

Single-Dose Dexamethasone Induces Whole-Body Insulin Resistance and Alters Both Cardiac Fatty Acid and Carbohydrate Metabolism

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Glucocorticoids impair insulin sensitivity. Because insulin resistance is closely linked to increased incidence of cardiovascular diseases and given that metabolic abnormalities have been linked to initiation of heart failure, we examined the acute effects of dexamethasone (DEX) on rat cardiac metabolism. Although injection of DEX for 4 h was not associated with hyperinsulinemia, the euglycemic-hyperinsulinemic clamp showed a decrease in glucose infusion rate. Rates of cardiac glycolysis were unaffected, whereas the rate of glucose oxidation following DEX was significantly decreased and could be associated with augmented expression of PDK4 mRNA and protein. Myocardial glycogen content in DEX hearts increased compared with control. Similar to hypoinsulinemia induced by streptozotocin (STZ), hearts from insulin-resistant DEX animals also demonstrated enlargement of the coronary lipoprotein lipase (LPL) pool. However, unlike STZ, DEX hearts showed greater basal release of LPL and were able to maintain their high heparin-releasable LPL in vitro. This effect could be explained by the enhanced LPL mRNA expression following DEX. Our data provide evidence that in a setting of insulin resistance, an increase in LPL could facilitate increased delivery of fatty acid to the heart, leading to excessive triglyceride storage. It has not been determined whether these acute effects of DEX on cardiac metabolism can be translated into increased cardiovascular risk. *Diabetes* 53:1790–1797, 2004

Glucocorticoids have widespread use as anti-inflammatory and immunosuppressive agents (1). However, chronic glucocorticoid therapy is often associated with adverse and serious side effects, including Cushing's syndrome, osteoporosis, gas-

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DCA, dichloroacetate; DEX, dexamethasone; HSPG, heparan sulfate proteoglycan; LPL, lipoprotein lipase; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; STZ, streptozotocin.

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trointestinal bleeding, and dyslipidemia (1). More importantly, both excess endogenous (2,3) and exogenous (4,5) glucocorticoids impair insulin sensitivity, contributing to generation of the metabolic syndrome, including insulin resistance, obesity, and hypertension. Incidence of this syndrome is closely linked to increased mortality from cardiovascular diseases (6).

Increasing evidence from clinical and experimental studies has established that metabolic abnormalities play a crucial role in the development of heart diseases (7,8). Heart acquires most of its energy from metabolism of glucose and fatty acid. Following glucose uptake and glycolysis, the pyruvate dehydrogenase complex (PDC) facilitates entry of pyruvate into the mitochondria, and changes in PDC activity alter glucose utilization. Thus, dephosphorylation by pyruvate dehydrogenase phosphatase activates, whereas phosphorylation by pyruvate dehydrogenase kinase (PDK) inactivates, PDC, with resultant augmentation or inhibition of glucose oxidation, respectively. Of the four different isoforms of PDK that have been identified, PDK2 and -4 are the main isoforms present in the heart (9). Diabetes and starvation upregulate cardiac PDK4 but not PDK2 expression (10,11). Although the effects of glucocorticoids on cardiac PDK are less well recognized, dexamethasone (DEX) was recently reported to increase expression of PDK4 in hepatoma cells (12).

Compared with glucose, fatty acids are the preferred substrate consumed by cardiac tissue (8), with hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase (LPL) positioned at the endothelial surface of the coronary lumen being suggested to be the principal source of fatty acid for cardiac utilization (13). Endothelial cells do not synthesize LPL, and hence the enzyme is synthesized in cardiomyocytes. Secreted LPL binds to myocyte cell-surface heparan sulfate proteoglycans (HSPGs) before it is translocated onto comparable HSPG binding sites on the luminal side of the vessel wall. Regulation of cardiac luminal LPL may be an important means whereby the heart is able to maintain its function during times of metabolic stress, such as diabetes that is characterized by inadequate glucose utilization (14). Hence, in the streptozotocin (STZ)-injected rat, with its attendant hypoinsulinemia and hyperglycemia, we demonstrated significantly elevated luminal LPL activity (15,16) and hypothesized that this may lead to metabolic switching, which provides

excessive fatty acid to the diabetic heart. Although the role of hypoinsulinemia in regulating cardiac LPL is now established, the influence of glucocorticoid-induced insulin resistance on cardiac LPL is unknown.

The objective of the present study was to determine the acute effects of DEX-induced reduction in insulin sensitivity on cardiac metabolism. We demonstrate that a single dose of DEX leads to whole-body insulin resistance and that in hearts from these animals, glucose oxidation is compromised due to augmentation of PDK4, whereas amplification of LPL increases lipoprotein triglyceride clearance, likely providing the heart with excessive fatty acids that are then stored as intracellular triglyceride.

RESEARCH DESIGN AND METHODS

The investigation conforms to the guide for the care and use of laboratory animals published by the National Institutes of Health and the University of British Columbia. Adult male Wistar rats (270–290 g) were obtained from the University of British Columbia Animal Care Unit and fed a standard laboratory diet (PMI Feeds, Richmond, VA) and water ad libitum. The synthetic glucocorticoid hormone DEX (1 mg/kg) or an equivalent volume of ethanol was administered by intraperitoneal injection and the animals killed 1–4 h later (plasma half-life of DEX is ~279 min). Previous studies have determined that this dose of DEX inhibits insulin-stimulated skeletal muscle glucose transport (17).

