

Perfused hearts from Type 2 diabetic (*db/db*) mice show metabolic responsiveness to insulin

Anne Dragøy Hafstad,¹ Geir Helge Solevåg,¹ David L. Severson,² Terje S. Larsen,¹ and Ellen Aasum¹

¹Department of Medical Physiology, Institute of Medical Biology, Faculty of Medicine, University of Tromsø, Tromsø, Norway; and ²Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

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Hafstad, Anne Dragøy, Geir Helge Solevåg, David L. Severson, Terje S. Larsen, and Ellen Aasum. Perfused hearts from Type 2 diabetic (*db/db*) mice show metabolic responsiveness to insulin. *Am J Physiol Heart Circ Physiol* 290: H1763–H1769, 2006. First published December 3, 2005; doi:10.1152/ajpheart.01063.2005.—Diabetic (*db/db*) mice provide an animal model of Type 2 diabetes characterized by marked in vivo insulin resistance. The effect of insulin on myocardial metabolism has not been fully elucidated in this diabetic model. In the present study we tested the hypothesis that the metabolic response to insulin in *db/db* hearts will be diminished due to cardiac insulin resistance. Insulin-induced changes in glucose oxidation (GLUox) and fatty acid (FA) oxidation (FAox) were measured in isolated hearts from control and diabetic mice, perfused with both low as well as high concentration of glucose and FA: 10 mM glucose/0.5 mM palmitate and 28 mM glucose/1.1 mM palmitate. Both in the absence and presence of insulin, diabetic hearts showed decreased rates of GLUox and elevated rates of FAox. However, the insulin-induced increment in GLUox, as well as the insulin-induced decrement in FAox, was similar or even more pronounced in diabetic than in control hearts. During elevated FA and glucose supply, however, the effect of insulin was blunted in *db/db* hearts with respect to both FAox and GLUox. Finally, insulin-stimulated deoxyglucose uptake was markedly reduced in isolated cardiomyocytes from *db/db* mice, whereas glucose uptake in isolated perfused *db/db* hearts was clearly responsive to insulin. These results show that, despite reduced insulin-stimulated glucose uptake in isolated cardiomyocytes, isolated perfused *db/db* hearts are responsive to metabolic actions of insulin. These results should advocate the use of insulin therapy (glucose-insulin-potassium) in diabetic patients undergoing cardiac surgery or during reperfusion after an ischemic insult.

myocardial metabolism; insulin action; glucose and fatty acid metabolism

DIABETES IS ASSOCIATED with a switch in cardiac substrate metabolism with an increase in fatty acid (FA) oxidation (FAox) and a concomitant decrease in glucose utilization (2, 4). This metabolic change has been referred to as a metabolic maladaptation (47) and may in particular have adverse consequences under conditions of reduced oxygen availability. The reason for this metabolic switch in diabetic hearts is partially explained by changes in circulating substrate levels and alterations in metabolic gene expression (17, 49). In addition, reduced insulin responsiveness has been assumed to contribute to decreased glucose utilization in Type 2 diabetic hearts. Reduced insulin responsiveness in skeletal muscle has been well documented and may be the major factor leading to

overall in vivo insulin resistance (43). The mechanism and degree of reduced responsiveness to insulin in the heart, however, have not been fully elucidated. For instance, studies in humans with Type 2 diabetes have demonstrated that glucose uptake in cardiac muscle is insulin sensitive, despite insulin resistance in skeletal muscle (22, 48). Furthermore, administration of glucose-insulin-potassium (GIK) has been demonstrated to be of particular benefit to patients with diabetes undergoing cardiac surgery (29, 32). It is not clear, however, whether this beneficial effect is related to altered metabolism, although it is well known that a metabolic shift toward glucose utilization improves myocardial recovery after ischemic injury in patients without diabetes (16, 31, 46).

The diabetic *db/db* mouse provides a model for severe Type 2 diabetes with obesity, hyperglycemia, hyperinsulinemia, and dyslipidemia (12). Both glucose tolerance tests (18, 24) and hyperglycemic-hyperinsulinemic clamps (8) also demonstrate a marked in vivo insulin resistance in *db/db* mice. Characterization of the metabolic phenotype of *db/db* hearts, assessed with isolated perfused hearts, has revealed that glucose utilization [glycolysis and glucose oxidation (GLUox)] was reduced and FA utilization was markedly enhanced (1, 4, 10, 50). Most studies addressing insulin responsiveness in Type 2 diabetic hearts have studied glucose uptake into isolated cardiomyocytes (13, 25, 33), and indeed a recent study reported that insulin-induced glucose uptake into cardiomyocytes from *db/db* hearts was reduced (9). The metabolic effect of insulin on oxidative metabolism in *db/db* hearts has, however, not been examined previously. The aim of the present study was to test the hypothesis that metabolic responses to insulin in Type 2 diabetic *db/db* hearts is impaired. Therefore, ex vivo working hearts from control and *db/db* mice were perfused with low glucose and palmitate concentrations (10 mM and 0.5 mM, respectively) with or without insulin. Because Type 2 diabetic hearts are exposed to elevated concentration of FA and glucose in vivo, we also measured the metabolic responses to insulin in hearts perfused with high glucose and palmitate (28 mM and 1.1 mM, respectively).

