of 100 mm Hg. Flow was then held constant for the duration of the experiment. The heart rate was maintained at 4 Hz by the right ventricular pacing electrode.

The third group (LVH) consisted of 18 hypertrophied hearts perfused with buffer containing a 5.5 mM glucose concentration. The fourth group (LVH/G+I) was composed of 11 hypertrophied hearts perfused with buffer containing 27.5 mM glucose and 100 mU/ml insulin. In both hypertrophied groups, coronary flow was adjusted to achieve a perfusion pressure of 125 mm Hg during the baseline period, and flow was then held constant. The higher perfusion pressure in the hypertrophied groups was used to achieve comparable coronary flows per gram left ventricle between hypertrophied and nonhypertrophied hearts. Heart rate was maintained at 4 Hz as in the nonhypertrophied groups.

Measurements Under Aerobic Conditions

After a 30-minute baseline period with oxygenated buffer, left ventricular pressure, coronary perfusion pressure, and coronary flow were measured. Arterial
and coronary venous perfusate samples were obtained for lactate determinations.

**Hypoxia and Reoxygenation Protocol**

After the 30-minute period of oxygenated perfusion, the perfusate in all groups was changed to a buffer equilibrated with 95% N₂-5% CO₂ and containing either a normal glucose concentration or high glucose with insulin per appropriate group. Hypoxic perfusion at constant coronary flow was maintained for 15 minutes. Repeat hemodynamic measurements were made at end hypoxia. Arterial and coronary venous samples for lactate determinations were collected at 1, 5, and 15 minutes of hypoxia.

At end hypoxia, wet-to-dry weight ratios were determined in eight hearts from the CTL group and in nine hearts from the LVH group by isolating the left ventricles and measuring weights immediately after hypoxic perfusion and again 1 week after drying in a 45°C oven to a constant weight. After hypoxic perfusion, the remaining hearts from the CTL and LVH groups and all hearts in the CTL/G+I and LVH/G+I groups were subjected to reoxygenation for 15 minutes by perfusion with buffer equilibrated with 95% O₂-5% CO₂. Repeat hemodynamic measurements were then performed.

Four additional groups of 12 hearts each were subjected to an identical protocol to the groups described above. Six hearts in each group were freeze-clamped at the end of the oxygenated perfusion with tongs cooled with liquid nitrogen. The remaining six hearts in each group were freeze-clamped at the end of 15 minutes of hypoxia. All hearts were stored in liquid nitrogen for subsequent analysis of tissue levels of glycogen, ATP, and creatine phosphate.

**Metabolic Measurements**

The arterial and coronary venous perfusate samples obtained were diluted 1:2 with 10% trichloroacetic acid and stored at ⁴°C for lactate determination by a specific enzymatic method.¹⁶ Lactate production was calculated by subtracting the arterial lactate concentration from the coronary venous lactate concentration and multiplying by coronary flow per gram left ventricular weight. Results are expressed in micromoles per minute per gram.

**High-Energy Phosphate and Glycogen Measurements**

Creatine phosphate and ATP levels were measured by an enzymatic technique and are expressed in micromoles per gram left ventricular dry weight. Glycogen levels were measured by an enzymatic technique and are expressed in milligrams of glucose equivalents per gram left ventricular wet weight.

**Data Analysis**

Statistical comparisons between groups studied under oxygenated and hypoxic conditions were made by analysis of variance (ANOVA). Lactate production over time was analyzed by ANOVA for repeated measures. If ANOVA indicated a significant effect of trials, groups, or interactions, differences between specific mean values were tested by the method of least significant differences.¹⁹ Differences between groups by the least significant differences method were considered significant only if the overall ANOVA indicated significant differences (i.e., protected method). Statistical differences were considered significant at the p<0.05 level. All data are reported as mean±SEM.

**Results**

Baseline characteristics of all groups are shown in Table 1. Tail-cuff blood pressures, left ventricular wet weights, and left ventricle/body weight ratios were higher in the hypertrophied than in the nonhypertrophied groups. Coronary flow per gram left ventricle was comparable in all groups.

