Dexamethasone Induces Posttranslational Degradation of GLUT2 and Inhibition of Insulin Secretion in Isolated Pancreatic β Cells

COMPARISON WITH THE EFFECTS OF FATTY ACIDS*

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GLUT2 expression is strongly decreased in glucose-unresponsive pancreatic β cells of diabetic rodents. This decreased expression is due to circulating factors distinct from insulin or glucose. Here we evaluated the effect of palmitic acid and the synthetic glucocorticoid dexamethasone on GLUT2 expression by in vitro cultured rat pancreatic islets. Palmitic acid induced a 40% decrease in GLUT2 mRNA levels with, however, no consistent effect on protein expression. Dexamethasone, in contrast, had no effect on GLUT2 mRNA, but decreased GLUT2 protein by about 65%. The effect of dexamethasone was more pronounced at high glucose concentrations and was inhibited by the glucocorticoid antagonist RU-486. Biosynthetic labeling experiments revealed that GLUT2 translation rate was only minimally affected by dexamethasone, but that its half-life was decreased by 50%, indicating that glucocorticoids activated a posttranslational degradation mechanism. This degradation mechanism was not affecting all membrane proteins, since the α subunit of the Na+/K+-ATPase was unaffected. Glucose-induced insulin secretion was strongly decreased by treatment with palmitic acid and/or dexamethasone. The insulin content was decreased (~55 percent) in the presence of palmitic acid, but increased (~180%) in the presence of dexamethasone. We conclude that a combination of elevated fatty acids and glucocorticoids can induce two common features observed in diabetic β cells, decreased GLUT2 expression, and loss of glucose-induced insulin secretion.

Development of non-insulin-dependent, type II diabetes mellitus is accompanied by a loss of glucose-stimulated insulin secretion (GSIS)† (1, 2). The primary causes of this secretory defect are not yet completely elucidated. However, in rodent models of diabetes, the loss of GSIS has been demonstrated to correlate with a reduced or suppressed expression of the β cell glucose transporter GLUT2 (3–6). Thus, in addition to a loss of GSIS, a decreased expression of GLUT2 is also a characteristic of diabetic β cells. In an attempt at identifying the causes of GLUT2-regulated expression, we previously performed islet cross-transplantation experiments. When control islets were transplanted in diabetic mice, GLUT2 expression was suppressed whereas when GLUT2 nonexpressing islets from diabetic animals were transplanted into control mice, a complete recovery of transporter expression was observed. These experiments led to the conclusion that circulating factors present in the diabetic environment, distinct from glucose and insulin, were responsible for the loss of GLUT2 expression (6). Furthermore, the down-expression of GLUT2 in transplanted islets correlated with a loss of GSIS (7). Identification of the circulating factors that control GLUT2 expression in β cells is therefore of critical importance, as they may be responsible for the functional alterations of β cells in diabetes.

Elevated circulating free fatty acids and triglycerides are part of the symptoms of both insulin-dependent and non-insulin-dependent diabetes mellitus (8, 9). Free fatty acids have been described for many years as being able to induce a state of insulin resistance in peripheral tissues by a glucose/fatty acid cycle that prevents a normal uptake of glucose (10). The effects of free fatty acids on the function of pancreatic islets have been studied both in vivo and in vitro experiments. These studies indicated that short term (1–3 h) exposure of pancreatic islets to free fatty acids had a stimulatory effect on GSIS (11–13), whereas longer exposure led to a suppression of insulin secretion (12–14). This inhibitory effect is also accompanied by a decrease in insulin biosynthesis, in glucose oxidation, and a reduction in pyruvate dehydrogenase activity with a parallel increase in pyruvate dehydrogenase kinase activity (15, 16). A major role for free fatty acids in the development of β cell glucose unresponsiveness has thus been proposed. This was further supported by the observation that circulating free fatty acid levels were increased a few weeks before development of hyperglycemia and loss of GLUT2 expression in male Zucker diabetic rats, while obese female Zucker rats, which do not develop hyperglycemia and do not lose GLUT2, did not show this increase in free fatty acids, even though they develop similar hypertriglyceridemia (17). Furthermore, incubation of pancreatic islets in the presence of free fatty acids induced an increase in low $K_m$ glucose usage (18) and an elevated basal insulin secretion rate (14, 18). However, no apparent regulation of GLUT2 expression by free fatty acids has been observed in islets maintained in tissue culture (9). Thus, although free fatty acids may lead to a number of β cell dysfunctions associated with diabetes, they apparently do not induce the decreased or suppressed expression of GLUT2. This therefore indicates that additional factors also participate in the induction of the β cells functional alterations in diabetes.