MATERIALS AND METHODS

Experimental animals. Male C57BL/KsJ-lepr^{db}/lepr^{db} (*db/db*) and lean control heterozygote (*db/+*) mice were purchased from Harlan. The mice were maintained at 23 ± 1°C and 55 ± 5% humidity, with a 12-h:12-h light-dark cycle and ad libitum access to water and a standard mouse diet [RM1 (E), Special Diets Services, Witham,

Address for reprint requests and other correspondence: E. Aasum, Dept. of Medical Physiology, Faculty of Medicine, Univ. of Tromsø, N-9037 Tromsø, Norway (e-mail: ellenaa@fagmed.uit.no).

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Essex, UK] containing 2.7% fat and 14% protein. All animals were treated according to the guidelines on accommodation and care of animals formulated by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes. The experimental protocol was approved by the University of Tromsø Welfare Committee. After the excision of hearts from animals at an age of 12–16 wk, a blood sample was taken from the body cavity, and plasma levels of glucose and FAs were determined by using kits from Boehringer-Mannheim (Mannheim, Germany) and Wako Chemicals (Neuss, Germany), respectively. Plasma levels of insulin were determined using an ELISA kit (Crystal Chem Chicago).

Isolated working heart perfusions. Cardiac metabolism and function were measured in isolated perfused hearts as previously described (2, 28). In brief, the aorta and left atrium were cannulated with an 18- and 16-gauge cannula, respectively. The preload pressure was 12.5 mmHg, and the afterload pressure was 50 mmHg. Hearts were allowed to beat spontaneously; peak systolic pressure was recorded in the aortic (afterload) line. Cardiac output was obtained from the sum of aortic and coronary flows. The hearts were perfused with a modified Krebs-Henseleit bicarbonate (KHB) buffer consisting of (in mM) 118.5 NaCl, 25 NaHCO₃, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, and 2.5 CaCl₂, gassed with 95% O₂-5% CO₂ (pH 7.4). The KHB buffer was supplemented with glucose and palmitate bound to 3% BSA (fraction V, Sigma A-8022). The buffer was recirculated with a total volume of 40 ml.

Insulin-induced changes in myocardial oxidative metabolism in isolated perfused hearts. To study insulin-induced changes in cardiac metabolism, we perfused hearts with 10 mM glucose and 0.5 mM palmitate (low-glucose and low-palmitate buffer; LGLP) or 28 mM glucose and 1.1 mM palmitate (high-glucose and high-palmitate buffer; HGHP) in the absence and in the presence of insulin (0.3 or 1 mU/ml). Glucose and palmitate oxidation were determined in separate groups of hearts by measuring ¹⁴CO₂ released from oxidation of [U-¹⁴C]glucose or [1-¹⁴C]palmitate, respectively (1). Trapping and measuring the ¹⁴CO₂ released required the use of an airtight perfusion apparatus. Released ¹⁴CO₂ was trapped by continuously bubbling the outflow air from the perfusion apparatus through 10 ml of hyamine hydroxide (1 M), from which samples (350 μl) were taken. The ¹⁴CO₂ remaining in the perfusion medium (in the form of bicarbonate anion) was released by injecting 0.75 ml of the perfusate sample into 0.75 ml of 9 N H₂SO₄ inside a sealed test tube, where the ¹⁴CO₂ released from the sample was trapped in a filter paper containing 300 μl of hyamine hydroxide. Quantitative ¹⁴CO₂ production was determined by adding the values for ¹⁴CO₂ obtained from the outflow air and perfusion sample. Heart metabolism was measured by taking samples of the perfusion buffer every 10 min. At the end of the perfusion, hearts were frozen between precooled metal clamps, and total dry mass was measured by freeze-drying the heart.

Insulin-induced deoxyglucose uptake into isolated cardiomyocytes. Cardiomyocytes were isolated by using collagenase digestion of Langendorff-perfused hearts. After aortic cannulation, *db/db* or *db/+* hearts were retrogradely perfused at 37°C for 5 min with a buffer consisting of (in mM) 120 NaCl, 5.4 KCl, 5 MgSO₄, 0.6 KH₂PO₄, 5.5 glucose, 20 NaHCO₃, 10 HEPES, 9.2 taurine, and 10 2,3-butanedione monoxime and gassed with 95% O₂-5% CO₂ (pH 7.3). Hearts were then perfused for 8–10 min at a constant flow (3 ml/min) with buffer containing 12.5 μM CaCl₂, 1% FA-free BSA, and 0.10–0.18 U/ml type II collagenase (Worthington). The ventricles were then removed and digested at 37°C for 5–10 min longer in the presence of collagenase. Dispersed myocytes were filtered (250-μm mesh), gently pelleted by centrifugation, and resuspended in collagenase-free buffer where BSA was substituted with 10% FCS. Calcium concentrations were then increased gradually to 1.0 mM in subsequent washings. Glucose uptake into the cardiomyocytes was measured using radiolabeled 2-deoxyglucose (9). Cardiomyocytes were plated (35 mm) and washed twice with glucose-free DMEM (GIBCO). The cells were then incubated for 40 min (37°C, with 95% O₂-5% CO₂ gassing) with

