Aldose reductase inhibition improves altered glucose metabolism of isolated diabetic rat hearts

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Trueblood, Nathan, and Ravichandran Ramasamy. Aldose reductase inhibition improves altered glucose metabolism of isolated diabetic rat hearts. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H75–H83, 1998.—Alterations in glucose metabolism have been implicated in the cardiovascular complications of diabetes. Previous work in this laboratory demonstrated that hearts from diabetic animals have an elevated cytosolic redox ratio (NADH/NAD+) and that this redox imbalance is probably due to elevated polyol pathway flux. We therefore hypothesized that 1) the elevated cytosolic redox ratio of diabetic hearts could result in inhibition of glycolytic enzymes sensitive to the redox state, 2) polyol pathway inhibition could restore the abnormal glucose metabolism of diabetic hearts, and 3) the relative incorporation of mixed substrates into hearts from diabetic animals would demonstrate less glycolytic and more fatty acid oxidation. Hearts from diabetic (BB/W) and nondiabetic control rats were perfused with buffers containing 13C-labeled substrates, and the metabolism of these hearts was analyzed using 13C NMR spectroscopy. Tissue samples were analyzed for metabolic levels using biochemical assay. Compared with controls, diabetic hearts had glyceraldehyde-3-phosphate levels that were four times greater than nondiabetic hearts and exhibited 91% less 13C labeling of lactate and 92% less 13C labeling of glutamate (P < 0.03). Aldose reductase inhibition with zopolrestat restored the metabolite labeling of diabetic hearts. Diabetic hearts perfused with a mixture of substrates used 53% more acetate than nondiabetic control hearts (P < 0.05), and aldose reductase inhibition lowered the acetate utilization of diabetic hearts by 9% (P < 0.05). These data suggest that glycolytic flux in diabetic hearts is inhibited at glyceraldehyde-3-phosphate dehydrogenase and that inhibition of the polyol pathway with zopolrestat increases glycolytic flux in these hearts. Furthermore, hearts from diabetic animals showed a marked dependence on fatty acids for substrate utilization compared with nondiabetic controls, consistent with inhibition of the pyruvate dehydrogenase complex in diabetic hearts.

carbon-13 nuclear magnetic resonance; NADH/NAD+; glyceraldehyde 3-phosphate dehydrogenase

CARDIOVASCULAR DISEASES and the complications of myocardial infarction are the major cause of morbidity and mortality in persons with diabetes (2). These complications may be due to abnormalities in glucose metabolism, such as the dramatic increase in polyol pathway flux (20, 26). In this pathway, glucose is reduced to sorbitol by aldose reductase in the presence of NADPH, and sorbitol is then oxidized by sorbitol dehydrogenase (SDH) to fructose at the cost of NAD+. Inhibition of aldose reductase thus conserves NADPH, NAD+, and glucose.

Because aldose reductase and the polyol pathway exhibit increased activity under conditions of elevated blood glucose, considerable attention has been focused on studying the benefits of aldose reductase inhibition in diabetes (20, 26). Previously published data from this laboratory, using diabetic and nondiabetic rat hearts and aldose reductase inhibition under ischemic conditions, demonstrated ATP preservation as well as a reduction in ischemic injury (20). In that study, aldose reductase inhibition was shown to lower sorbitol levels in diabetic hearts by approximately fourfold and to lower the abnormally elevated cytosolic redox state (NADH/NAD+) of diabetic hearts by approximately ninefold (20). Lowering of the cytosolic redox state was presumably due to conservation of NAD+ by limiting the levels of sorbitol, the substrate for SDH (Fig. 1). Furthermore, NADH/NAD+ for diabetic hearts under baseline conditions was shown to be more than three times higher than nondiabetic hearts, which could have profound effects on the various glycolytic enzymes sensitive to the redox state (20). If the decrease in glycolytic flux of diabetic hearts is secondary to the redox effects of increased polyol pathway flux, then aldose reductase inhibition, by lowering the redox state, should normalize glycolysis and increase glycolytic substrate available for incorporation into the tricarboxylic acid (TCA) cycle. In fact, Obrosova et al. (19) have presented evidence supporting this link between polyol pathway flux and glucose metabolism in their galactose-fed rats. Therefore the goal of this study was to test the hypothesis that inhibition of aldose reductase would improve glycolysis and enhance entry of glycolytic end products into the TCA cycle of diabetic rat hearts. To test our hypothesis, we perfused diabetic rat hearts with 13C-labeled substrates and measured changes in glycolytic intermediates and entry of substrates into the TCA cycle using 13C NMR spectroscopy and biochemical assay.