Euglycemic-hyperinsulinemic clamp. Whole-animal insulin resistance was assessed using a euglycemic-hyperinsulinemic clamp, as described previously (18,19). This procedure involves the simultaneous intravenous infusion of insulin (to inhibit endogenous hepatic glucose production) and glucose; the quantity of exogenous glucose required to maintain euglycemia is a reflection of the net sensitivity of target tissues (mainly skeletal muscle) to insulin. Briefly, after injection of vehicle or DEX for 4 h, animals were anesthetized with sodium pentobarbital (Somnotol; 65 mg/kg) and a cannula inserted into the left jugular vein. Surgical insertion of the cannula was rapid (~10 min for each animal). Insulin (Humulin R; $3 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and D-glucose (50%) were continuously delivered for 3 h, with the glucose infusion started 4 min after commencement of insulin infusion. Insulin and glucose were dissolved in 0.9% saline before infusion. At regular intervals, a small amount of blood taken from the tail vein was analyzed for glucose (using a glucometer: AccuSoft Advantage) and fatty acid. The glucose infusion rate was adjusted accordingly to maintain euglycemia.

Cardiac glucose metabolism. When metabolized, glucose passes through the glycolytic pathway to be oxidized to CO_2 or nonoxidatively catabolized to lactate and alanine. To measure glucose oxidation, isolated hearts were perfused for 30 min with Krebs-Henseleit buffer in the working mode at a preload of 11.5 mmHg and an afterload of 80 mmHg, as previously described (20). The buffer solution contained 0.4 mmol/l palmitate, 2.0 mmol/l CaCl_2 , 5.5 mmol/l glucose, 0.5 mmol/l lactate, 100 mU/l insulin, and 3% BSA (pH 7.4). Rates of glucose oxidation (quantitative collection of $^{14}\text{CO}_2$ liberated from [^{14}C]glucose at the pyruvate dehydrogenase reaction and in the citric acid cycle) and glycolysis (^{14}C -labeled products, [^{14}C]lactate and [^{14}C]pyruvate) were determined as previously described (20). On transport into cardiac cells, glucose disposal can also occur via its conversion into glycogen, which serves as the primary storage form of glucose. Cardiac glycogen was determined as glucose residues by a glucose kinase method after acid hydrolysis (21).

The rate of glucose oxidation is dependent on mitochondrial PDC. Phosphorylation via PDK inhibits PDC. PDK gene expression was measured in the indicated groups using RT-PCR. Briefly, total RNA from hearts (100 mg tissue) was extracted using Trizol (Invitrogen). After spectrophotometric quantification and resolving of RNA integrity using a formaldehyde agarose gel, reverse transcription was carried out using an oligo (dT) primer and superscript II RT (Invitrogen). First-strand cDNA was amplified using PDK2- and PKD4-specific primers. PDK2: 5'-TCTACCTCAGCCGCATCTCT-3' (left) and 5'-GTTGGTG GCATGACTTCTCT-3' (right); PDK4: 5'-CCTTTGGCTGGTTTTGGTTA-3' (left) and 5'-CACCAGTCATCAGCCTCAGA-3' (right); the β -actin gene was amplified as an internal control using 5'-TTGTAACCAACTGGGACGATATGG-3' (left) and 5'-GATCTTGATCTTCATGGTGCTAGG-3' (right). The linear range was determined to be between 15 and 40 cycles. The amplification parameters were set at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min (40 cycles total). The PCR products were electrophoresed on a 1.7% agarose gel containing ethidium bromide. Expression levels were represented as the ratio of signal intensity for PDK mRNA relative to β -actin mRNA.

To determine whether changes in PDK4 mRNA are translated to an

increase in protein expression, Western blot analysis for PDK4 was carried out in mitochondria isolated by a previously described method (10). Mitochondrial proteins (5 μg) were fractionated by 11% SDS-PAGE and transferred onto nitrocellulose membranes. Blots were probed with polyclonal rabbit anti-PDK4 antisera (1:500) (generously provided by Dr. K.M. Popov), followed by incubation with goat anti-rabbit horseradish peroxidase linked secondary antibody (1:3,000). Reaction products were visualized using chemiluminescence and quantified by densitometry.

Heart function. Isolated heart function (rate pressure product – heart rate [in bpm] \times peak systolic pressure [in mmHg]) was recorded using a Direwin physiograph (Raytech) (20). Mean arterial pressure was measured in vivo using a cannula inserted into the carotid artery.

LPL activity. At varying times (1–4 h) following DEX, hearts were perfused retrogradely through the aorta by the nonrecirculating Langendorff technique (16). To measure endothelium-bound LPL, perfusion solution was changed to buffer containing 1% fatty acid-free BSA and heparin (5 units/ml). Coronary effluent was collected in timed fractions over 10 min and assayed for LPL activity by measuring the hydrolysis of a sonicated [^3H]triolein substrate emulsion (16).

To compare DEX effects on LPL with those previously observed following hypoinsulinemia, β -cell death was induced with a single dose of STZ (55 mg/kg i.v.) (15). Diabetic rats were kept for 4 days after STZ injection, at which time they were killed and hearts removed. To determine whether DEX- and STZ-treated animals can maintain their augmented LPL activity in vitro, hearts from control and STZ- and DEX-treated animals were either perfused with heparin, immediately upon removal (0 min), or perfused with heparin-free buffer for 60 min. During this 60-min perfusion with heparin-free buffer, LPL activity in the buffer reservoir (total volume 30 ml) was determined at various intervals. Finally, a 10-min perfusion with heparin was carried out to determine the extent of residual LPL at the coronary lumen.

Immunolocalization of LPL following DEX. Control and DEX (4 h) hearts were fixed in 10% formalin overnight. Blocks were embedded in Paraplast and sectioned at 5 μm . For immunostaining, sections were incubated with affinity-purified chicken anti-bovine LPL polyclonal antibody (1:400). Samples were then incubated with the secondary biotinylated rabbit anti-chicken IgG (1:150; Chemicon), followed by incubation for 1 h with streptavidin-conjugated Cy3 fluorescent probe (1:1,000). Slides were visualized using a Biorad 600 Confocal Microscope at 630 \times magnification.

