Glucose and insulin improve cardiac efficiency and postischemic functional recovery in perfused hearts from type 2 diabetic (db/db) mice

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Hafstad AD, Khalid AM, How OJ, Larsen TS, Aasum E. Glucose and insulin improve cardiac efficiency and postischemic functional recovery in perfused hearts from type 2 diabetic (db/db) mice. Am J Physiol Endocrinol Metab 292: E1288–E1294, 2007. First published January 9, 2007; doi:10.1152/ajpendo.00504.2006.—Hearts from type 2 diabetic (db/db) mice demonstrate altered substrate utilization with high rates of fatty acid oxidation, decreased functional recovery following ischemia, and reduced cardiac efficiency. Although db/db mice show overall insulin resistance in vivo, we recently reported that insulin induces a marked shift toward glucose oxidation in isolated perfused db/db hearts. We hypothesize that such a shift in metabolism should improve cardiac efficiency and consequently increase functional recovery following low-flow ischemia. Hearts from db/db and nondiabetic (db/+ ) mice were perfused with 0.7 mM palmitate plus either 5 mM glucose (G), 5 mM glucose and 300 μU/ml insulin (GI), or 33 mM glucose and 900 μU/ml insulin (HGHII). Substrate oxidation and postischemic recovery were only moderately affected by GI and HGHII in db/+ hearts. In contrast, GI and particularly HGHII markedly increased glucose oxidation and improved postischemic functional recovery in db/db hearts. Cardiac efficiency was significantly improved in db/db, but not in db/+ hearts, in the presence of HGHII. In conclusion, insulin and glucose normalize cardiac metabolism, restore efficiency, and improve postischemic recovery in type 2 diabetic mouse hearts. These findings may in part explain the beneficial effect of glucose-insulin-potassium therapy in diabetic patients with cardiac complications.

There has been a long-standing interest in metabolic modulation as a means to improve functional recovery following myocardial ischemia, and administration of glucose and insulin, as part of glucose-insulin-potassium (GIK) treatment, has gained new interest in the last decade (19, 20, 25, 26, 28, 32). Although cardioprotective effects of high glucose and/or insulin have been demonstrated in experimental studies using nondiabetic models (7, 37), few studies have examined if this treatment exerts a cardioprotective effect in type 2 diabetic models.

We have previously reported that ex vivo perfused hearts from type 2 diabetic db/db mice show enhanced FAox and reduced glucose utilization (1, 5, 6), as well as reduced tolerance to ischemia–reperfusion (2). More recently, we have also shown that db/db hearts exhibit reduced cardiac efficiency [elevated unloaded myocardial oxygen consumption (MV02); see Ref. 13]. It is reasonable to suggest that reduced ischemic tolerance of diabetic hearts is causally related to lower cardiac efficiency, since lower efficiency will increase oxygen utilization and thus exacerbate the energy shortage in the ischemic myocardium. Moreover, several lines of evidence have linked cardiac efficiency to the choice of metabolic fuel. Elevated rates of FA uptake and oxidation increased MV02 in nondiabetic hearts (12, 29), and decreased cardiac efficiency has been associated with elevated rates of FA utilization in diabetic- and/or insulin-resistant hearts in both experimental (13, 34) and clinical studies (33). Accordingly, interventions aimed to improve cardiac metabolism in diabetic hearts should potentially also increase cardiac efficiency and improve ischemic tolerance.

Interestingly, we have recently found that ex vivo perfused hearts from the db/db mouse show a marked shift toward higher glucose oxidation (Gox) at the expense of FAox in response to acutely administered insulin (11), despite the fact that db/db mice show severe insulin resistance in vivo (18). McNulty (27) has pointed out that this observation agrees with reports of cardiac insulin responsiveness in human diabetes (14, 36). Because the cardioprotective role of insulin in a type 2 diabetic model has not previously been investigated, the aim of the present study was to examine whether an acute metabolic intervention (high glucose and insulin) will improve cardiac efficiency and postischemic recovery following low-flow ischemia in perfused db/db hearts.