MATERIALS AND METHODS

Acute diabetic and nondiabetic rats. All animal studies were performed with the approval of the University of California, Davis, Animal Research and Care Committee. Spontaneously diabetic Bio-Bred (BB/W) rats and their age-matched littersmates from the colony maintained at the University of Massachusetts Medical Center, Worcester, MA, were used in this study. Like human insulin-dependent diabetes mellitus (IDDM), diabetes spontaneously appears in BB/W rats during adolescence, with abrupt onset of symptoms including weight loss, hypoinsulinemia, hyperglycemia, and ketonuria (3, 7). The BB/W rats used were 3–4 mo old and weighed 300–350 g. Animals that spontaneously developed diabetes received daily insulin therapy (which was discontinued 24 h before heart isolation), and the duration of diabetes in this acute model was 14 days. Diabetic animals had mean blood glucose levels of 386 ± 36 mg/dl and...
nondiabetic animals had mean blood glucose levels of 112 ± 18 mg/dl.

Isolated perfused heart model. Experiments were performed using an isovolumetric isolated rat heart preparation (Langendorff model) as described by Schaefer et al. (22). Rats were pretreated with heparin (1,000 U ip), followed by pentobarbital sodium (65 mg/kg ip). After deep anesthesia was achieved, as determined by the absence of a foot reflex, the hearts were rapidly excised and placed into iced buffer for cold cardioplasia. Within 1 min, the arrested hearts were retrogradely perfused through the aorta at 12.5 ml/min. Myocardial function was monitored with a latex balloon retrogradely perfused through the aorta at 12.5 ml/min. All hearts received 10 min of nonrecirculating perfusion with a modified Krebs-Henseleit (KH) buffer (containing, in mM, 118 NaCl, 4.7 KCl, 1.2 CaCl_2, 1.2 MgSO_4, 25 NaHCO_3, and 11 glucose), followed by perfusion with 13C-labeled KH buffer for 50 min. The perfusion time of 50 min was chosen to ensure steady-state incorporation of the 13C label (4, 16). To detect the various glycolytic intermediates and to determine the contribution of 13C label into the TCA cycle, there were two separate perfusion groups: a glucose-perfused group designed to investigate glycolytic flux and a mixed substrate-perfused group designed to investigate substrate selection into the TCA cycle. The 13C-labeled KH buffer contained either 11 mM [1-13C]glucose (glucose-perfused heart group) or 0.25 mM [1,2-13C]acetate, 1 mM [3-13C]lactate, and 11 mM nonlabeled glucose (mixed substrate-perfused heart group). Eight diabetic and eight nondiabetic hearts were used in each of the two perfusion groups. Half of the hearts from all groups were exposed to 1 µM zopolrestat during perfusion with the 13C-labeled KH buffer. This concentration of zopolrestat has been shown to significantly lower sorbitol and fructose levels of diabetic hearts, which in the untreated state are approximately ninefold higher than in nondiabetic hearts (20). After perfusion, hearts were freeze-clamped in liquid nitrogen and extracted in perchloric acid, followed by neutralization to pH 7.2. Heart extracts were then lyophilized and brought up in 700 µl of deuterium oxide (D_2O) for NMR spectroscopy.

13C NMR spectroscopy. 13C NMR spectroscopy is a powerful technique for simultaneously measuring glycolytic and TCA cycle intermediates (4, 15). Differentiating between labeled and unlabeled metabolites allows the fractional enrichment of labeled metabolites to be determined. The region between 1.6 and 1.1 ppm reveals resonances from the methyl protons of [3-13C]lactate. Because only half of the lactate originating from [1-13C]glucose will contain the 13C label (because fructose 1,6-bisphosphate is split in half by aldolase), the actual enrichment from our exogenous [1-13C]glucose is equal to twice the ratio of the areas of [3-13C]lactate to [3-12C]lactate (5) and therefore twice the values presented here.

The region between 1.6 and 1.1 ppm reveals resonances from the methyl protons of [3-13C]lactate as well as satellites from [3-13C]lactate. Resolution of the 1H satellites corresponding to [3-13C]lactate from the 1H resonances corresponding to unlabeled lactate allows determination of the fractional enrichment of lactate. Because only half of the lactate originating from [1-13C]glucose will contain the 13C label (because fructose 1,6-bisphosphate is split in half by aldolase), the actual enrichment from our exogenous [1-13C]glucose is equal to twice the ratio of the areas of [3-13C]lactate to [3-12C]lactate (5) and therefore twice the values presented here.