The effects of hypoxia on diastolic function are shown in Table 2 and Figure 1. In the groups perfused with a normal glucose concentration, LVEDP after 15 minutes of hypoxia was higher in hypertrophied (LVH) than in nonhypertrophied (CTL) hearts (65±6 vs. 44±4 mm Hg, respectively; p<0.05). Compared with perfusion with a normal glucose concentration, perfusion with a high glucose concentration and insulin (G+I) resulted in a lower LVEDP in both groups at the end of hypoxia. The effect of G+I to prevent the rise in LVEDP during

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**Table 1. Baseline Characteristics of Isolated Hypertrophied and Nonhypertrophied Rat Hearts**

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>LVH</th>
<th>CTL/G+I</th>
<th>LVH/G+I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail-cuff SBP (mm Hg)</td>
<td>121±4</td>
<td>192±8*</td>
<td>128±4</td>
<td>189±12*</td>
</tr>
<tr>
<td>LV wet weight (g)</td>
<td>0.83±0.03</td>
<td>1.02±0.05*</td>
<td>0.90±0.05</td>
<td>1.09±0.05*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>417±7</td>
<td>348±8*</td>
<td>416±22</td>
<td>365±10*</td>
</tr>
<tr>
<td>LV/body weight (g/kg)</td>
<td>2.0±0.1</td>
<td>2.9±0.1*</td>
<td>2.2±0.1</td>
<td>3.0±0.1*</td>
</tr>
<tr>
<td>Coronary flow/g LV</td>
<td>(ml/min)/g</td>
<td>23±1</td>
<td>21±1</td>
<td>24±1</td>
</tr>
</tbody>
</table>

CTL, nonhypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); LVH, hypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); CTL/G+I, nonhypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM) with insulin (100 mU/ml); LVH/G+I, hypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM) with insulin (100 mU/ml); SBP, systolic blood pressure; LV, left ventricle (ventricular).

*p<0.05 vs. CTL.
TABLE 2. Hemodynamics at Baseline and in Response to 15 Minutes of Hypoxia

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dev P (mm Hg)</th>
<th>Dev P/g LV (mm Hg/g)</th>
<th>LVEDP (mm Hg)</th>
<th>CPP (mm Hg)</th>
<th>CVR (mm Hg/[(ml/min)/g])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>142±3</td>
<td>174±7</td>
<td>10±1</td>
<td>99±1</td>
<td>3.9±0.2</td>
</tr>
<tr>
<td>LVH</td>
<td>177±7*</td>
<td>179±9</td>
<td>10±1</td>
<td>125±1*</td>
<td>5.8±0.3*</td>
</tr>
<tr>
<td>CTL/G+I</td>
<td>135±11</td>
<td>152±15</td>
<td>10±1</td>
<td>101±3</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>LVH/G+I</td>
<td>179±3*</td>
<td>169±9</td>
<td>10±1</td>
<td>125±1*</td>
<td>5.8±0.3*</td>
</tr>
<tr>
<td>End hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>25±2</td>
<td>31±3</td>
<td>44±4</td>
<td>103±10*</td>
<td>2.7±0.5</td>
</tr>
<tr>
<td>LVH</td>
<td>21±2</td>
<td>20±2*</td>
<td>65±6*</td>
<td>129±7*</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>CTL/G+I</td>
<td>31±2</td>
<td>35±3</td>
<td>32±4</td>
<td>89±10</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>LVH/G+I</td>
<td>38±1*</td>
<td>36±2†</td>
<td>26±2†</td>
<td>102±4†</td>
<td>3.8±0.3</td>
</tr>
</tbody>
</table>

Dev P, left ventricular developed pressure; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; CPP, coronary perfusion pressure; CVR, coronary vascular resistance; CTL, nonhypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); LVH, hypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); CTL/G+I, nonhypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM); LVH/G+I, hypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM) with insulin (100 mU/ml).

*p<0.05 vs. CTL.
†p<0.05 vs. LVH.