LPL gene expression following DEX. LPL gene expression was measured using RT-PCR as described previously (22).

Treatments. Acute (90 min) treatment of STZ-induced diabetic rats with rapid-acting insulin reduces heparin-releasable LPL activity to normal levels (15). To determine whether insulin could also influence LPL activity following DEX, insulin was infused over 3 h via the euglycemic-hyperinsulinemic clamp and cardiac LPL activity subsequently measured. For comparison, some STZ rats were treated intravenously with a rapid-acting insulin (8 units/animal); rats were killed after 180 min, and LPL activity was determined. Some hearts from DEX-treated animals were also perfused for 60 min with dichloroacetate (DCA; 1 mmol/l) for quantification of LPL. DCA, by inhibiting PDK, stimulates pyruvate dehydrogenase and promotes glucose utilization (23).

Plasma measurements. Control rats were injected with DEX at 10:00 a.m. (fed state). Following DEX, blood samples from the tail vein were collected at varying intervals in heparinized glass capillary tubes. Blood samples were immediately centrifuged, and plasma was collected and assayed. Diagnostic kits were used to measure glucose, triglyceride (Sigma), nonesterified fatty acid (Wako), and insulin (Linco).

Electron microscopy. To assess whether DEX causes accumulation of lipid droplets, morphological evaluation of hearts was carried out using transmission electron microscopy. Briefly, left ventricular tissue was fixed in 1.5% glutaraldehyde and paraformaldehyde, cut into small blocks (~1 \times 0.5 \times 0.2 mm), and fixed for 8 h at 4°C. After washing, tissue was postfixated with 1% osmium tetroxide and further treated with 1% uranyl acetate and dehydrated using increasing concentrations of ethanol (50–100%). Blocks were embedded in epoxy resin and sectioned at ~90 nm. Sections were stained with 1% uranyl acetate and Reynolds lead citrate. Images of the longitudinal sections were obtained with a Hitachi H7600 electron microscope.

[^3H]triolein was purchased from Amersham Canada. Heparin sodium injection (Hapalean; 1,000 USP units/ml) was obtained from Organon Teknika. All other chemicals were obtained from Sigma Chemical.

Statistical analysis. Values are means \pm SE. LPL activity in response to heparin perfusion over time was analyzed by multivariate (two-way) ANOVA using the Number Cruncher Statistical System. Wherever appropriate, one-way ANOVA followed by the Tukey or Bonferroni tests or the unpaired and paired Student's *t* test were used to determine differences between group mean values. The level of statistical significance was set at $P < 0.05$.

TABLE 1
General characteristics of the animals

| | Control | DEX |
|---|----------------|----------------|
| Plasma glucose (mmol/l) | 8.7 ± 0.2 | 8.7 ± 0.1 |
| Plasma insulin (ng/ml) | 3.2 ± 0.5 | 3.3 ± 0.7 |
| Heart weight (g) | 1.4 ± 0.1 | 1.2 ± 0.001 |
| Heart rate (bpm) | 313 ± 10 | 312 ± 8 |
| Rate pressure product (bpm × mmHg/1,000) | 32 ± 1 | 34 ± 1 |
| Mean arterial pressure (mmHg) | 122 ± 6/93 ± 4 | 125 ± 6/93 ± 6 |

Data are means ± SE for six animals in each group. DEX (1 mg/kg) was administered by intraperitoneal injection into control rats and the animals killed 4 h later. Hearts were perfused in the working mode for 1 h at a preload of 11.5 mmHg and an afterload of 80 mmHg. Mean arterial pressure was measured by an in vivo cannula inserted into the carotid artery.

RESULTS

Acute DEX treatment induces whole-body insulin resistance. Injection of DEX for 4 h was not associated with either hyperinsulinemia or hyperglycemia (Table 1). However, by using the euglycemic-hyperinsulinemic clamp, a direct measure of insulin sensitivity, the unfavorable effect of DEX on glucose metabolism was established. Accordingly, our clamp data revealed that the glucose infusion rate necessary to maintain euglycemia was lower after DEX administration (Fig. 1). Development of insulin resistance was not linked to any change in blood pressure (Table 1). Additionally, mechanical function of hearts isolated from DEX-treated animals remained unchanged throughout the perfusion period (Table 1).

Cardiac glucose metabolism is altered following DEX. Insulin resistance involves multiple organs and various mechanisms. Thus, although DEX is known to inhibit insulin-stimulated skeletal muscle glucose transport, its

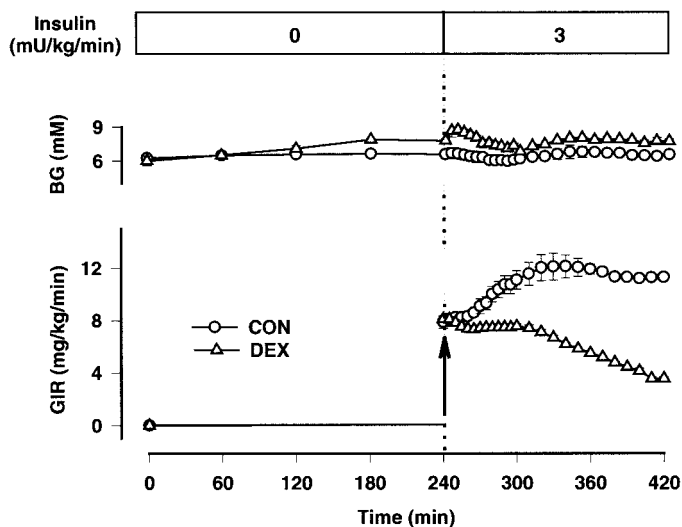


FIG. 1. Effects of DEX on whole-animal insulin resistance. Following injection of vehicle or DEX for 4 h, whole-animal insulin resistance was assessed using an euglycemic-hyperinsulinemic clamp. Insulin (HumulinR; 3 mU · min⁻¹ · kg⁻¹) and D-glucose (50%) were continuously delivered (by a cannula inserted into the left jugular vein) for 3 h, with the glucose infusion started 4 min after commencement of insulin infusion. At regular intervals, blood samples taken from the tail vein were analyzed for glucose using a glucometer. The glucose infusion rate (GIR) was adjusted accordingly to maintain euglycemia. BG, blood glucose; CON, control.