MATERIALS AND METHODS

Animals, C57BL/KsJ-lep+/-lep+/- diabetic mice (db/db); body wt 45.7 ± 0.7 g, n = 30) of mixed gender (12–14 wk old), as well as their

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non-diabetic heterozygote littermates (db/+; body wt 26.6 ± 0.7 g, n = 29), were purchased from Harlan (Bicester, UK) or M&B (Ry, Denmark) and housed in a room maintained at 23°C and 55% humidity with a 12:12-h light-dark cycle. The mice were given ad libitum access to food and water and treated in accordance to the guidelines on accommodation and care of animals formulated by the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes. The experiments were approved by the Animal Welfare Committee of the University of Tromsø. Plasma glucose, determined in blood samples taken at the day of death (catalog no. 1442449; Boehringer Mannheim, Mannheim, Germany), revealed marked hyperglycemia in db/db (48.5 ± 2.6 mM, n = 16) compared with db/+ (18.9 ± 0.8 mM, n = 11) mice.

Measurements of cardiac metabolism and postischemic functional recovery. Heparin (100 units ip) was administered 5 min before the mice were anesthetized (10 mg ip injection of plastic pentobarbital sodium). Hearts were quickly excised, and the aorta was cannulated with an 18-gauge cannula. The left atrium was cannulated with a steel cannula (1.5 mm outer diameter, 1.0 mm inner diameter) connected to a preload reservoir (12.5 mmHg), and hearts were perfused in the working mode with the left ventricle ejecting against an afterload of 55 mmHg using a modified Krebs-Henseleit bicarbonate buffer supplemented with 0.4 mM palmitate bound to 3% BSA. All hearts were allowed to beat spontaneously. The concentration of endogenous free fatty acids in the 3% BSA solution was 0.3 mM, so that the total FA concentration in the perfusion buffer was 0.7 mM. In addition to FAs, the perfusion buffer was also supplemented with either 5 mM glucose (G), 5 mM glucose and 300 μU/ml insulin (low glucose and insulin, GI), or 33 mM glucose and 900 μU/ml of insulin (high glucose and high insulin, HGH). Measurements of intraventricular pressure were obtained by inserting a 2-Fr micromanometer-tipped catheter (SPR 407; Millar), via the atrial steel cannula, into the left ventricle. In some cases, introduction of the catheter resulted in a marked drop in aortic flow, presumably because of interference with the function of the mitral valves. We therefore decided to run the protocol without the intraventricular pressure recording if the introduction of the catheter caused a decrease in the aortic flow of >0.5 ml/min. Coronary flow was measured by timed collections of the effluent dripping from the heart, whereas aortic flow was determined using a drop counter (with infrared detection) at the outlet of the afterload line. Cardiac output was calculated as the sum of the coronary and aortic flow. Following 30 min preischemic perfusion, hearts were subjected to 40 min low-flow ischemia (3.1 ml·g dry wt⁻¹·min⁻¹, which is ~3% of their baseline coronary flow), followed by 5 min reperfusion in Langendorff mode and 30 min in working mode. Hearts that did not produce pressure exceeding that of the afterload column were perfused in an “assisted” mode to hold the perfusion pressure (i.e., the height of the afterload column was maintained by supplying it with freshly oxygenated buffer). Postischemic recovery of ventricular function was measured after 35 min reperfusion relative to baseline (preischemic) values. Glucose and palmitate oxidation were determined simultaneously by measuring 14CO2 and 3H2O released by the oxidation of [U-14C]glucose and [9,10-3H]palmitate, respectively (2, 5). Measurements were performed for all three perfusion groups (G, GI, and HGH) both in the pre- and postischemic periods.

Measurements of cardiac efficiency and ventricular function. Cardiac efficiency was determined in a separate series of experiments by measuring the relationship between cardiac work (pressure-volume area, PVA) and MV˙O2 (12, 13). A 1.4-Fr micromanometer-conductance catheter (Millar Instruments, Houston, TX) was inserted in the left ventricle through the apex. Fiber-optic oxygen probes (FOXY-AL300; Ocean Optics, Duiven, Netherlands) were placed in the left atrial cannula (adjacent to the heart) and in the pulmonary trunk for on-line recordings of the partial oxygen pressure (P02). MV˙O2 was calculated by the following equation: MV˙O2 = [P02 (oxygenated perfusate) – P02 (coronary effluent)] × Bunsen solubility coefficient of O2 × coronary flow. Finally, electrodes were connected to the right atrium for electrical pacing of the heart (10% above the intrinsic heart rate). Hearts were exposed to different workloads by changing preload (from 3 to 8 mmHg) and afterload (from 35 to 50 mmHg), and steady-state values of PVA and MV˙O2 were calculated at each workload. The PVA-MV˙O2 relationship was first determined in hearts perfused with low glucose (G buffer). Thereafter, the concentrations of glucose and insulin were elevated to 33 mM and 900 μU/ml, respectively (HGH buffer), and another set of PVA-MV˙O2 relationships was determined following stabilization. The PVA-MV˙O2 regression allows the myocardial oxygen cost to be separated in the following two parts: unloaded MV˙O2 (γ-intercept of the PVA-MV˙O2 relationship) and contractile efficiency (the inverse slope of the PVA-MV˙O2 relationship). Unloaded MV˙O2 is a measure of the energy cost of excitation-contraction coupling and basal metabolism, whereas contractile efficiency reflects the amount of metabolic energy that is consumed during the process of systolic work. Cardiac output was calculated as the sum of the coronary and aortic flow. Following