hypoxia was greater in hypertrophied (26±2 vs. 65±6 mm Hg, LVH/G+I vs. LVH, respectively; p<0.05) than in nonhypertrophied (32±4 vs. 44±4 mm Hg, CTL/G+I vs. CTL, respectively; p=NS) hearts. Additionally, perfusion with high glucose and insulin abolished the difference in LVEDP at end hypoxia between hypertrophied and nonhypertrophied hearts (26±2 vs. 32±4 mm Hg, LVH/G+I vs. CTL/G+I, respectively; p=NS). LVEDP was also measured at zero coronary flow to directly assess the myofiber contribution to diastolic pressure. In the presence of a normal glucose concentration, LVEDP at zero coronary flow in hypertrophied hearts after 15 minutes of hypoxia was higher than that in nonhypertrophied hearts (28±5 vs. 14±2 mm Hg, LVH vs. CTL, respectively; p<0.05). Perfusion with high glucose and insulin resulted in a lower LVEDP at zero coronary flow at end hypoxia in hypertrophied (8±1 vs. 28±5 mm Hg, LVH/G+I vs. LVH, respectively; p<0.05) and in nonhypertrophied hearts (6±1 vs. 14±2 mm Hg, CTL/G+I vs. CTL, respectively; p=NS). There was no difference in LVEDP at zero coronary flow at end hypoxia between the hypertrophied and nonhypertrophied hearts that received high glucose and insulin (8±1 vs. 6±1 mm Hg, LVH/G+I vs. CTL/G+I, respectively; p=NS). When LVEDP at zero coronary flow was "normalized" by expressing it as millimeters mercury per gram left ventricle to take into account differences in left ventricular mass between groups, the relative relation between groups did not significantly change (Figure 2). These findings at zero coronary flow indicate that the differences in diastolic function between hypertrophied and nonhypertrophied hearts during hypoxia are not due solely to differences in the coronary turgor contribution to diastolic pressure but, rather, to differences in the myofiber contribution to diastolic pressure. Thus, high glucose and insulin modified the behavior of hypertrophied rat hearts such that diastolic function during hypoxia was similar to that of nonhypertrophied hearts. Perfusion with high glucose and insulin also resulted in better recovery of diastolic function after 15 minutes of reoxygenation in both nonhypertrophied (LVEDP: 16±2 vs. 33±10 mm Hg, CTL/G+I vs. CTL, respectively; p<0.05) and hypertrophied (LVEDP: 13±2 vs. 23±6 mm Hg, LVH/G+I vs. LVH, respectively; p=0.15) hearts.
To assess whether the protective effect of high glucose and insulin on diastolic function during hypoxia was due to increased osmolarity from the high glucose concentration, we compared additional concurrent groups of hypertrophied (n=5) and nonhypertrophied (n=5) hearts subjected to the same hypoxic perfusion protocol using buffer containing either 5.5 mM glucose and 400 mg/dl mannitol or an equiosmolar high glucose concentration (27.5 mM) and insulin. At end hypoxia, LVEDP was higher in the hypertrophied group perfused with mannitol compared with the hypertrophied group perfused with the high glucose concentration (44±3 vs. 34±1 mm Hg, respectively; p<0.05). The LVEDP at end hypoxia also tended to be higher in the hypertrophied group perfused with mannitol compared with the nonhypertrophied group perfused with the high glucose concentration (40±3 vs. 28±4 mm Hg, respectively; p=0.07). Thus, an equiosmotic mannitol perfusate did not confer the same degree of protection against hypoxic diastolic dysfunction as did high glucose and insulin.

Systolic function among groups is shown in Table 2. Developed pressure during oxygenated perfusion with a normal glucose concentration was higher in hypertrophied than in nonhypertrophied hearts (177±7 vs. 142±3 mm Hg, LVH vs. CTL, respectively; p<0.05). Perfusion with high glucose and insulin did not alter developed pressure during oxygenated perfusion in either hypertrophied (179±3 vs. 177±7 mm Hg, LVH/G+I vs. LVH, respectively; p=NS) or nonhypertrophied (135±11 vs. 142±3 mm Hg, CTL/G+I vs. CTL, respectively; p=NS) hearts. When developed pressure was normalized by expressing it as millimeters mercury per gram left ventricle, there was no significant difference between any of the groups. Developed pressure at end hypoxia (Figure 3) expressed as a percentage of baseline values during oxygenated perfusion with a normal glucose concentration was significantly lower in hypertrophied than in nonhypertrophied hearts (11±1% vs. 18±1% of baseline, LVH vs. CTL, respectively; p<0.05). Perfusion with high glucose and insulin resulted in an improvement in developed pressure at end hypoxia in both hypertrophied (21±1% vs. 11±1% of baseline, LVH/G+I vs. LVH, respectively; p<0.05) and nonhypertrophied (24±2% vs. 18±1% of baseline, CTL/G+I vs. CTL, respectively; p<0.05) hearts. Additionally, perfusion with high glucose and insulin abolished the greater depression of developed pressure at end hypoxia between hypertrophied and nonhypertrophied hearts (21±1% vs. 24±2% of baseline, LVH/G+I vs. CTL/G+I, respectively; p=NS). Thus, similar to diastolic function, glucose and insulin changed the response of hypertrophied hearts to hypoxia such that systolic function was similar to that of nonhypertrophied hearts. High glucose and insulin resulted in better recovery of systolic function during reoxygenation in both nonhypertrophied (developed pressure: 83±4% vs. 66±6% of baseline, CTL/G+I vs. CTL, respectively; p<0.05) and hypertrophied (developed pressure: 86±3% vs. 74±5% of baseline, LVH/G+I vs. LVH, respectively; p<0.05) hearts.
insulin had no effect on coronary vascular resistance during oxygenated conditions in either the hypertrophied or nonhypertrophied hearts. Coronary vascular resistance fell to comparable levels in both hypertrophied and nonhypertrophied hearts during hypoxia regardless of whether a normal glucose concentration or high glucose and insulin were used.