Dexamethasone administration in humans and in animals as well as hypercortisolism in Cushing syndrome are known to

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†The abbreviations used are: GSIS, glucose-stimulated insulin secretion; BSA, bovine serum albumin; PBS, phosphate-buffered saline.
induce a state of insulin resistance. This is also usually accompanied by changes in β cell functions, in particular an increase in basal but a decrease in stimulated insulin secretion, an increase in proinsulin mRNA, a decrease in islet insulin stores, and an hyperplasia and hypertrophy of the β cells (19–23). Dexamethasone administration to rats does not, however, induce a decrease in β cell GLUT2 (24). Only if diabetes is induced, as for example following repeated injections of high doses of dexamethasone to Wistar rats or of relatively lower doses to Zucker fa/fa rats, is a decrease in GLUT2 expression observed (22, 25). The exact role of dexamethasone on pancreatic β cells is, however, difficult to evaluate when administered to the intact animal. In vitro, exposure of RINm5F cells to dexamethasone increased proinsulin mRNA levels but did not alter the insulin secretion rate at any glucose concentrations (26). In HIT cells and isolated β cells dexamethasone induces a decrease in insulin secretion and mRNA (27). The inhibitory effect on mRNA levels can, however, be completely prevented by increases in intracellular CAMP (28).

Here we studied the effect of palmitic acid and dexamethasone alone, or in combination on the expression of GLUT2, on GSIS and on insulin mRNA levels in in vitro cultured rat pancreatic islets. We demonstrate that a combination of both GSIS and on insulin mRNA levels in one alone, or in combination on the expression of GLUT2, on one transporter detected with a rabbit antibody raised against a peptide corresponding to amino acids 513–522 of rat GLUT2 (31) (dilution 1:2,000) and a horseradish peroxidase-coupled donkey anti-rabbit immunoglobulin antibody (dilution 1:8,000). Detection was with the enhanced chemiluminescence detection technique. For the α subunit of the Na+/K+ pump, an antibody against the purified α subunit of the Bufo marinus Na+/K+ ATPase was used (34).

Biochemical Labeling—For pulse-chase experiments, islets were first treated for 48 h with or without 1 μM dexamethasone. After washing twice with PBS, they were incubated 30 min in RPMI 1640 medium depleted of methionine and complemented with 10% dialyzed fetal calf serum and labeled with 80 μCi/ml [35S]methionine for 5 min at 37°C. Cells were then washed twice with PBS and lysed in PBS containing 1% Triton X-100 and 5 mM EDTA for 10 min at 4°C. Nuclei and cells debris were pelleted by a 15-min centrifugation at 13,000 rpm in a tabletop centrifuge, and the supernatant was recovered. Incorporated radioactivity was quantitated with trichloroacetic acid precipitation, and samples containing equivalent amounts of radioactivity were immunoprecipitated with 3 μl of anti-GLUT2 antibodies, raised against peptides corresponding to amino acids 513–522 and 47–60 of the rat GLUT2, as described (33). Immunoprecipitates were collected with 30 μl of protein A-Sepharose beads for 20 min at room temperature. After washings, they were resuspended in sample buffer and analyzed on SDS-containing 7.5% polyacrylamide gel, exactly as described (33). Gels were then treated 15 min in glacial acetic acid, dried, and visualized with 120 μCi/ml of [3H]glucose and 90 min in H2O before being dried and exposed to x-ray films at −70°C.

For determination of GLUT2 half-life, islets were first treated with or without 1 μM dexamethasone for 24 h. Islets in groups of ~200 were then pulse-labeled as described above except that the pulse was for 3 h in the presence of 200 μCi/ml [35S]methionine. Islets were then washed and either lysed directly or returned to the normal culture medium containing 1% Triton X-100 and chased at 37°C for 6, 12, or 24 h with or without dexamethasone. Islets were lysed in 100 μl of a buffer consisting of 1% SDS in PBS and protease inhibitors. After further dilution of the lysate in 400 μl of PBS containing 1.25% Triton X-100, GLUT2 was immunoprecipitated from identical amounts of total cellular proteins and analyzed by gel electrophoresis as described above. Quantitation of band intensity was by laser scanning densitometry.

RESULTS

Effects of Palmitic Acid on GLUT2 Expression—Pancreatic islets were kept in tissue culture for 48 h in the presence of 2.8 mM glucose or 30 mM glucose and increasing concentrations of palmitic acid. Total RNA was isolated from batches of 40 islets, and GLUT2 and actin mRNA levels were evaluated by North-
ern blot analysis. Quantitation of GLUT2 was always expressed as the ratio of GLUT2 to actin mRNA. Fig. 1A shows that increasing the glucose concentration from 2.8 to 30 mM led to an increase in GLUT2 mRNA, as expected (35, 36), and that addition of 0.6 mM palmitic acid induced a decrease in GLUT2 mRNA. Fig. 1B shows a quantitation of the time-dependent modulation of the GLUT2 to actin mRNA ratio in pancreatic islets incubated in the presence of 0.6 mM palmitic acid for different periods of time (for each point, n ≥ 4). C, quantitation of the GLUT2 to actin mRNA ratio in pancreatic islets incubated in the presence of different concentrations of palmitic acid for 48 h (for each point, n ≥ 4).