DMEM containing 0.1% FA-free BSA and 5.0 mM pyruvate, in the absence and in the presence of insulin (0.2–20 μU/ml). Twenty microliters of a solution containing 220 μl of glucose-free DMEM, 30 μl of a 20 mM 2-deoxyglucose solution, 30 μl of a 20 mM L-glucose solution, 5 μCi of 2-deoxy-D-[2,6-³H]glucose, and 3 μCi of L-[1-¹⁴C]glucose were added to each dish, and the incubation was continued for 20 min. Deoxyglucose transport was terminated by aspiration of the buffer, followed by two washes with cold PBS. Cells were lysed in 500 μl of 0.25 M NaOH. A 300-μl sample from each dish (after addition of 30 μl 3 M HCl) was used for determining radioactivity. Protein assay was performed with 30 μl of the lysate by use of a Lowry protein assay. Glucose uptake was calculated on the basis of 2-deoxy-D-[2,6-³H]glucose uptake after correction for passive diffusion of glucose from L-[1-¹⁴C]glucose measurements (25).

Insulin-induced glucose uptake into isolated perfused hearts. In isolated working hearts, glucose uptake was determined by measuring the amount of ³H₂O released from the metabolism of [2-³H]glucose. In these experiments, glucose and palmitate concentrations were 5 and 0.5 mmol/l, respectively. Each heart was initially perfused for 20 min with no insulin in the buffer, followed by a 30-min perfusion period where insulin (0.3 mU/ml) was added to the buffer. Separation of [2-³H]glucose and ³H₂O in the perfusate samples was performed by vacuum sublimation of a 400-μl aliquot (1).

Measurement of glycogen content. Glycogen content was measured in hearts at the end of perfusions, after extraction of ~10 mg (dry wt) frozen heart tissue in trichloroacetic acid (37). After acid hydrolysis, glycogen was measured as glucose units with a standard kit from Boehringer-Mannheim (Mannheim, Germany).

Measurement of tissue levels of protein kinase B. The insulin-stimulated phosphatidylinositol 3-kinase pathway results in phosphorylation of protein kinase B (PKB)/Akt at Thr-308, which results in enzyme activation (44). Total and phosphorylated levels of PKB/Akt were determined by immunoblotting tissue from *db/+* and *db/db* hearts after perfusion in the absence or presence of insulin. Boiled samples of tissue homogenates were subjected to SDS-PAGE in gels containing 8% acrylamide and transferred to nitrocellulose membranes. These membranes were blocked in 5% skim milk in PBS-0.1% Tween 20 (PBS-T) and then immunoblotted at 1:800 dilution with either rabbit anti-phospho-Akt (Thr-308) or rabbit anti-Akt (Cell Signaling Technology) overnight at 4°C to detect phospho-PKB and total PKB, respectively. After being washed extensively, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody in 5% milk in PBS-T followed by further washing in PBS-T.

Statistical analysis. Data are expressed as means ± SE. Differences in cardiac function, myocardial substrate oxidation, glycogen, and PKB phosphorylation were determined by ANOVA, followed by an unpaired Student's *t*-test or a Mann-Whitney rank sum test if the normality test failed. Bonferroni's method was applied to adjust for multiple comparisons. Animal characteristics were analyzed by unpaired Student's *t*-test. Differences between means were regarded as statistically significant when the *P* value was <0.05.

RESULTS

Animal characteristics. The general characteristics of the nondiabetic (*db/+*) and diabetic (*db/db*) mice are shown in Table 1. The *db/db* mice exhibited obesity as well as elevated plasma levels of glucose, FAs, and insulin, confirming previous reports of profound insulin resistance as evidenced by concomitant hyperglycemia and hyperinsulinemia (2, 4), as well as impaired glucose tolerance (24, 35). There was no significant difference in heart weight between *db/+* and *db/db* mice.

Effect of insulin on GLUox and FAox in perfused hearts. The effect of insulin on myocardial oxidative metabolism was

Table 1. Characteristics of control (*db/+*) and diabetic (*db/db*) mice

	Body Wt, g	Heart Dry Wt, mg	Plasma Glucose, mM	Plasma FA, mM	Plasma Insulin, μ U/ml
<i>db/+</i>	29.3 \pm 0.4	28.4 \pm 0.7	15.7 \pm 0.7	1.07 \pm 0.11	50 \pm 5
<i>db/db</i>	44.0 \pm 1.0*	29.0 \pm 0.4	44.2 \pm 2.3*	1.50 \pm 0.08*	177 \pm 9*

Values are means \pm SE of 12–19 in each group. Blood samples ($n = 12$) were taken from animals at the time of death. FA, free fatty acids. * $P < 0.05$ vs. *db/+* mice.