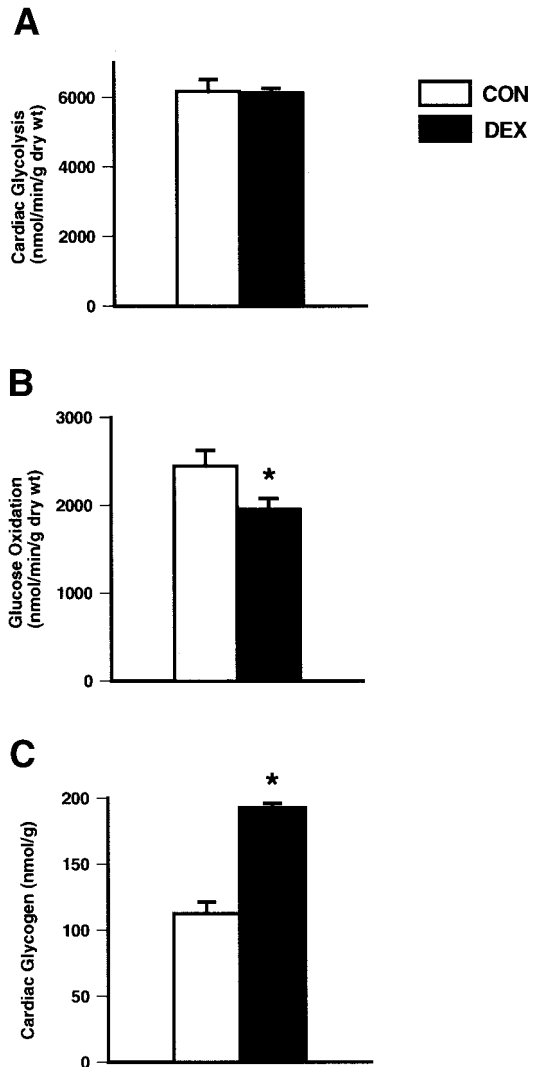


FIG. 2. Glycolysis, glucose oxidation, and glycogen in DEX-treated hearts. Four hours after control (CON) rats were treated with DEX, animals were killed and hearts collected. Isolated hearts were perfused in the working mode for 1 h (preload 11.5 mmHg, afterload 80 mmHg) and rates of glycolysis (A) and glucose oxidation (B) determined as described in RESEARCH DESIGN AND METHODS. Mean steady-state rates of glycolysis and glucose oxidation were determined from data obtained during the initial portion of the heart perfusion. C: Glycogen content in rat ventricular muscle. Cardiac glycogen was determined as glucose residues by a glucose kinase method after acid hydrolysis. Values are the means ± SE for five rats in each group. **P* < 0.05 vs. control.

influence on cardiac metabolism is unknown. Upon transport into cardiac cells, glucose disposal occurs via oxidative metabolism to CO₂, nonoxidative metabolism to lactate and alanine, or conversion into glycogen. Myocardial glucose metabolism in control and DEX-treated hearts is summarized in Fig. 2. Mean steady-state rates of glycolysis and glucose oxidation were determined from data obtained during the initial portion of the heart perfusion. Compared with control, although rates of glycolysis were unaffected in DEX-treated hearts (Fig. 2A), the rate of cardiac glucose oxidation following DEX treatment was significantly decreased (Fig. 2B). In a separate experiment, cardiac glycogen was also evaluated from whole hearts after injection of DEX for 4 h. Myocardial glycogen content in DEX hearts increased approximately twofold compared with control (Fig. 2C).

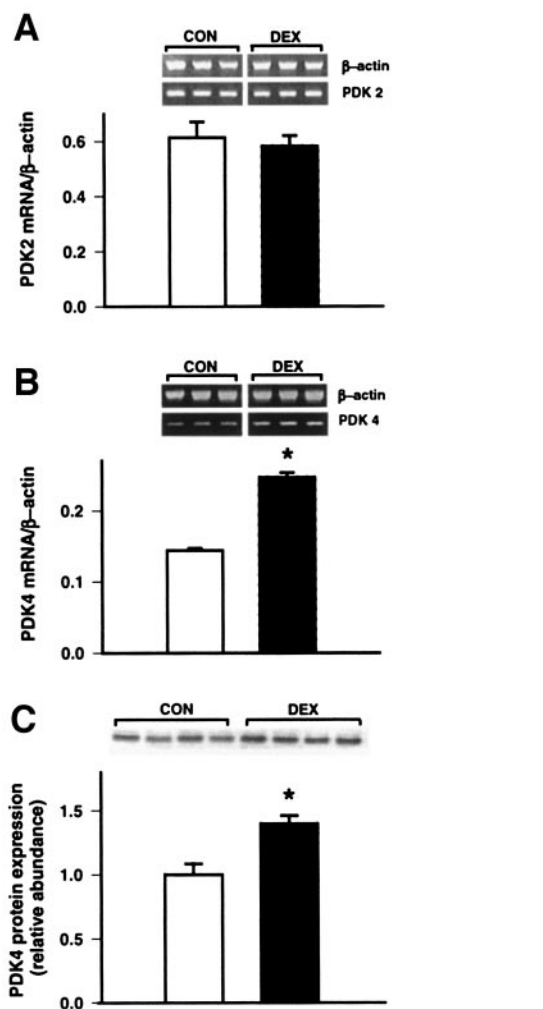


FIG. 3. PDK mRNA and protein expression in hearts from insulin-resistant rats. PDK2 (A) and PDK4 (B) gene expression were measured using RT-PCR, and total RNA was extracted from 100 mg heart tissue. Expression levels were represented as the ratio of signal intensity for PDK mRNA relative to β -actin mRNA. Western blot analysis for PDK4 (C) was carried out in isolated mitochondria. Results are the means \pm SE of three to four animals in each group. * $P < 0.05$ vs. control. CON, control.