Lactate production during hypoxia is shown in Figure 4. Lactate production during the entire hypoxic period was significantly lower in the hypertrophied (LVH) than in the nonhypertrophied (CTL) hearts when perfusate with a normal glucose concentration was used. When hearts were perfused with high glucose and insulin, lactate production during hypoxia was greater and was comparable in hypertrophied (LVH/G+I) and nonhypertrophied (CTL/G+I) hearts concomitant with the improvement in diastolic and systolic function.

Linear regression of LVEDP at end hypoxia plotted as a function of lactate production at end hypoxia for all hearts in all four groups (n=50) showed a significant inverse correlation (r=−0.63, p<0.001). This inverse linear relation is illustrated in Figure 5 in which mean values of LVEDP at end hypoxia are plotted as a function of mean lactate production values at end hypoxia. Linear regression of developed pressure at end hypoxia plotted as a function of lactate production at end hypoxia for all hearts in all four groups (n=50) showed a significant correlation (r=0.66, p<0.001). This direct linear relation is illustrated in Figure 6 by graphing group mean values of relative developed pressure at end hypoxia as a function of lactate production at end hypoxia.

Results of tissue high-energy phosphate and glycogen determinations are shown in Table 3. There are no significant differences in the values of myocardial creatine phosphate, ATP, or glycogen content between any of the groups after oxygenated perfusion except for a lower creatine phosphate level in the LVH group compared with the CTL group. At the end of hypoxia, creatine phosphate, ATP, and glycogen levels had fallen to comparable levels in all the groups.

Wet-to-dry weight ratios were similar in nonhypertrophied and hypertrophied hearts at the end of hypoxic perfusion (3.8±0.1 vs. 3.9±0.1, respectively).

**Discussion**

Pressure-overload cardiac hypertrophy is often associated with diastolic dysfunction and impairment in relaxation, although the exact mechanism responsible for this phenomenon is unclear. Previous studies have shown that hypertrophied hearts...
exhibit an increased sensitivity toward developing hemodynamic dysfunction in response to hypoxia and ischemia. Enhancement of glycolytic metabolism by provision of a high glucose concentration has been shown to preserve mechanical function, especially diastolic function, in nonhypertrophied hearts. Conversely, glycolytic blockade can accelerate the development of diastolic dysfunction. These studies suggest that alterations in glycolytic metabolism in hypertrophied hearts could contribute to the tendency of hypertrophied hearts to develop more profound hypoxic dysfunction.

This study was designed to assess whether differences in glycolytic metabolism between hypertrophied and nonhypertrophied hearts play a role in the greater susceptibility of hypertrophied hearts to develop hypoxia-induced dysfunction. Using a hypertensive rat model with left ventricular hypertrophy, we found that both the decrease in left ventricular diastolic distensibility and developed pressure were worse in hypertrophied hearts during 15 minutes of hypoxic perfusion with a normal glucose concentration, consistent with prior observations. A novel finding of the current study is that this greater dysfunction was associated with lower lactate production in hypertrophied hearts although high-energy phosphate and glycogen levels at end hypoxia were not different between the groups. Perfusion with high glucose and insulin resulted in less deterioration in systolic and diastolic function in both hypertrophied and nonhypertrophied groups during hypoxia. Furthermore, the greater hemodynamic dysfunction during hypoxia in hypertrophied compared with nonhypertrophied hearts was abolished by perfusing with a high glucose concentration and insulin. Additionally, the deficient degree of hypoxic lactate production in hypertrophied hearts relative to controls during perfusion with a normal glucose concentration was eliminated when high glucose and insulin were provided. Thus, the use of a high glucose concentration and insulin changed the behavior of hypertrophied hearts such that hemodynamic and metabolic function during hypoxia became similar to that of nonhypertrophied hearts. These findings implicate an impairment in glycolytic metabolism as an important contributor to the increased sensitivity of hypertrophied hearts to hypoxia.