**Fig. 1. Rates of preischemic and postischemic glucose oxidation and palmitate oxidation in isolated perfused hearts from non-diabetic (db/+; open bars) and diabetic (db/db, filled bars) mice. Hearts were perfused with buffer supplemented with 0.7 mM fatty acids in addition to either 5 mM glucose (G), 5 mM glucose and 300 μU/ml insulin (GI), or 33 mM glucose and 900 μU/ml insulin (HGH). Results are means of 6–9 mice in each group. **P < 0.025 vs. G. †P < 0.05 vs. db/+ at the same perfusion condition.**
Statistical analysis. Data are expressed as means ± SE. Differences in cardiac function, myocardial substrate oxidation, and recovery of functional parameters were determined by a two-way ANOVA followed by Holm-Sidak’s method to adjust for multiple comparisons. For analysis of regression (correlation between cardiac recovery and preischemic oxidation rates), a Person Product Moment Correlation was used. Between- and within-group differences in cardiac efficiency and cardiac function were analyzed by an unpaired and paired Student’s t-test, respectively. The overall significance level was 0.05.

RESULTS

Effect of HGHI on cardiac metabolism. Figure 1 shows the metabolic response to elevated insulin and glucose in nondiabetic (db/db) hearts and from type 2 diabetic (db/db) mice. In line with previous results (2, 5), FAox was significantly increased with a corresponding decline in Gox in db/db hearts under baseline preischemic conditions (0.7 mM FA, 5 mM G). Addition of insulin (300 μU/ml, GI) or high glucose and insulin (33 mM glucose, 900 μU/ml insulin, HGHI) had no significant effect on rates of Gox or FAox in control db/db hearts. In db/db hearts, however, addition of insulin (GI) increased rates of Gox (P < 0.01) and decreased rates of FAox (P < 0.001). An even higher response was observed during HGHI perfusion, which resulted in a 3.5-fold elevation in Gox (P < 0.001) and a 78% reduction in the FAox rate (P < 0.001). In fact, under HGHI conditions, there was no difference in the rates of substrate oxidation between db/db and db/+ hearts (Fig. 1).

The mechanical function of perfused working hearts is shown in Table 1. Hearts from db/db mice showed reduced ventricular function, as indicated by reduced aortic flow, cardiac output, and cardiac power, consistent with previous studies (2, 5). There were, however, no differences in cardiac function between the various subgroups (G, GI, and HGHI) for neither db/db nor db/+ hearts, indicating that the observed metabolic effects of glucose and insulin (Fig. 1) were not because of differences in cardiac performance. The effects of glucose and insulin on cardiac metabolism were also maintained during the postischemic perfusion period (Fig. 1) in db/db hearts. Again, there was no difference in Gox or FAox rates between db/+ and db/db hearts when perfused under HGHI conditions.

Effect of HGHI on postischemic functional recovery. Figure 2 shows postischemic functional recovery following low-flow ischemia and reperfusion in all perfusion groups. In accordance with Aasum et al. (2), db/db hearts showed reduced functional recovery following ischemia under baseline conditions, as indicated by reduced recovery of cardiac output, aortic flow, stroke volume, and cardiac power. GI and HGHI only moderately improved postischemic recovery in control hearts (Fig. 2), whereas all functional parameters were significantly improved by GI and HGHI in db/db hearts. In the presence of HGHI postischemic recovery of db/db hearts was not different from that of db/+ hearts. In Fig. 3, postischemic recovery of aortic flow is plotted relative to preischemic Gox and FAox rates. There was no significant relationship between these parameters for db/+ hearts. In diabetic hearts, on the other
hand, recovery of aortic flow was positively correlated to $G_{\text{ox}}$ rates ($r = 0.67, P < 0.001, n = 23$) and negatively correlated to palmitate oxidation rates ($r = -0.62, P < 0.001, n = 23$).