DEX induces expression of PDK4 but not PDK2 in the heart. Inhibition of cardiac glucose oxidation by DEX could occur through inactivation of PDC by PDK. As PDK2 and -4 are the main isoforms present in the heart, their expressions were determined following acute DEX administration. DEX increased PDK4 (Fig. 3B) but not PDK2 mRNA (Fig. 3A). More importantly, the increased PDK4 mRNA was associated with augmented PDK4 protein, as measured by Western blot analysis (Fig. 3C).

Changes in coronary lumen LPL following DEX persist in vitro. LPL-mediated hydrolysis of circulating triglyceride-rich lipoproteins at the coronary lumen provides the heart with fatty acid. To measure this LPL activity, retrograde perfusion of hearts with heparin results in the release of LPL into the coronary perfusate (Fig. 4A). In a preliminary study, to determine the kinetics of LPL regulation at the vascular lumen, hearts from some fed control animals treated with a single injection of DEX were isolated at 1–4 h and LPL activity measured. Interestingly, increase in LPL activity became apparent as early as 1 h

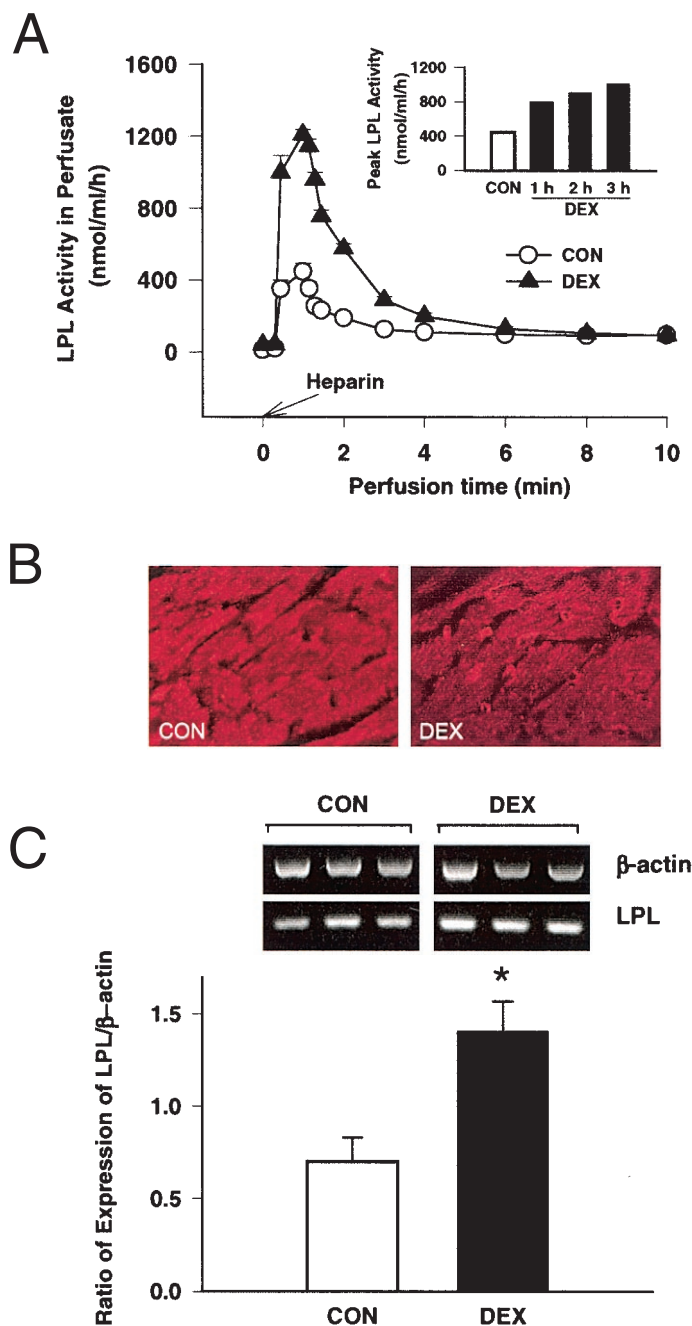


FIG. 4. Effects of DEX on luminal LPL and cardiac mRNA expression. Coronary luminal LPL activity was determined in vitro by heparin perfusion (over 10 min) of hearts isolated from animals treated with DEX (A). Hearts were perfused in the retrograde mode with heparin (5 units/ml) and fractions of perfusate collected and analyzed for LPL activity as described previously. The inset represents peak LPL activity at various intervals (1–3 h of a single representative experiment), whereas the graph shows LPL activity after 4 h of DEX ($n = 6$). Changes in LPL activity in response to heparin perfusion, over time, were analyzed by multivariate ANOVA followed by the Newman-Keul's test using the Number Cruncher Statistical System. B: Representative photograph showing the effect of DEX (4 h) on LPL immunofluorescence, as visualized by fluorescent microscopy. Heart sections were fixed and then incubated with the polyclonal chicken antibody against bovine LPL, followed by incubations with biotinylated rabbit anti-chicken IgG and streptavidin-conjugated Cy3 fluorescent probe. C: LPL mRNA gene expression as measured using RT-PCR. Results are the means \pm SE of three rats in each group. * $P < 0.05$ vs. control. \square , control (CON); \blacksquare , DEX.

after injection of DEX (Fig. 4A, *inset*) and was maintained for an additional 4 h (Fig. 4A). The DEX-induced increase in heparin-releasable LPL activity at the vascular lumen after 4 h was substantial compared with control (approximately threefold) (Fig. 4A). Additionally, immunofluorescence microscopy of myocardial sections from DEX hearts confirmed a more intense LPL immunofluorescence in blood vessels (Fig. 4B).