The region between 2.6 and 2.0 ppm reveals proton resonances from C-4 of glutamate. Although the proton resonances corresponding to [4-13C]glutamate are resolvable, the 1H satellites corresponding to [4-13C]glutamate are obscured by other nearby peaks. Accordingly, the fractional enrichment of glutamate cannot be determined by measuring the areas of the proton peaks bound to labeled and nonlabeled carbons, as is the case with lactate (10). However, the enrichment of glutamate C-4 may be determined from the increase in intensity of C-4 glutamate proton resonances after heteronuclear decoupling, since these resolvable resonances then correspond to the sum of protons from labeled and nonlabeled glutamate (10). The fraction of glucose that originated from exogenously supplied [1-13C]glucose was determined by similar technique to that described for glutamate enrichment.

Biochemical assay for glyceroldehyde 3-phosphate. After deproteinization with perchloric acid and neutralization, the
RESULTS

Effects of aldose reductase inhibition on myocardial function in diabetic and nondiabetic rat hearts. Table 1 illustrates the myocardial function of all four heart groups with both perfusate types (glucose-perfused and substrate mix-perfused hearts). There were no significant differences in developed pressure between the heart groups or between perfusate types. The heart rates of the diabetic control (DC) groups were significantly lower with both perfusate types than the nondiabetic control (C) group and nondiabetic group treated with zopolrestat (Z), with both perfusate types (P < 0.05). The heart rates of the diabetic hearts treated with zopolrestat (DZ) perfused with the substrate mix buffer were also significantly lower than the C and Z groups (P < 0.05), but the heart rates of the glucose-perfused DZ group were not significantly different from those of the C or Z groups (Table 1).

Effects of aldose reductase inhibition on glucose metabolism in diabetic and nondiabetic rat hearts. Tissue levels of glyceraldehyde 3-phosphate were assayed spectrophotometrically in heart extracts using enzymatic procedures. The glyceraldehyde 3-phosphate contents (in nmol/g wet wt) were 5.1 ± 6, 3.9 ± 1.4, 21.8 ± 4.1, and 6.8 ± 1.8, in C, Z, DC, and DZ groups, respectively. DC hearts contained significantly greater glyceraldehyde 3-phosphate than all other groups (n = 6, P < 0.001; Fig. 2). The DZ group had glyceraldehyde 3-phosphate levels that were not significantly different from those of the C or Z groups.

13C NMR spectra of the extracts from C hearts perfused with [1-13C]glucose were characterized by the incorporation of 13C label from glucose into C-3 of lactate, at 21 ppm, and into C-2, C-3, and C-4 of glutamate at 55.2, 27.6, and 34.2 ppm, respectively (Fig. 3). The appearance of [13C]lactate indicated metabolism of glucose via glycolysis, whereas the labeling in glutamate was consistent with the entry of glycolytic end products into the TCA cycle via the pyruvate dehydrogenase complex (PDHC).

The 13C NMR spectra of extracts from diabetic hearts perfused under the same conditions (n = 4) exhibited significantly decreased 13C labeling in lactate (C-3) and glutamate (C-4). The ratio of [3-13C]lactate to [1-13C]glucose was 0.044 ± 0.011 and 0.004 ± 0.0009 in the C and DC group hearts, respectively (P < 0.02). The ratio of [4-13C]glutamate to [1-13C]glucose was 0.077 ± 0.016 and 0.0058 ± 0.00065 in C and DC groups, respectively (P < 0.03), as seen in Figs. 3 and 4. To summarize, DC group hearts exhibited 90.9% less labeling of lactate and 92.5% less labeling of glutamate than the C group hearts.

The 13C NMR spectrum from a [1-13C]glucose-perfused DZ heart (Fig. 2) exhibited significantly increased 13C labeling of lactate and glutamate compared with the results for the DC heart. The ratios of [3-13C]lactate to [1-13C]glucose and [4-13C]glutamate to [1-13C]glucose were 0.017 ± 0.0032 and 0.0187 ± 0.004, respectively (n = 4, P < 0.03, DZ vs. DC), as seen in Fig. 4. To summarize, zopolrestat treatment of diabetic hearts resulted in 76.47% more lactate labeling and 68.98% more glutamate labeling.