studied in isolated hearts perfused with LGLP and HGHP. During LGLP conditions, basal rates of glucose oxidation (GLUox) were 75% lower in *db/db* compared with *db/+* hearts (Fig. 1A). In contrast, basal FAox was twofold higher in *db/db* hearts. Insulin was found to have a clear stimulatory effect on GLUox and an inhibitory effect on FAox both in control and diabetic hearts under LGLP. Surprisingly, we found that insulin (1 mU/ml) caused an increment of GLUox of 1.12 μ mol

\cdot min $^{-1}$ \cdot g $^{-1}$ in *db/db* hearts compared with 0.74 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$ in *db/+* hearts. The corresponding insulin-induced FAox decrements were 0.34 and 0.17 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$ in *db/db* and *db/+* hearts, respectively. Despite the fact that the insulin-induced changes in FAox and GLUox were greater in *db/db* than in *db/+* hearts, absolute rates of GLUox were still lower and rates of FAox higher in diabetic hearts in the presence of insulin.

In control hearts, elevation in the supply of glucose and palmitate (HGHP) caused a small but significant decrease in the basal rate of GLUox and a slight increase ($P = 0.015$) in the basal FAox rate. HGHP did not, however, alter basal rates of GLUox and FAox in *db/db* hearts. Also under these conditions, basal GLUox was significantly lower and basal FAox higher in *db/db* compared with *db/+* hearts. Insulin caused a more pronounced increment of GLUox in control hearts under HGHP conditions (increment of 1.16 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$) compared with LGLP conditions. The ability of insulin to inhibit FAox in *db/+* hearts was, however, slightly reduced (decrement of 0.12 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$). The metabolic responsiveness of *db/db* hearts to insulin was maintained at HGHP; the insulin-induced increment in GLUox was 0.69 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$, while the insulin-induced decrement in FAox was 0.18 μ mol \cdot min $^{-1}$ \cdot g $^{-1}$. Nevertheless, absolute rates of GLUox were still markedly decreased in diabetic hearts in the presence of insulin and HGHP, whereas FAox was moderately elevated compared with control hearts.

On the basis of the substrate oxidation rates, we calculated the relative contribution of GLUox and FAox to acetyl-CoA production for *db/+* and *db/db* hearts (Fig. 1B). The theoretical yield of acetyl-CoA was calculated on the basis of the measured substrate oxidation rates by using a stoichiometric ratio of 2 and 8 moles acetyl-CoA per mole glucose and palmitate being metabolized, respectively. With LGLP and no insulin, *db/db* hearts showed a clear preference for FA as energy substrate; 83% of acetyl-CoA production was derived from FAox compared with 36% for control hearts. The same pattern of substrate utilization was observed for HGHP perfusions; *db/db* hearts preferentially utilized FA compared with control hearts (77 vs. 51%, respectively). In the presence of insulin (1 mU/ml), the contribution of FAox to energy production was reduced under LGLP as well as HGHP conditions both in control (13 and 28%) and diabetic (36 and 52%) hearts. It is clear, therefore, that insulin reduced the strong dependence on FAox for energy production in diabetic hearts.

Contractile function. Contractile function of isolated hearts perfused under LGLP and HGHP conditions (\pm insulin) are summarized in Table 2. Diabetic hearts exhibited contractile dysfunction, with reduced peak systolic pressure, cardiac output, and cardiac work. There was no difference in coronary flow between *db/+* and *db/db* hearts. Elevation of glucose and