Work by Lorell et al using the same model showed that the rise in diastolic pressure was greater in hypertrophied rat hearts during a brief 3-minute period of hypoxia despite comparable rates of coronary flow per gram of left ventricle. The responsible mechanisms for these differences between hypertrophied and nonhypertrophied hearts were different.

### Table 3. High-Energy Phosphate and Glycogen Levels in Hypertrophied and Nonhypertrophied Rat Hearts

<table>
<thead>
<tr>
<th>Groups</th>
<th>ATP (μmol/g dry LV wt)</th>
<th>Creatine phosphate (μmol/g dry LV wt)</th>
<th>Glycogen (mg of glucose eq/g LV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prehypoxia</td>
<td>End hypoxia</td>
<td>Prehypoxia</td>
</tr>
<tr>
<td>CTL (n=6)</td>
<td>20.0±1.4</td>
<td>10.7±0.4</td>
<td>25.7±2.0</td>
</tr>
<tr>
<td>LVH (n=6)</td>
<td>16.7±0.9</td>
<td>9.2±1.1</td>
<td>19.2±1.8*</td>
</tr>
<tr>
<td>CTL/G+I (n=6)</td>
<td>19.9±0.9</td>
<td>10.8±1.3</td>
<td>24.3±0.8</td>
</tr>
<tr>
<td>LVH/G+I (n=6)</td>
<td>17.7±1.0</td>
<td>11.2±0.5</td>
<td>21.2±1.2</td>
</tr>
</tbody>
</table>

LV, left ventricle (ventricular); CTL, nonhypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); LVH, hypertrophied hearts perfused with buffer containing a normal glucose concentration (5.5 mM); CTL/G+I, nonhypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM) with insulin (100 μU/ml); LVH/G+I, hypertrophied hearts perfused with buffer containing a high glucose concentration (27.5 mM) with insulin (100 μU/ml).

*p < 0.05 vs. CTL.
phied and nonhypertrophied hearts were not delineated. The current study showed that, during 15 minutes of hypoxia, the greater rise in isovolumic LVEDP in hypertrophied hearts was due to the myofiber component of diastolic pressure and not simply due to a more marked rise in the coronary turgor component of diastolic pressure. Wexler et al.\textsuperscript{8} using \textsuperscript{31}P NMR spectroscopy to study this greater rise in diastolic pressure in hypertrophied hearts during prolonged hypoxia, showed that the differences in diastolic dysfunction between hypertrophied and control hearts were not explained by differences in the decline in myocardial ATP and creatine phosphate levels or by changes in intracellular pH. This study, however, did not rule out differences in the rate of ATP production and utilization as a potential contributing factor to this phenomenon since the measurement of steady-state levels of high-energy phosphates may not reflect the dynamic nature of ATP production and utilization. Additionally, compartmentalization of ATP generated by anaerobic glycolysis as suggested by Weiss and Hilbrand\textsuperscript{22} and others\textsuperscript{23,24} could also contribute to these observed differences in hypoxia-induced dysfunction between hypertrophied and nonhypertrophied hearts. Using different methodology, our study confirms the findings of Wexler et al.\textsuperscript{8} in that there were no differences in myocardial levels of ATP and creatine phosphate at the end of hypoxia between hypertrophied and control hearts. However, our novel finding of lower lactate production in hypertrophied hearts relative to controls during hypoxic perfusion with normal glucose strongly suggests that a lower rate of glycolytic ATP production and utilization contributed to the greater hemodynamic deterioration in the hypertrophied hearts. During perfusion with high glucose and insulin, an increase in lactate production in hypertrophied hearts to a level similar to that in nonhypertrophied hearts occurred and was associated with improvement in systolic and diastolic function to control levels, although myocardial high-energy phosphate levels were not significantly affected. This finding provides further support for the concept that a lower rate of glycolytic ATP generation and utilization may contribute to the increased sensitivity of hypertrophied hearts to develop greater mechanical dysfunction during hypoxia.

Our findings are consistent with previous work. Brooks et al.\textsuperscript{25} used papillary muscles from spontaneously hypertensive rats to study the response to hypoxia in hypertrophied and nonhypertrophied hearts. They found that the rise in resting tension and decrease in active tension development during hypoxia was greater in papillary muscles from hypertrophied than from nonhypertrophied hearts. These differences in mechanical function were not related to any differences in ATP levels although there appeared to be a correlation with total energy charge. Superfusion with a high glucose concentration abolished the differences in mechanical function between hypertrophied and nonhypertrophied muscles during hypoxia. However, the effect of increasing the glucose concentration on lactate production as an indicator of glycolytic ATP production during hypoxia was not examined.