The 13C NMR spectrum from a [1-13C]glucose-perfused Z heart demonstrates significantly increased lactate levels (3.95 times greater) compared with that for the untreated C heart (n = 4, P < 0.002). The increase in glutamate intensity, seen in Fig. 4, was not significant compared with the C group hearts. The ratios of [3-13C]lactate to [1-13C]glucose, and [4-13C]glutamate to [1-13C]glucose for Z group hearts were 0.174 ± 0.004, 0.191 ± 0.016, and 0.011 ± 0.002, respectively.

To define the amount of glucose, lactate, and glutamate derived from exogenously supplied [1-13C]glucose (specific activity/fracti onal enrichment), 1H NMR spectroscopy was performed on all heart extracts (spectra not shown) as described in MATERIALS AND METHODS and by others (5, 10).

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Table 1. Myocardial function in diabetic and nondiabetic rat hearts

<table>
<thead>
<tr>
<th>Group + Perfusate</th>
<th>Heart Rate, beats/min</th>
<th>Developed Pressure, mmHg</th>
</tr>
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<tr>
<td>C + glucose</td>
<td>278 ± 15</td>
<td>108 ± 11</td>
</tr>
<tr>
<td>C + mix</td>
<td>281 ± 17</td>
<td>107 ± 9</td>
</tr>
<tr>
<td>Z + glucose</td>
<td>262 ± 18</td>
<td>116 ± 14</td>
</tr>
<tr>
<td>Z + mix</td>
<td>260 ± 14</td>
<td>118 ± 8</td>
</tr>
<tr>
<td>DC + glucose</td>
<td>184 ± 26*</td>
<td>86 ± 15</td>
</tr>
<tr>
<td>DC + mix</td>
<td>191 ± 16*</td>
<td>122 ± 17</td>
</tr>
<tr>
<td>DZ + glucose</td>
<td>206 ± 30</td>
<td>104 ± 11</td>
</tr>
<tr>
<td>DZ + mix</td>
<td>193 ± 12*</td>
<td>114 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE. C, nondiabetic controls; Z, zopolrestat perfused, nondiabetic; DC, diabetic controls; DZ, zopolrestat perfused, diabetic; + glucose, hearts perfused with glucose buffer; + mix, hearts perfused with buffer containing lactate, acetate, and glucose. *DC + glucose, DC + mix, and DZ + mix were significantly lower than controls, P < 0.05.
There was no significant difference in the fractional enrichment of glucose between groups, as seen in Table 2.

The fractional enrichment of lactate was significantly lower in diabetic compared with nondiabetic hearts (11.9 ± 1.6 vs. 28.3 ± 2.5% in DC and C groups, respectively, P < 0.01), as seen in Table 2. Zopolrestat normalized the fractional enrichment of lactate (for DZ, 24.4 ± 3.4%, NS vs. C group). Nondiabetic hearts treated with zopolrestat (Z group) exhibited a signifi-
significant increase in the fractional enrichment of lactate compared with all groups (42.6 ± 4.5%, P < 0.001 vs. DC, and P < 0.01 vs. DZ and C). These results are consistent with increased enrichment of lactate from exogenously supplied glucose in hearts perfused with zopolrestat.

The fractional enrichment in glutamate was significantly lower in both diabetic groups compared with corresponding controls (P < 0.03 and P < 0.001 vs. DZ and DC, respectively), as seen in Table 2. There was a moderate but nonsignificant increase in glutamate enrichment with zopolrestat.

Effects of aldose reductase inhibition on incorporation of multiple substrates into TCA cycle of diabetic and nondiabetic hearts. The [1,13C]glucose experiments suggested increased 13C incorporation in glutamate of diabetic hearts treated with zopolrestat. This evidence suggested that, in the absence of competing substrates, aldose reductase inhibition increased glycolysis and flux via PDHc in diabetic hearts. Glucose, however, is not the only substrate available to the heart, and furthermore, the entry of nonglycolytic substrate into the TCA cycle is known to inhibit glycolysis and glucose oxidation (9, 21). We therefore performed 13C NMR experiments using glucose plus other substrates to determine 1) the effect of zopolrestat and mixed substrates on flux through PDHc and 2) the relative substrate incorporation of lactate and acetate into the TCA cycle of diabetic hearts.