We have previously demonstrated that hypoinsulinemia also causes a substantial increase in coronary LPL activity and immunofluorescence at the vascular lumen (22). To determine whether this high LPL activity can be maintained *in vitro*, hearts from STZ and DEX animals were perfused with normal Krebs buffer for 1 h. Four days of STZ-induced diabetes caused a decline in plasma insulin (control 2.3 ± 0.4 , diabetic 0.9 ± 0.1 ng/ml; $P < 0.05$) with ensuing hyperglycemia (control 8.7 ± 0.2 , diabetic 14 ± 1.1 mmol/l; $P < 0.05$). Interestingly, STZ but not DEX hearts demonstrated a decline in heparin-releasable LPL activity to control values, presumably through dissociation of the enzyme from HSPG binding sites into the perfusate (Fig. 5). The mechanism by which DEX hearts are able to maintain high LPL *in vitro* suggests either an increased recruitment from myocytes or decreased displacement from the coronary lumen. To further investigate this process, basal LPL activity (in the absence of heparin) was determined in the buffer reservoir over time. DEX hearts showed greater basal release of LPL throughout the perfusion, suggesting that these hearts were able to maintain coronary lumen LPL through accelerated transfer from the myocytes (Fig. 5, *inset*).

Alterations in cardiac LPL are reversed by exogenous insulin in STZ but not DEX animals. Acute treatment of STZ rats with a rapid-acting insulin reduced hyperglycemia (STZ 18.7 ± 1.7 , STZ + insulin 6.4 ± 0.8 mmol/l; $P < 0.05$). Acute insulin treatment also lowered peak heparin-releasable LPL activity to control levels (Fig. 6, *inset*). Unlike insulin reversal of LPL in STZ rats, LPL activity remained high even after 3 h of insulin perfusion during the clamp, suggesting ongoing resistance to the action of insulin. DCA, a stimulator of glucose oxidation, was also unable to reverse the DEX-induced increase in cardiac LPL (data not shown). This was not surprising given the evidence that PDK2 is more sensitive to inhibition by DCA, whereas PDK4 is insensitive (9).

DEX augments expression of cardiac LPL. Previously, we have reported that STZ-induced diabetes does not influence LPL mRNA and that increases in cardiac heparin-releasable LPL activity are likely due to a posttranslational mechanism (22). To determine whether the change in LPL activity following DEX is related to augmented synthesis, we measured LPL mRNA in heart homogenates. Four hours of DEX increased LPL mRNA levels (Fig. 4C).

DEX lowers plasma levels of triglyceride but not fatty acid. Cardiac-specific overexpression of LPL has been suggested to be an important determinant of plasma triglyceride levels in mice (24). Given the increase of LPL at the coronary lumen, we evaluated both plasma triglyceride and fatty acid at varying times (1–4 h) following DEX. Interestingly, although plasma triglyceride declined progressively, there was no increase on plasma fatty acid (Fig. 7A).

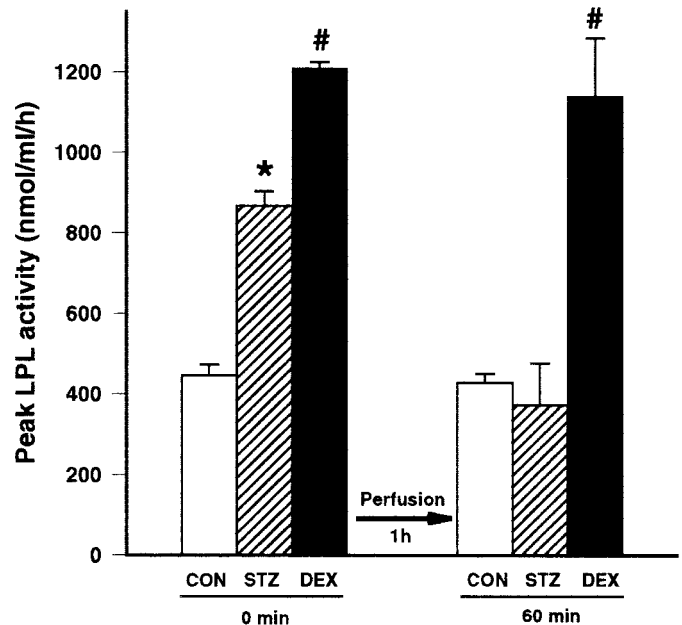
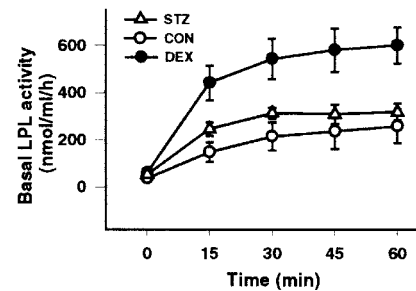


FIG. 5. Differential effects on coronary LPL in isolated hearts from STZ and DEX animals that are perfused *in vitro* for 1 h. On immediate removal of hearts from STZ (4 days) and DEX (4 h) rats, peak LPL activity was determined after perfusion with heparin (0 min). In a separate experiment, hearts from different groups were first perfused for 60 min with Krebs' buffer. During the 60-min perfusion, basal LPL activity (in the absence of heparin) was determined in the buffer reservoir over time (*inset*). Subsequently, LPL was displaced by heparin and activity determined. Results are the mean \pm SE of four rats in each group. * $P < 0.05$ vs. control; # $P < 0.05$ vs. all other groups. CON, control.