**Effect of HGHI on cardiac efficiency.** To study the effect of HGHI on cardiac efficiency, the relationship between ventricular PVA and $\dot{M}V_{\text{O}_2}$ was determined in hearts that were first perfused with G buffer and thereafter by HGHI buffer. Table 2 gives the $y$-intercept (unloaded $\dot{M}V_{\text{O}_2}$) and slope of the regression lines obtained in individual experiments, as well as the group means. In accordance with How et al. (13) hearts from $db/db$ mice perfused under baseline conditions showed decreased cardiac efficiency revealed as a 36% increase in unloaded $\dot{M}V_{\text{O}_2}$ compared with $db/+$. Contractile efficiency (the inverse of the slope of the regression line) was, however, not significantly altered. HGHI improved cardiac efficiency significantly in $db/db$ hearts by decreasing unloaded $\dot{M}V_{\text{O}_2}$. In contrast, HGHI did not influence efficiency in $db/+$. Contractile efficiency (slope) was not altered by the addition of HGHI, neither in $db/db$ nor in $db/+$.  

**Functional effects of HGHI.** Analysis of P-V loops obtained under baseline loading conditions confirmed ventricular dysfunction in $db/db$ hearts, as indicated by reduced cardiac output and elevated left ventricular end-diastolic pressure (LVEDP; Table 3). To study the functional effect of HGHI, P-V loops were compared just before and after the elevation of glucose and insulin. HGHI slightly, but significantly, reduced LVEDP and increased $dP/dt_{\text{max}}$ in both $db/+$ and $db/db$ hearts. In $db/db$ hearts, HGHI also significantly increased left ventricular end-systolic pressure and $dP/dt_{\text{min}}$.

**DISCUSSION**

Hearts from type 2 diabetic $db/db$ mice showed high reliance on FAox for energy production, mechanical dysfunction, reduced efficiency, and reduced functional recovery following ischemia. We demonstrated that acute administration of HGHI in the perfusate of $db/db$ hearts not only normalized cardiac metabolism but also restored cardiac efficiency and improved recovery of contractile function following low-flow ischemia.
It is well established that elevation of myocardial FA supply and oxidation impairs functional recovery following ischemia in normal hearts (21). On the other hand, pharmacological inhibition of FAox (23) or stimulation of Gox (37) improves postischemic recovery. Although growing evidence supports the notion that dysregulation of metabolism contributes to the development of cardiac dysfunction and/or reduced ischemic tolerance in diabetic hearts (3, 6), no studies have examined the effect of acute administration of glucose and/or insulin in type 2 diabetes. However, we recently demonstrated that acute administration of insulin caused a marked metabolic shift toward Gox in perfused type 2 diabetic (db/db) mouse hearts (11). In accordance with this finding, the present study showed that insulin significantly increased Gox and decreased FAox in db/db hearts. After high glucose and insulin administration, cardiac metabolism was completely normalized. We also found that administration of insulin and HGHI improved postischemic recovery. Interestingly, the postischemic functional recovery (aortic flow) was positively correlated to preischemic Gox and negatively correlated to FAox rates in the diabetic hearts. These findings support the notion that the metabolic status of the heart, when entering the ischemic condition, is a determinant of the functional outcome of the ischemic insult (23, 35, 37). The lack of correlation between cardiac substrate oxidation and recovery in control hearts was most likely because of a much lower effect of insulin and glucose on fuel selection, plus the fact that these hearts were only modestly damaged by the ischemic insult, so that the window for protection was limited. Although our data show that insulin and glucose were protective only in diabetic hearts, it should be noted that the ischemic stress used in the present protocol was adjusted to give a reasonable dysfunction in the diabetic hearts and, not surprisingly, this degree of stress caused only a minor functional loss in the control hearts. Thus one should not categorically exclude that insulin and glucose would have had beneficial effects also in control hearts, given that these hearts were subjected to a more severe stress.

Clearly, there are controversies in the literature with respect to the cardioprotective effect of GIK in the setting of ischemia-reperfusion or recovery from cardiac surgery in humans.