Hearts were perfused with [1,2-13C]acetate, [3-13C]lactate, and unlabeled glucose. Lactate was chosen because it is an end product of glycolysis and its entry into the TCA cycle can demonstrate flux through PDHc. Unlabeled glucose (11 mM) was added to control for aldose reductase activity, which is sensitive to glucose levels. Because the cytosolic glutamate pool is NMR visible and in equilibrium with α-ketoglutarate (a mitochondrial TCA cycle intermediate), 13C label that ends up in cytosolic glutamate represents the incorporation of exogenous substrates with 13C labels into acetyl-CoA and the TCA cycle (15). Specifically, the 13C NMR spectrum of C-4 of glutamate is an index of acetyl-CoA incorporation, because the pattern of labeling from differentially 13C-labeled exogenous substrates into glutamate gives a predictable spectrum as follows: the incorporation of carbons from [3-13C]lactate into glutamate yields either a 13C carbon at position 4 only, which results in the singlet (S), or 13C carbons at positions 3 and 4 (given recycling of the label through the TCA cycle), resulting in the doublet D34 due to J coupling; the incorporation of carbons from [1,2-13C]acetate into glutamate results in 13C carbons at positions 4 and 5, resulting in the doublet D45 (due to different J coupling than at D34), or 13C carbons at positions 3, 4, and 5, resulting in the quartet (Q), which is a doublet of doublets.

As a consequence of this predictable labeling pattern, we determined the relative incorporation of lactate and acetate into the TCA cycle by comparing the areas of the respective peaks from the spectrum of C-4 of glutamate. Specifically, lactate incorporation is measured by summing the areas of the S and D34, whereas acetate incorporation is measured by summing the areas of the Q and D45 (16, 23). Figure 5, A and B, shows the 13C spectra generated from C-4 of glutamate from a control heart and from a diabetic heart, respectively. Figure 6 is graphic presentation of the relative 13C label incorporation of lactate and acetate into C-4 of glutamate in both diabetic and nondiabetic hearts.

C rat hearts used 45.2 ± 3.6% acetate and 54.8 ± 3.6% lactate (Fig. 6). These data on nondiabetic control hearts are in dose agreement with those shown by

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**Table 2. Percent enrichment from exogenously supplied [1-13C]glucose**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose, %</th>
<th>Lactate, %</th>
<th>Glutamate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>83.7 ± 3.8</td>
<td>28.3 ± 2.5*</td>
<td>42.35 ± 2.45§</td>
</tr>
<tr>
<td>Z</td>
<td>85.7 ± 2.9</td>
<td>42.6 ± 4.9†</td>
<td>43.05 ± 2.05§</td>
</tr>
<tr>
<td>DC</td>
<td>81.9 ± 2.0</td>
<td>11.9 ± 1.6</td>
<td>15.34 ± 1.75</td>
</tr>
<tr>
<td>DZ</td>
<td>82.8 ± 3.6</td>
<td>24.4 ± 3.4‡</td>
<td>30.55 ± 1.95</td>
</tr>
</tbody>
</table>

Values are means ± SE. Comparisons by ANOVA: *P < 0.01 vs. DC; †P < 0.01 vs. DZ, DC, and C; ‡P < 0.02 vs. DC. §P < 0.01 vs. DZ and DC.
lactate into the TCA cycle of diabetic hearts (restat), there was a 9% increase in the incorporation of acetate. Furthermore, when flux through the polyol pathway is altered, there was almost exclusive incorporation of acetate into the TCA cycle (97.9 ± 3.1%, P < 0.0001), and acetate utilization in DC hearts is markedly greater than in C hearts (P < 0.0001). Lactate utilization in DC hearts is significantly greater than DC hearts, and lactate utilization is significantly lower in DZ than in DC hearts (P < 0.05). Zopolrestat treatment had no significant effect on lactate or acetate utilization in C hearts.