Accumulation of cardiac lipid droplets following DEX. Electron micrographs illustrate increases lipid-like vacuoles in myocytes following DEX treatment (Fig. 7B, arrows). These spherical lipid droplets were closely associated with the mitochondria. No other ultrastructural difference was detected between control and DEX hearts.

DISCUSSION

Chronic DEX treatment induces insulin resistance, hyperinsulinemia, and hyperglycemia (25,26). In our study, a single bolus dose of DEX had no influence on either plasma insulin or glucose after 4 h. Nevertheless, exploiting the euglycemic-hyperinsulinemic clamp, we demonstrate that DEX induced whole-body insulin resistance at this time point. Although this insulin resistance could embrace metabolic abnormalities in multiple organs (27), the effects of DEX on insulin-sensitive cardiac tissue have not been entirely elucidated. Assessment of cardiac glycolytic rates revealed no change following DEX, whereas

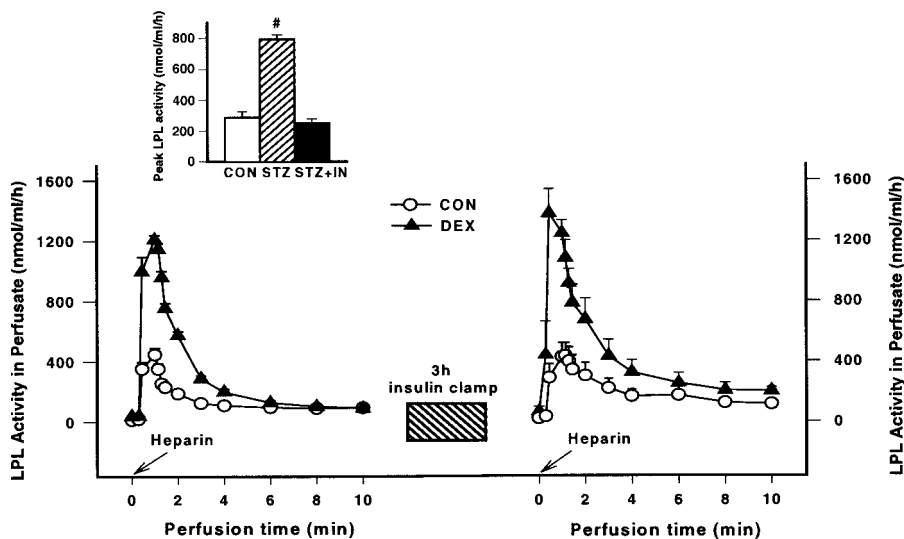


FIG. 6. Responses of exogenous insulin on cardiac LPL activity. STZ-induced diabetic rats were injected with a rapid-acting insulin (8 units; IN) 4 days after diabetes induction, the rats killed 180 min later, and heparin-releasable LPL activity determined in perfused hearts (*in-set*). Heparin-releasable LPL activity was also determined before and after termination of the 3-h euglycemic-hyperinsulinemic clamp. Results are the means \pm SE of four rats in each group. # $P < 0.05$ vs. all other groups. CON, control.

glucose oxidation decreased significantly. Moreover, cardiac glycogen content increased almost twofold after DEX. These data suggest that in the short term, DEX is capable of inducing insulin resistance and switching cardiac glucose disposal from oxidation to storage, likely compromising energy production in the heart.

Glucose utilization provides the heart with $\sim 30\%$ of its energy requirements. After glucose uptake and conversion to pyruvate, PDC facilitates subsequent entry and oxida-

tion of pyruvate in the mitochondria. By phosphorylating PDC, PDK can decrease the rate of glucose oxidation. In Morris hepatoma cells, DEX is known to stimulate PDK4 but not PDK2 expression (12). Because these two PDKs are the major isoforms in the heart, we examined their cardiac expression. DEX increased the expression of PDK4 but was without effect on the high basal level of PDK2. Our data suggest that acute DEX could lower cardiac glucose oxidation through augmentation of PDK4 gene and protein expression. Whether pyruvate dehydrogenase phosphatase also plays a role in explaining the effect of DEX on cardiac glucose metabolism, as suggested in STZ-induced diabetes (28), is currently unknown.

Typically, fatty acids provide most of the energy required by the normal heart, with the balance coming from oxidation of other substrates like glucose. Since cardiac glucose oxidation is impaired following DEX, we predicted that fatty acid consumption would make up the energy deficit. Based on reports that 1) LPL is the major fatty acid supplier to the heart and 2) glucocorticoids are known to enhance LPL activity in postheparin serum (29), heart homogenates (30), and isolated myocytes (31), we examined LPL regulation following DEX. The present study is the first to report a rapid increase in LPL activity and protein at its functionally relevant location, the coronary lumen. Given the observation that cardiac LPL is a major determinant of plasma triglyceride (24), the increase in cardiac luminal LPL could be associated with the decline in circulating triglyceride. However, as no apparent change was noted in plasma fatty acid levels, our data suggest that following LPL-mediated triglyceride hydrolysis, fatty acid can be taken up rapidly and directly into tissues. In support of this suggestion, cardiac- and skeletal muscle-specific overexpression of LPL decreased plasma triglyceride and elevated triglyceride storage in muscle tissue but was without effect when plasma fatty acid was measured (32). In this study, visualization using electron microscopy also revealed high triglyceride storage in DEX-treated hearts.