Table 2. Individual values and group means of the y-intercept and slope of the PVA-MV˙O2 relationship obtained in isolated perfused hearts from control (db/+ ) and diabetic (db/db) mice before and after addition of high glucose and insulin

<table>
<thead>
<tr>
<th>Value No.</th>
<th>G</th>
<th>HGHI</th>
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<tbody>
<tr>
<td></td>
<td>y-Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td>1</td>
<td>1.16</td>
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</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>2.61</td>
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<td>4</td>
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<td>5</td>
<td>2.52</td>
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</tr>
<tr>
<td>6</td>
<td>1.55</td>
<td>2.86</td>
</tr>
<tr>
<td>db/+</td>
<td>2.00±0.25</td>
<td>2.94±0.31</td>
</tr>
<tr>
<td>1</td>
<td>2.47</td>
<td>4.16</td>
</tr>
<tr>
<td>2</td>
<td>3.53</td>
<td>4.46</td>
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<tr>
<td>3</td>
<td>2.85</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>2.64</td>
<td>3.15</td>
</tr>
<tr>
<td>6</td>
<td>2.60</td>
<td>2.34</td>
</tr>
<tr>
<td>db/db</td>
<td>2.71±0.19†</td>
<td>3.31±0.39</td>
</tr>
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</table>

Values are means ± SE. For each heart, the pressure-volume area (PVA)-myocardial oxygen consumption (MV˙O2) relationship was initially determined in perfusions with buffer containing 5 mM glucose (G) and thereafter in perfusions with elevated glucose and insulin (33 mM and 900 µU/ml, respectively, HGHI). y-Intercept represents MV˙O2 for unloaded hearts, expressed as l-beat-1 g dry heart wt-1 (10-3). Slope is dimensionless.*P < 0.05 vs. G. †P < 0.05 vs. db/+ at the same perfusion condition.

Table 3. Cardiac function in isolated perfused hearts from control (db/+ ) and diabetic (db/db) mice, before and after addition of high glucose and insulin

<table>
<thead>
<tr>
<th></th>
<th>db/+ (n = 6)</th>
<th>db/db (n = 6)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>HGHI</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>12.9±0.9</td>
<td>13.3±0.8</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>2.9±0.2</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>Stroke volume, µl</td>
<td>27.8±2.3</td>
<td>28.4±2.0</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>62.8±1.3</td>
<td>63.7±1.5</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>4.3±0.5</td>
<td>3.9±0.5*</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>5.026±223</td>
<td>5.418±255*</td>
</tr>
<tr>
<td>dP/dtmin, mmHg/s</td>
<td>-4.273±338</td>
<td>-4.423±324</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>15±2</td>
<td>15±2</td>
</tr>
<tr>
<td>Stroke work, mmHg×µl</td>
<td>1,641±181</td>
<td>1,712±163</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of hearts. Data were obtained at a baseline workload of 8 mmHg preload and 50 mmHg afterload. All hearts were paced at 10% above their intrinsic heart rate (468±10 and 390±13 in db/+ and db/db hearts, respectively). LVESP, left ventricular end systolic pressure. The relaxation constant Tau (glanz) is the regression of dP/dt vs. pressure. Statistical comparison of dP/dt and Tau between db/+ and db/db hearts was not performed because of differences in heart rate. The hearts are the same as those in Table 2. *P < 0.05 vs. G. †P < 0.05 vs. db/+ at the same perfusion condition.
Cardiac efficiency was, however, markedly improved in the presence of HGHI, supporting earlier findings of improved cardiac efficiency following stimulation of glucose metabolism with GIK in chronic ovine diabetes (34). The increased cardiac efficiency can only partly be explained by the switch in fuel consumption, since a maximum of 12% decrease in MV\(\dot{O}_2\) can occur when changing from 100% FA\(\dot{O}_2\) to 100% G\(\dot{O}_2\). Therefore, additional mechanisms must contribute to the reduced unloaded MV\(\dot{O}_2\) in the \(db/db\) hearts, for instance reduced cost of excitation-contraction coupling (resulting from improved calcium handling and increased calcium sensitivity), less mitochondrial uncoupling (30), and less turnover of intracellular futile triglyceride-FA cycles (31). Because reduced cardiac efficiency may have deleterious consequences, particularly under conditions of decreased oxygen supply, the improved ischemic tolerance in glucose- and/or insulin-perfused \(db/db\) hearts is most likely related to the resulting improvement in cardiac efficiency. In support of this view, recent results from our own laboratory (unpublished observations) show that normalization of cardiac metabolism and lowering of unloaded MV\(\dot{O}_2\) following PPAR\(\gamma\) treatment are associated with an improved ischemic tolerance of \(db/db\) hearts.

In summary, the present study shows that HGHI normalize myocardial metabolism, restore efficiency, and improve postischemic functional recovery in hearts from a type 2 diabetic animal model, a finding that may explain the particular beneficial effects of GIK therapy in diabetic patients with cardiac complications.

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