Sherry et al. (23). There was no significant effect of zopolrestat on relative acetate and lactate use in nondiabetic hearts (41.4 ± 2.7% acetate and 58.6 ± 2.7% lactate in group Z). DC hearts used 97.9 ± 1.9% acetate and 2.08 ± 1.9% lactate, whereas DC utilized significantly less acetate (89.1 ± 3.1%, P < 0.05) and more lactate (10.9 ± 3.1%, P < 0.05; Fig. 6). DC hearts demonstrated that there was almost exclusive incorporation of acetate into the TCA cycle (97.9 ± 1.9% acetate in group DC, 45.2 ± 3.6% in group C, P < 0.01), when provided with a mixture of lactate and acetate. These data are consistent with inhibition of PDHc in diabetic hearts. Furthermore, when flux through the polyol pathway was reduced (by inhibiting aldose reductase with zopolrestat), there was a 9% increase in the incorporation of lactate into the TCA cycle of diabetic hearts (P < 0.05). By use of 1H NMR with and without heteronuclear 13C decoupling (described in MATERIALS AND METHODS and in Ref. 10), the contribution of nonlabeled substrates to acetyl-CoA was determined. The total of the labeled and nonlabeled substrate incorporation into acetyl-CoA must equal one. Accordingly, the fractional enrichment data for C-4 of glutamate (13C/12C) was combined with the relative incorporation data from lactate and acetate to define the substrate contribution to acetyl-CoA for these substrate mix experiments. As seen in Table 3, the nonlabeled substrate incorporation in acetyl-CoA was significantly greater in both diabetic groups compared with the control group (P < 0.05). There was no significant effect of zopolrestat on nonlabeled substrate incorporation.

**DISCUSSION**

In this paper we have demonstrated, for the first time, a relationship between increased polyol pathway flux and altered glucose metabolism of acute, spontaneously diabetic rat hearts. Our 13C NMR results demonstrated significantly reduced labeling of lactate and glutamate from exogenously supplied [13C]glucose in diabetic hearts, which is in agreement with that reported in other models of diabetes (9, 12). Our results (using a spontaneously diabetic model) and those of Chatham and Forder (using a streptozotocin-induced diabetic model; Refs. 4, 6) were consistent both with inhibition of glycolysis and substrate entry into the TCA cycle via PDHc. The new findings that we have presented here support a specific mechanism for decreased glycolysis in diabetic hearts. In addition, these experiments provide a mechanism for the beneficial effects of aldose reductase inhibition on the diabetic heart.

Aldose reductase activity and cytosolic redox state. Previous findings in our laboratory demonstrated that increased aldose reductase activity in diabetic animals was correlated with increased NADH/NAD+ (20). Specifically, the sorbitol and fructose levels of diabetic hearts were approximately ninefold higher, and NADH/NAD+ for diabetic hearts was approximately fourfold higher than that measured in nondiabetic hearts. Furthermore, 1 µM zopolrestat (concentration also used in present study) was sufficient to significantly lower the sorbitol and fructose levels and to lower this elevated redox ratio by approximately ninefold (20).

Inhibition of glycolysis at glyceraldehyde 3-phosphate dehydrogenase. We observed a reduction of 13C incorporation into lactate and glutamate from [13C]glucose in diabetic hearts compared with nondiabetic controls. These data reinforce previous studies in diabetic hearts (4) and lenses from galactose-fed rats (19).

**Table 3. Substrate contribution to Acetyl-CoA**

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactate, %</th>
<th>Acetate, %</th>
<th>Non-13C-Enriched Substrates, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>35.3 ± 3.6</td>
<td>29.1 ± 3.7</td>
<td>35.6 ± 4.2*</td>
</tr>
<tr>
<td>Z</td>
<td>37.2 ± 2.7</td>
<td>26.3 ± 4.1</td>
<td>36.5 ± 3.4</td>
</tr>
<tr>
<td>DC</td>
<td>1.2 ± 0.9†</td>
<td>58 ± 3.8‡</td>
<td>40.7 ± 4.1</td>
</tr>
<tr>
<td>DZ</td>
<td>6.3 ± 2.7†</td>
<td>51.8 ± 4.3‡</td>
<td>41.9 ± 5.6</td>
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</table>

Values are means ± SE. Comparisons by ANOVA: *P < 0.05 vs. DC and DZ; †P < 0.0002 vs. C and Z; ‡P < 0.005 vs. C and Z.
We also measured significant elevations in glyceraldehyde 3-phosphate, a glycolytic intermediate, in diabetic hearts compared with nondiabetic controls. Glyceraldehyde 3-phosphate accumulation was also observed in rat hearts by Obrosova et al. (19), who used galactose feeding to increase polyol pathway flux. This marked accumulation of glyceraldehyde 3-phosphate over the amount seen in control nondiabetic hearts, combined with the absence of 13C labeling in downstream glycolytic intermediates (such as lactate) in diabetic hearts, suggests that glycolysis is being limited at glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an enzyme requiring NAD+.