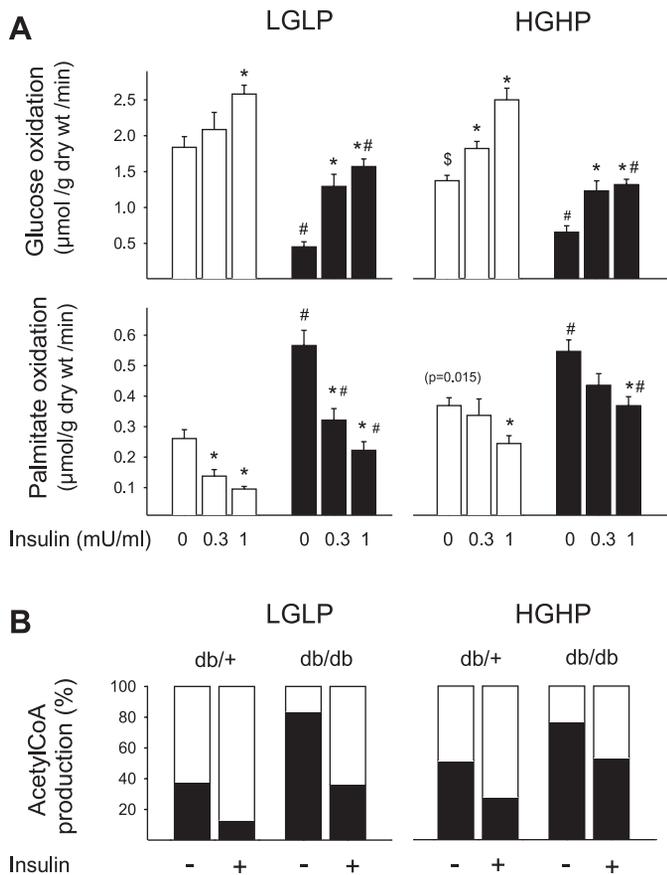


Fig. 1. A: rates of glucose oxidation and fatty acid oxidation in isolated perfused working hearts from control (*db/+*, open bars) and diabetic (*db/db*, filled bars) mice. Values are means \pm SE of 5–8 hearts in each group. Hearts were perfused with Krebs-Henseleit bicarbonate (KHB) buffer containing either 10 mM glucose and 0.5 mM palmitate [low glucose and low palmitate (LGLP)] or 28 mM glucose and 1.1 mM palmitate [high glucose and high palmitate (HGHP)] in the absence or presence of 0.3 or 1 mU/ml insulin. * $P < 0.012$ vs. hearts within the same group perfused in the absence of insulin. # $P < 0.012$ vs. *db/+* hearts perfused at similar conditions. \$ $P < 0.012$ vs. hearts within the same group perfused with LGLP. B: relative contribution of glucose oxidation (open portion of bars) and fatty acid oxidation (filled portion of bars) to acetyl-CoA production in the absence (-) or presence (+) of insulin (1 mU/ml) as calculated from metabolic rates shown in A. The theoretical yield of acetyl-CoA was calculated by using a stoichiometric ratio of 2 and 8 moles acetyl-CoA per mole of glucose and palmitate being metabolized, respectively.

Table 2. Mechanical function of isolated perfused working hearts from control (*db/+*) and diabetic (*db/db*) mice

	Insulin, mU/ml	HR, beats/min	CO, ml/min	CF, ml/min	CW, mmHg·ml·min ⁻¹
LGLP					
<i>db/+</i>	0	348±12	14.2±0.7	2.9±0.1	854±46
	0.3	363±13	12.7±0.6	2.8±0.1	765±44
	1	334±9	14.7±0.7	2.9±0.1	891±37
<i>db/db</i>	0	308±12*	11.6±0.5*	3.0±0.1	695±39
	0.3	324±18	9.9±0.6*	2.7±0.1	593±42*
	1	316±14	11.5±0.5*	2.8±0.1	690±28*
HGHP					
<i>db/+</i>	0	351±11	13.4±0.5	2.9±0.1	838±34
	0.3	357±10	12.3±0.6	2.8±0.1	745±43
	1	352±14	14.1±0.9	2.9±0.1	870±60
<i>db/db</i>	0	303±12*	10.3±0.4*	2.5±0.1	622±33*
	0.3	322±14	10.2±0.6*	2.7±0.1	616±43*
	1	317±9	10.4±0.6*	2.7±0.1	634±44*

Values are means ± SE of 11–17 hearts in each group. LGLP, low (10 mM) glucose and low (0.5 mM) palmitate; HGHP, high (28 mM) glucose and high (1.1 mM) palmitate; HR, heart rate; CF, coronary flow; CO, cardiac output; CW, cardiac work (peak systolic pressure × CO). **P* < 0.012 vs. *db/+* hearts perfused at similar conditions.

palmitate in HGHP perfusions did not affect cardiac function in *db/+* and *db/db* hearts. Moreover, insulin had no effect on contractile function in *db/+* and *db/db* hearts.

Myocardial glycogen content. Myocardial glycogen content in *db/+* and *db/db* hearts was measured at the end of the perfusion period (Fig. 2). In the absence of insulin, *db/db* hearts showed a lower glycogen content than *db/+* hearts for both LGLP (127 ± 5 vs. 181 ± 7 μmol/g dry wt, *P* < 0.001) and HGHP perfusions (121 ± 7 vs. 160 ± 11 μmol/g dry wt, *P* < 0.001). Inclusion of insulin significantly (*P* < 0.001) increased glycogen content in *db/db* hearts to 202 ± 7 (LGLP) and 186 ± 14 (HGHP) μmol/g dry wt. In *db/+* hearts, glycogen content did not change significantly in response to insulin during LGLP conditions (211 ± 11 μmol/g dry wt), whereas it increased to 251 ± 7 μmol/g dry wt during HGHP perfusions (*P* < 0.025).

Immunoblot analysis of total and phosphorylated PKB. Insulin markedly increased PKB phosphorylation (Thr-308) in both *db/+* and *db/db* hearts (Fig. 3). There was no difference in the PKB phosphorylation level between the two groups, either in the absence or presence of insulin both during LGLP and HGHP conditions. PKB phosphorylation in isolated car-

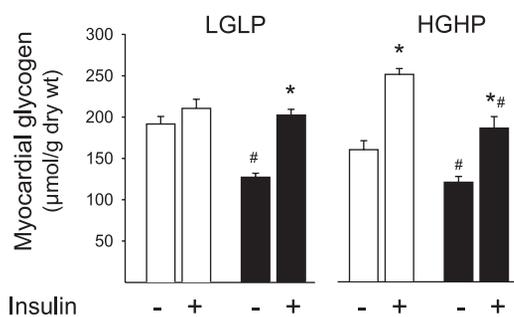


Fig. 2. Myocardial glycogen content in control *db/+* (open bars) and diabetic *db/db* (filled bars) hearts after 40 min perfusion with buffer as described in Fig. 1 in the absence (–) or presence (+) of insulin (1 mU/ml). Values are means ± SE of *n* = 7–13. **P* < 0.025 vs. hearts perfused in the absence of insulin. #*P* < 0.025 vs. *db/+* hearts perfused at similar conditions.