The mechanism whereby lower glycolytic ATP production may result in greater diastolic and systolic mechanical dysfunction during hypoxia may be due to impairment in energy-dependent calcium homeostatic mechanisms.\textsuperscript{26–31} Testing this hypothesis would require techniques that directly measure and compare intracellular calcium transients during hypoxia in working hypertrophied and nonhypertrophied hearts. Such techniques may be feasible in the future as calcium-indicator techniques become more refined.\textsuperscript{32,33}

The changes in glycolytic metabolism that appear to occur with hypertrophy are not defined by this study. The most obvious explanation would be that lower glycogen levels in hypertrophied hearts result in lower glycolytic ATP production during hypoxia unless exogenous sources of glucose are supplied. Our data failed to detect any differences in glycogen levels to support this explanation. However, our methodology is not sensitive enough to detect differences in glycogen utilization between the epicardium and endocardium that may be important in hypertrophied hearts.\textsuperscript{34} An alternative explanation is that alterations in glycolytic enzyme expression may occur during hypertrophy and lead to a modification of glycolytic enzyme levels or in their kinetics resulting in an altered capacity to recruit anaerobic glycolysis during hypoxia. Lastly, differences in glucose transport across cell membranes may be different between hypertrophied and nonhypertrophied hearts and could have contributed to our observations.

Strengths and Limitations of the Study

Our isolated isovolumic heart model provides an ideal model to study differences in function between hypertrophied and nonhypertrophied hearts. This model is free of autonomic reflexes, and the isovolumic nature of the preparation keeps loading conditions constant, which could otherwise confound the metabolic and hemodynamic response to hypoxia. Our experiments were also done under physiological conditions of heart rate and temperature that were maintained constant throughout the course of the experiment. Additionally, the ability to maintain constant coronary flow in our model prevents the superimposition of ischemia during hypoxic perfusion. However, despite this constant flow nature of our system, we cannot be absolutely certain that there is not a mild component of relative subendocardial ischemia due to regional redistribution of coronary flow\textsuperscript{35,36} contributing to our findings. The comparable fall in coronary vascular resistance during hypoxia in all groups would seem to make this explanation unlikely.

Buffer perfusion does require high coronary flow rates to maintain adequate tissue oxygenation. This can enhance the coronary turgor contribution to
LVEDP and predispose to the development of edema. Measuring LVEDP after reducing coronary flow to zero has been shown to allow separation of coronary turgor and myofiber contributions to diastolic pressure. Our study demonstrated that the greater rise in LVEDP in hypertrophied compared with nonhypertrophied hearts when perfused with a normal glucose concentration was in large part due to the myofiber component of LVEDP and was not due solely to the coronary turgor contribution to LVEDP. Perfusion with increased glucose and insulin improved and equalized not only the absolute LVEDP in response to hypoxia between the groups but also had the same effect on the myofiber component of LVEDP. The comparable wet-to-dry weight ratios at the end of hypoxia in hypertrophied and nonhypertrophied hearts indicate that differences in the degree of tissue edema were unlikely to be contributing to our findings. Additionally, we observed that perfusion with mannitol added to the perfusate to achieve a comparable osmolarity to the high glucose perfusate failed to show the same protective effect against hypoxia-induced diastolic dysfunction. This finding suggests that the protective effect of high glucose and insulin is mediated through the stimulation of anaerobic glycolysis and not through a passive osmotic effect. The finding that mannitol failed to show the same protective effect on mechanical function during hypoxia as a high glucose concentration is what would have been predicted given previous studies comparing high glucose and mannitol in different experimental models of hypoxia and ischemia.

In conclusion, our study demonstrates that the greater sensitivity of hypertrophied hearts to develop hemodynamic dysfunction during hypoxia is associated with reduced myocardial hypoxic lactate production relative to nonhypertrophied hearts. Perfusion with high glucose and insulin results in a higher level of lactate production such that the hypertrophied hearts become comparable with the nonhypertrophied hearts. Also, high glucose and insulin abolish the greater degree of mechanical dysfunction in response to hypoxia in the hypertrophied hearts. These results are consistent with the hypothesis that the hypertrophied heart has an impaired capacity to recruit anaerobic glycolysis to preserve mechanical function during hypoxia. Defining the exact changes in glycolytic metabolism in hypertrophied hearts responsible for our observations will require further study.

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