As the consequence of an increased cytosolic redox state under baseline conditions, it has been speculated that glycolysis would be impaired in diabetic tissues due to a reduced availability of NAD+. For enzymes requiring the cofactor (19, 20, 26). An earlier study from this laboratory demonstrated the profound effect of the polyol pathway on NADH/NAD+ in the heart and that both are significantly elevated in diabetes (20). Moreover, Mochizuki and Neely (17) clearly demonstrated inhibition of cardiac GAPDH with increasing concentrations of NADH. In this current study, we present data consistent with the increased activity of the polyol pathway in diabetic animals inhibiting glycolysis due to a decrease in the NAD+ dependent flux from glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.

The evidence presented here and that presented by Obrosova et al. (19) for the inhibition of glycolysis at GAPDH come from the differential accumulation of intermediates. Levels of intermediates, alone, however, do not give a complete picture. It is possible that phosphofructokinase 1 (PFK), PDHc, or a combination of other regulatory enzymes could be the primary enzyme(s) affected by diabetes. However, PFK is “upstream” of GAPDH, and glyceraldehyde 3-phosphate accumulation would not therefore be an expected result from PFK inhibition. A possible explanation for the lack of labeling in glutamate from [13C]glucose is a potential inhibition of the mitochondrial shuttles involved. However, the substrate mix-perfused hearts (Fig. 5) demonstrated 13C labeling of glutamate from [13C]-labeled TCA intermediates, which suggests that the shuttles are in fact intact and active in both diabetic and nondiabetic groups.

Another possible explanation for glyceraldehyde 3-phosphate accumulation could be from the previously demonstrated inhibition of PDHc in diabetic hearts (4, 6). However, if PDHc were the only glycolytic enzyme inhibited with diabetes, then lactate accumulation should not have been significantly decreased in the DC group, as shown here.

Although the lack of insulin with diabetes would clearly result in decreased numbers of GLUT-4 glucose transporters in the sarcolemma, 1) it has been established that glucose transport is in general not limiting in diabetes (8), and 2) all heart groups used in this study were perfused with 11 mM glucose, which has been repeatedly shown to provide sufficient cytosolic glucose concentrations to the heart without the use of insulin (4, 13).

It is possible that the elevated NADH/NAD+ could cause degradation of GAPDH. Knecht and Roche (11) demonstrated that the reduced forms of cofactors, such as NADH and NADPH, accelerate proteolysis of GAPDH and that the oxidized forms of these cofactors decrease the proteolysis of GAPDH. From this effect of redox state on GAPDH, it is plausible that GAPDH may not only be inhibited by NADH/NAD+ but may also be in lower quantities. Additional support for this possibility, is the effect of insulin on GAPDH. Alexander et al. (1) showed that insulin increased GAPDH gene expression. With the autoimmune destruction of pancreatic β-cells, as in human IDDM and the BB/W rat, comes the potential of decreased GAPDH enzyme in the cytosol.

Finally, the accumulation of glyceraldehyde 3-phosphate could also be explained by the inhibition of GAPDH caused by the accumulation of endogenous aldehydes. Novotny et al. (18) have shown significant inhibition of GAPDH and other glycolytic enzymes due to elevated levels of endogenous aldehydes that are found with diabetes. Clearly, diabetes and metabolic regulation are complex issues, and there are several possible explanations for the elevated glyceraldehyde 3-phosphate that we demonstrate here in the acutely diabetic heart. However, the evidence presented here supports the mechanism that the elevation of glyceraldehyde 3-phosphate in diabetic hearts is due to inhibition of GAPDH secondary to effects of elevated polyol pathway flux on the cytosolic redox state (Fig. 1). Certainly, however, more studies are required to demonstrate altered kinetics and quantities of the various enzymes involved.

Aldose reductase inhibition improves glycolysis. The data presented here from diabetic rat hearts treated with zopolrestat demonstrated that the 13C label from glucose was incorporated into lactate and glutamate and that the glyceraldehyde 3-phosphate concentrations were significantly reduced compared with the untreated diabetic group. Furthermore, a previous study in this laboratory demonstrated that inhibiting aldose reductase with zopolrestat conserves NAD+ (20). These effects of zopolrestat in diabetic hearts are consistent with restoration of glycolysis due to greater availability of NAD+ for GAPDH, secondary to decreased use of NAD+ by SDH. Furthermore, a similar effect of aldose reductase inhibition on glycolysis has been shown in lens tissue from galactose-fed rats (19). The data presented here indicate the dramatic influence of polyol pathway flux on diabetic glucose metabolism and suggest a mechanism for correcting some of the metabolic abnormalities of diabetes.