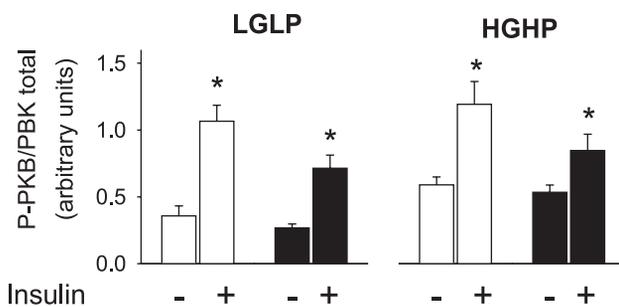


Fig. 3. Insulin-induced phosphorylation of protein kinase B (PKB; threonine 308) in cardiac tissue obtained from control *db/+* (open bars) and diabetic *db/db* (filled bars) hearts. Total PKB, as well as phosphorylation of Thr-308, was measured by immunoblotting extracts of cardiac tissue from hearts perfused with LGLP and HGHP buffers as described in Fig. 1, in the absence or presence of insulin (0.3 mU/ml). Results are expressed as the ratio of phospho-PKB (P-PKB) to total PKB. Values are means ± SE of 4–6 hearts. **P* < 0.025 vs. hearts in the same group in the absence of insulin.

diomyocytes from *db/+* and *db/db* hearts was also found not to be different (data not shown).

Effect of insulin on deoxyglucose uptake in cardiomyocytes and glucose uptake in isolated perfused hearts. Because of the somewhat surprising result of a clear metabolic effect of insulin in diabetic hearts, we also measured glucose uptake in isolated cardiomyocytes by using trapping of radiolabeled deoxyglucose. We also measured insulin-induced glucose uptake in isolated perfused hearts using [2-³H]glucose. In the latter experiments, the glucose concentration was reduced to 5 mM to enhance the effect of insulin. Deoxyglucose uptake in cardiomyocytes from *db/+* and *db/db* mice is shown in Fig. 4. There was no difference in basal deoxyglucose uptake between the two groups. The insulin-stimulated uptake was, however, significantly reduced in *db/db* cardiomyocytes; whereas cardiomyocytes from *db/+* mice exhibited a fivefold increase in glucose uptake at the highest insulin concentration, cardiomyocytes from *db/db* mice expressed only a twofold increase. In isolated perfused hearts, basal and insulin-stimulated glucose uptake were found to be reduced in *db/db* hearts [42% and 30% below the corresponding values in *db/+* hearts, respectively (Fig. 5)]. It is worth noticing, however, that the absolute insulin-induced increment in glucose uptake in *db/db* and *db/+* hearts was similar (5.2 vs. 4.3 μmol·min⁻¹·g⁻¹, respectively).

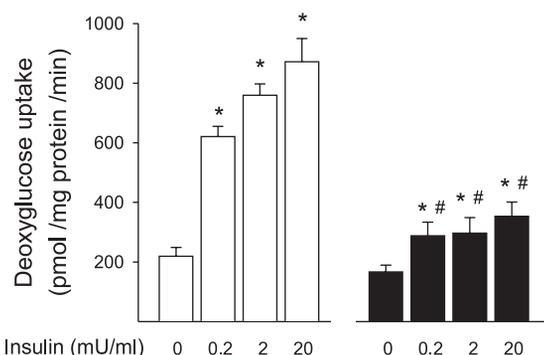


Fig. 4. Insulin-stimulated deoxyglucose uptake into cardiomyocytes from control (*db/+*, open bars, *n* = 8) and diabetic (*db/db*, filled bars, *n* = 10) hearts. Cardiomyocytes were preincubated for 40 min with the indicated concentrations of insulin before determination of 2-deoxy-[³H]glucose uptake. Results are means ± SE. **P* < 0.01 vs. basal (no insulin) deoxyglucose uptake. #*P* < 0.01 vs. cardiomyocytes from *db/+* mice.

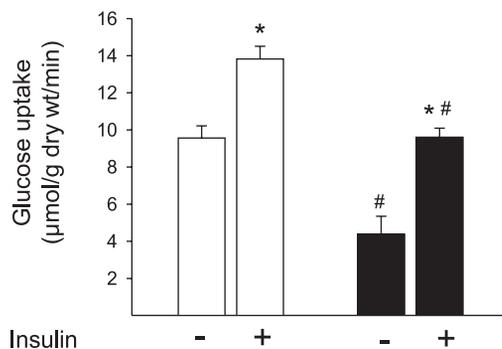


Fig. 5. Glucose uptake in isolated perfused working mouse hearts from control (*db/+*, open bars, $n = 6$) and diabetic (*db/db*, filled bars, $n = 6$) mice. Hearts were initially perfused in the absence of insulin for 20 min and thereafter in the presence of 0.3 mU/ml insulin for 30 min. Values are means \pm SE. * $P < 0.025$ vs. basal (no insulin) glucose uptake. # $P < 0.025$ vs. *db/+* hearts.