In hypoinsulinemic and hyperglycemic STZ rats, we reported elevated cardiac luminal LPL activity (15,16) that was independent of shifts in mRNA levels, suggesting a posttranslational increase of LPL at this location (22).

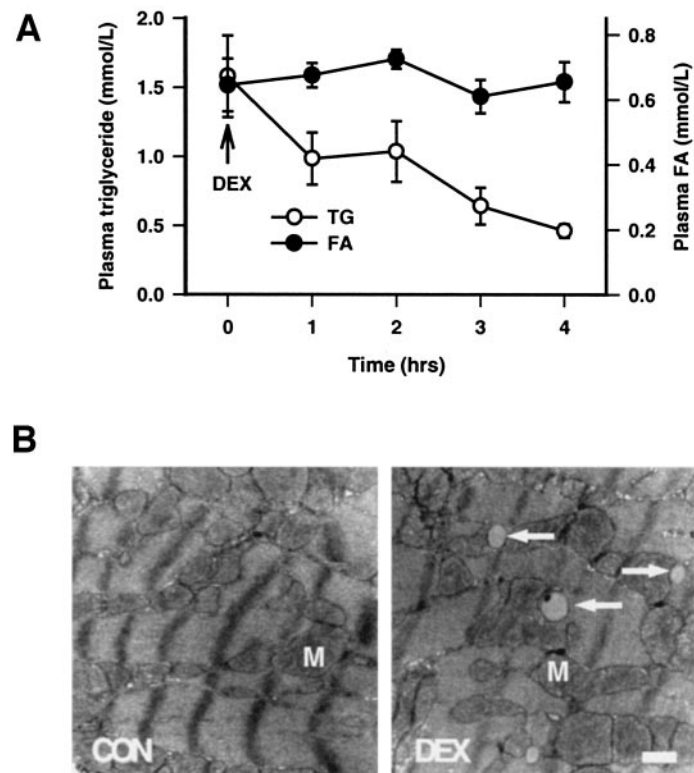


FIG. 7. Lipid homeostasis following DEX injection. Animals were treated with DEX (1 mg/kg i.p.) and blood samples from the tail vein collected at 60-min intervals for determination of triglyceride (TG) and fatty acid (FA) (A). Results are the means \pm SE of four rats in each group. After 4 h, cardiac morphology was evaluated using a transmission electron microscope. B: Representative electron micrograph of hearts from control (CON) and DEX animals. The scale bar represents 500 nm. M, mitochondria; white arrows, lipid-like vacuoles.

With insulin resistance induced by DEX, although comparable results were observed when luminal LPL activity was measured, in this instance, changes in LPL activity are coordinated to an increase in LPL mRNA. Another interesting dissimilarity between STZ-mediated hypoinsulinemia and DEX-induced insulin resistance is when hearts are perfused in vitro with heparin-free buffer. STZ hearts lose the augmented pool of heparin-releasable LPL at the coronary lumen, implicating hypoinsulinemia as an important reason for this effect in vivo, with continuous displacement of the enzyme in its absence in vitro. It should be noted that we are unable to detect any alteration in basal enzyme displacement between control and STZ hearts due to the large volume (30 ml) into which LPL was being shifted. In contrast, hearts from DEX animals continued to demonstrate augmented basal and heparin-releasable LPL activity in vitro, implying that the rate of displacement is overcome by intrinsic and enduring changes, likely an increased synthesis and transfer of enzyme, that act to keep LPL high at this location.

Regulation of cardiac luminal LPL may be an important means for maintaining cardiac function during metabolic stress by providing excess fatty acid to the heart. Acute treatment (180 min) of 4-day STZ-induced diabetic rats with a rapid-acting insulin reduced peak heparin-releasable LPL activity to control levels. It is likely that in vivo, insulin, by facilitating glucose entry and utilization, overcomes any energy deficit, eventually decreasing the requirement for LPL. DEX is considered a long-acting steroid, with a single dose lasting ~2–2.5 days, and an “every other day” schedule is recommended for treatment (33). Interestingly, estimation of cardiac LPL from DEX hearts revealed an increase in enzyme, both immediately and following 3 h of insulin infusion (during clamp). These data suggest that unlike hypoinsulinemia, provision of exogenous insulin during DEX-induced insulin resistance is unable to normalize LPL. It should be noted that in humans, insulin secretagogues like sulfonylureas are also ineffective in overcoming glucocorticoid-induced insulin resistance (34).

In summary, our studies suggest that under circumstances where glucose utilization is compromised due to a glucocorticoid-induced increase of PDK4, augmented LPL will amplify triglyceride hydrolysis and the fatty acids supplied to the heart are used as additional sources of substrate to maintain continuous energy production. On the other hand, intracellular availability of fatty acid could regulate glucose metabolism, as suggested by the “glucose–fatty acid cycle” (12,35,36). Accordingly, DEX effects on glucose oxidation are prevented by Acipimox, which reduces plasma fatty acid (37), and in Morris hepatoma cells, fatty acids directly increase PDK4 expression (12). Irrespective of the mechanisms that increase LPL or decrease glucose oxidation, a role for LPL in cardiac pathology has been demonstrated in transgenic mouse lines overexpressing human LPL in skeletal and cardiac muscle (24,38). These animals exhibited insulin resistance, severe myopathy characterized by muscle fiber degeneration, and extensive proliferation of mitochondria and peroxisomes. In a more recent study using genetically engineered mice that specifically overexpressed cardiomyocyte surface-bound LPL, lipid oversupply and impaired

contractile function (cardiomyopathy) were observed (39). It has not been determined whether these effects of DEX on cardiac metabolism can be translated into increased cardiovascular risk (40).

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