Nondiabetic hearts treated with zopolrestat demonstrated significantly increased 13C labeling in lactate compared with all groups (P < 0.05). This result suggests that anaerobic glycolysis is increased in nondiabetic hearts treated with zopolrestat. Under ischemic conditions, anaerobic glycolysis is the only ATP source for the heart, giving heightened importance to...
interventions that increase glycolysis in diabetic and nondiabetic individuals susceptible to ischemic heart disease or myocardial infarction (25).

Mixed substrate utilization and diabetes. The relative substrate utilization of diabetic hearts, as shown here, is dramatically shifted toward the use of fatty acids (Figs. 5 and 6). In addition to the obvious detriment of decreased glycolytic flux under ischemic conditions, this reliance of diabetic hearts on fatty acids as substrates may in fact increase the susceptibility of these hearts to ischemic injury (14).

Treating diabetic hearts with zopolrestat significantly lowered the acetate use and increased the lactate use of diabetic hearts. Greater lactate incorporation with zopolrestat in the presence of competing substrates is consistent with increased flux through PDHc and suggests altered regulation of PDHc. The mechanism of partial PDHc normalization with zopolrestat in diabetic hearts could be due to increased lactate dehydrogenase activity secondary to redox state correction, thereby increasing the substrate available for PDHc. Furthermore, pyruvate has been shown to have negative effects on PDH kinase and to therefore increase the active (dephosphorylated) form of PDHc (21). The increase in pyruvate availability for PDHc with zopolrestat (secondary to increased glycolysis) is supported by our earlier study (20). The metabolism of lactate through PDHc could also be attributed to the lowered redox state of the cytosol affecting the mitochondrial redox state and therefore PDHc activity. Finally, although there is no evidence suggesting a direct effect of zopolrestat on PDHc, our data could indicate altered sensitivity of PDHc to its known regulators, such as ATP, NADH, pyruvate, and acetyl-CoA. Data from this study, however, cannot distinguish these possibilities.

Aldose reductase and NADPH. Zopolrestat is the most specific aldose reductase inhibitor (ARI) available (24). However, despite its specificity, there are other potential effects of aldose reductase inhibition that should be kept in mind. For instance, cytosolic NADH/NAD$^+$ is not the only redox couple affected by ARI. Aldose reductase, itself, requires NADPH and generates NADP$^+$. Potentially, then, ARI could have secondary effects on the cell by affecting flux through other enzymes that are affected by the NADP$^+$-NADPH couple. Therefore glucose-6-phosphate dehydrogenase (of pentose phosphate pathway), glutathione reductase, and nitric oxide synthase could all be potentially affected secondarily by ARI. Further investigation of these possibilities should yield information regarding the relationship between ARI and these other important enzymes.

Diabetes, zopolrestat, and cardiac function. As seen in Table 1, the only diabetic group with a mean heart rate not significantly lower than corresponding controls was the glucose-perfused diabetic hearts treated with zopolrestat. The lower heart rate in diabetic control hearts was expected and is similar to that shown in other laboratories (4, 13). That zopolrestat treatment increased the heart rate of glucose-perfused diabetic hearts to insignificant difference from its nondiabetic control suggests improved function and increased oxygen consumption with aldose reductase inhibition but may also be due to a large SE. Developed pressure was not different between any of the groups, as also shown by Lopaschuk et al. (13).

In conclusion, the significant new findings presented in this paper are the following: 1) diabetic rat hearts have glycolysis inhibited at GAPDH secondary to elevated polyol pathway flux; 2) zopolrestat inhibition of aldose reductase, which lowers NADH/NAD$^+$, normalizes flux via GAPDH; 3) when perfused with lactate and acetate, diabetic hearts use 98% acetate and nondiabetic hearts use 45% acetate; and 4) zopolrestat increased the incorporation of $^{13}$C-labeled lactate into the TCA cycle of diabetic hearts in the presence of competing substrates, indicating improved flux through PDHc.

These findings, in total, are consistent with aldose reductase inhibition correcting abnormal glycolytic metabolism in diabetic hearts. Based on the known effects of aldose reductase inhibition on the cytosolic redox state, one likely component of this effect is normalization of NADH/NAD$^+$. The proposed mechanism by which aldose reductase inhibition affects the substrate metabolism of the diabetic heart is seen in Fig. 1. In essence, inhibition of this pathway of glucose metabolism normalizes the redox state of the cell, indirectly increasing substrate flux via GAPDH, and PDHc.

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