DISCUSSION

In accordance with previous studies (2, 4, 5), we found that basal rates of GLUox were reduced and of FAox were elevated in diabetic (*db/db*) compared with control (*db/+*) hearts. In the presence of insulin, *db/db* hearts showed reduced GLUox and elevated FAox compared with absolute rates in control hearts. Diabetic hearts were, however, clearly responsive to the metabolic actions of insulin, as judged by the insulin-induced increment in GLUox or decrement in FAox. In fact, insulin produced a more prominent shift in myocardial metabolism in *db/db* hearts than in control hearts. Under the LGLP condition, the contribution of FAox to myocardial acetyl-CoA production was reduced from 83% in the absence of insulin to 36% in the presence of insulin; in comparison, the contribution of FAox was reduced from 36% to 13% in *db/+* hearts. These data are in marked contrast to studies on Type 1 diabetic rat hearts (39), where the strong reliance of FAox for energy production was unaffected by insulin as well as alterations in the substrate supply.

Because the myocardial supply of energy substrates for diabetic hearts in vivo differs markedly from the supply for control hearts, we also studied the metabolic effects of insulin under HGHP supply. A striking result was that this simultaneous (and proportional) increase of palmitate and glucose concentrations only had modest effects on basal glucose and FAox in control hearts. These findings are in contrast to experiments where only the FA concentration was elevated, which resulted in a marked increase in FAox with a concomitant decrease in GLUox (5, 20, 27, 33). This difference in the metabolic effect, obtained by elevating only FA compared with our results obtained by elevating both glucose and FA, can be explained in terms of the classical Randle effect (38). In contrast to the present study, Sakamoto et al. (39) found a twofold increase in FAox and more than 50% reduction in GLUox in nondiabetic rat hearts perfused with elevated glucose as well as FA, indicating that there might be species differences with regard to the influence of substrate supply on myocardial metabolism.

We found that an elevated palmitate and glucose supply had little or no effect on basal GLUox and FAox in *db/db* hearts. Insulin, however, still significantly stimulated GLUox and inhibited FAox, although these responses were somewhat blunted compared with LGLP conditions. Our data are in

contrast to those of Mazumder et al. (33), who examined the effect of insulin on cardiac metabolism in isolated *ob/ob* heart. Although the metabolic phenotype of *ob/ob* hearts perfused under basal conditions was found to be similar to the *db/db* phenotype (decreased GLUox and increased FAox), these authors found no effect of insulin on GLUox and FAox both under basal and elevated FA concentrations (33). However, they reported marked differences in contractile function among the various perfusion groups; changes in cardiac function will certainly influence the absolute rate of metabolic fluxes and thus influence the interpretation of the metabolic action of insulin and altered substrate supply. In contrast, cardiac function in control and *db/db* hearts was not affected by changes in substrate levels (LGLP vs. HGHP perfusions) or by the addition of insulin in the present study (Table 2). Finally, it should also be noted that although *ob/ob* mice exhibit a less severe diabetic phenotype in vivo compared with the *db/db* mice, the degree of cardiac insulin resistance (complete abrogation of insulin-stimulated glucose uptake) in *ob/ob* cardiomyocytes (33) was much greater than that observed with *db/db* cardiomyocytes (Refs. 9, 11; Fig. 4).

It is generally accepted that diabetic hearts show a marked preference for FA as a source for oxidative energy production, due partly to elevated FA supply (4, 45, 47). Despite this, only a few studies (33, 39) on isolated perfused diabetic hearts have taken altered substrate conditions into account. In the present study, it might be argued that one should actually compare control hearts perfused with LGLP to *db/db* hearts perfused with HGHP because these two perfusates represent more closely the in vivo substrate supply for control and diabetic hearts, respectively. Interestingly, the insulin-induced increment in GLUox, as well as the insulin-induced decrement in FAox in *db/db* hearts perfused with HGHP, was very similar to the metabolic changes measured in control hearts perfused with LGLP (GLUox increment: 0.74 vs. 0.69 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$; and FAox decrement: 0.17 vs. 0.18 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$). Moreover, the GLUox rate in *db/db* hearts perfused with HGHP and insulin (diabetic-like condition) was only 25% below the GLUox rate for *db/+* hearts perfused with LGLP and no insulin (control-like condition), i.e., 1.37 ± 0.08 vs. 1.84 ± 0.15 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ($P = 0.031$). For comparison, GLUox in *db/db* hearts was 75% below the value for *db/+* hearts under LGLP conditions without insulin. Interestingly, rates of FAox were not different between control hearts perfused with LGLP and no insulin and *db/db* hearts perfused with HGHP and insulin (0.26 ± 0.03 vs. 0.37 ± 0.03 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$; $P = 0.030$). These data suggest that previously reported overreliance on FAox for energy production in diabetic hearts (10) should be extrapolated to the in vivo situation with care because these results were obtained with control and diabetic hearts perfused under similar conditions.

Although the role of insulin on myocardial gluoregulation has been extensively studied, the inhibitory effect of insulin on myocardial FAox is less described. In accordance with previous reports (3, 26, 33), we found that insulin inhibited myocardial FAox measured with both perfusion conditions. A number of mechanisms could be responsible for this insulin-induced reduction in rates of FAox, including 1) a direct effect of insulin on translocation of FA transporters and subsequent shunting of FA to TG esterification; 2) a direct effect of insulin to inhibit AMP kinase; and/or 3) an indirect effect of insulin

due to enhanced glucose uptake and utilization resulting in malonyl-CoA inhibition of carnitine palmitoyltransferase I, thereby reducing the entry of FA into mitochondria for oxidation (10). The observation that insulin only reduced FAox in cardiomyocytes incubated with glucose (11) is consistent with this third mechanism above, but this conclusion has to be qualified once again because of the use of quiescent cardiomyocytes as the experimental system. A mechanistic investigation into the mechanisms whereby insulin reduces FAox in beating mouse hearts is an important goal for future investigations.

The relatively strong influence of insulin on myocardial oxidative metabolism in isolated *db/db* hearts was somewhat surprising in light of previous results with isolated cardiomyocytes from these animals, showing clearly reduced insulin-induced uptake of deoxyglucose (9, 11). We therefore performed additional experiments where we studied glucose uptake in isolated cardiomyocytes using deoxyglucose-uptake measurements, as well as in isolated perfused hearts using [^3H]glucose. Both techniques have been used frequently for studying the insulin-induced responses in various diabetic models (11, 15, 21, 33). Impaired insulin-induced deoxyglucose uptake was confirmed in diabetic cardiomyocytes. In contrast to these results but in accordance with insulin-induced alteration in oxidative metabolism, glucose uptake was found to be clearly stimulated by insulin in perfused hearts from diabetic mice (Fig. 5); the insulin-induced increment of glucose uptake was similar in *db/db* and *db/+* hearts. A direct comparison of the effect of insulin on (deoxy)glucose uptake obtained in isolated cells and isolated hearts is not easy because of marked methodological differences, including different substrate conditions (cardiomyocytes were incubated in the presence of 5 mM pyruvate, while isolated hearts were perfused with 0.5 mM palmitate). A major factor may also be related to the much higher energy demand of beating hearts compared with quiescent cardiomyocytes (14, 34). Finally, the contribution of endothelial cells to the metabolic responses to insulin in whole perfused hearts cannot be excluded. Regardless of the limitations connected to the interpretation of the glucose uptake data, they do emphasize the fact that insulin-induced glucose uptake data can depend markedly on the technique and model that are used.

PKB/Akt activation is a key step in the insulin signaling cascade (7, 40), and previous studies have shown decreased PKB activation in skeletal muscle and adipose tissue from *db/db* mice (19, 42). In contrast, the present study shows that insulin-stimulated PKB phosphorylation was not reduced in hearts from *db/db* mice. Our data are supported by previous results obtained with Type 2 diabetic rat hearts, demonstrating no reduction in insulin-induced PKB activation (15, 21).

A limitation in the present experimental approach is that metabolic responses to insulin were examined with only two exogenous substrates (glucose and palmitate) in the perfusate, thereby neglecting the role of pyruvate, lactate (30), and ketone bodies (23). We also studied only one FA metabolic fate (oxidation). Therefore, future experiments should also measure the effect of insulin on FA uptake and esterification, as well as its effect on the overall metabolic capacity in control and *db/db* hearts. Furthermore, although the present study aimed to study metabolic changes under substrate conditions mimicking normal and diabetic conditions, an exact match of the *in vivo* condition (with regard to substrate levels, as well as insulin

levels) is not easy, as this will obviously change depending on the feeding state of the animals. An important goal for future investigations will, therefore, also be to apply tracer methodologies to control and *db/db* mice to study cardiac metabolism *in vivo* (36). Another limitation was that we were unable to perform a detailed dose-response relationship with respect to the effect of insulin on myocardial oxidative metabolism. This would have required a large number of perfusions because only one insulin dose could be applied for each heart because of the relatively long perfusion time required to obtain steady-state metabolic rates.

In conclusion, the present study demonstrates that despite profound overall insulin resistance exhibited by Type 2 diabetic *db/db* mice *in vivo*, isolated *db/db* hearts are metabolically responsive to insulin, as reflected by elevated rates of glucose uptake and GLUox, reduced rates of FAox, increased glycogen content, and preserved PKB activation. These results imply that insulin can produce a pronounced shift in myocardial metabolism and diminish the predominant contribution of FA to oxidative energy production in the diabetic heart. Moreover, the present results should advocate use of insulin therapy (for instance, GIK) in diabetic patients undergoing cardiac surgery or during reperfusion after an ischemic insult.

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