FIG. 1. Palmitic acid regulation of GLUT2 mRNA in isolated pancreatic islets. A, Northern blot analysis of GLUT2 and actin mRNAs in pancreatic islets incubated for 24 and 48 h in the presence of 2.8 mM glucose or 30 mM glucose in the presence or absence of 0.6 mM palmitic acid. B, quantitation of the GLUT2 to actin mRNA ratio in pancreatic islets incubated in the presence of 0.6 mM palmitic acid for different periods of time (for each point, n ≥ 4). C, quantitation of the GLUT2 to actin mRNA ratio in pancreatic islets incubated in the presence of different concentrations of palmitic acid for 48 h (for each point, n ≥ 4).

mRNA levels (Fig. 2, A and B) except for an apparently significant increase at 10 nM (142.6 ± 16.2%, mean ± S.E. of control value (n = 4), p < 0.05). At the protein level, however, dexamethasone induced a strong decrease in GLUT2 expression (Fig. 3, A and B). A significant effect was already observed at 10 nM, and the maximal inhibitory effect was reached at 1 µM dexamethasone (34.9 ± 10.7% of the control value (n = 5)). Time course experiments showed that this maximal effect was already observed after 24 h (Fig. 4, A and B).

Dexamethasone is thought to exert its cellular effects by binding to and activating glucocorticoid receptors. Activation of these receptors can be inhibited by the antagonist RU-486. We thus assessed whether the effect of 0.1 µM dexamethasone could be blocked by increasing concentrations of RU-486. Fig. 5 shows that the decreased expression of GLUT2 could indeed be completely prevented by RU-486.

To determine whether the effect of dexamethasone was dependent on the presence of high glucose concentrations in the medium, we incubated islets in the presence of 1 µM dexamethasone and different glucose concentrations and measured GLUT2 expression by Western blot analysis. At 2.8 mM glucose dexamethasone had no effect on GLUT2 protein expression (109.9 ± 21.5% of control value (n = 7)), but at higher glucose concentrations, dexamethasone induced a decrease in GLUT2 protein: 67.7 ± 9.2% of control at 5.6 mM glucose (n = 6) and 42.6 ± 9.2% of control at 30 mM glucose (n = 7) (Fig. 6).

Since dexamethasone decreased GLUT2 protein expression without modifying mRNA levels, we next determined whether the observed effect was at the translational or posttranslational level by performing biosynthetic labeling experiments. Islets previously treated for 48 h with 1 µM dexamethasone were pulse-labeled for 5 min with [35S]methionine, lysed, and GLUT2 was immunoprecipitated and analyzed by gel electrophoresis. In parallel, an aliquot of the biosynthetically labeled mRNA levels (Fig. 2, A and B) except for an apparently significant increase at 10 nM (142.6 ± 16.2%, mean ± S.E. of control value (n = 4), p < 0.05). At the protein level, however, dexamethasone induced a strong decrease in GLUT2 expression (Fig. 3, A and B). A significant effect was already observed at 10 nM, and the maximal inhibitory effect was reached at 1 µM dexamethasone (34.9 ± 10.7% of the control value (n = 5)). Time course experiments showed that this maximal effect was already observed after 24 h (Fig. 4, A and B).

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isletswasdirectlylysedintheelectrophoresissamplebufferfor quantitative analysis of GLUT2 by Western blot analysis. These experiments showed that GLUT2 synthesis rate was only slightly decreased (81.2 ± 5.9% of control (n = 3)), while at the same time total GLUT2 levels were decreased to 35.2 ± 5.7% of control (n = 2) (Fig. 7, A and B). This indicated that dexamethasone had little effect on GLUT2 translational rate. To assess whether the half-life of GLUT2 was decreased, batches of 200 islets were first treated with or without 1 μM dexamethasone for 24 h and then pulse-labeled for 3 h with [35S]methionine. At the end of the pulse the islets were washed and either lysed or returned to a normal culture medium containing an excess of cold methionine and incubated at 37°C for 6, 12, or 24 h with or without dexamethasone. After immunoprecipitation and separation by gel electrophoresis, GLUT2 was quantitated by laser scanning densitometry. Fig. 7C shows that the half-life of GLUT2 was decreased from 20 to 10 h in the presence of dexamethasone, indicating a major effect of glucocorticoids on transporter stability. Finally, to determine whether the dexamethasone effect was specific for GLUT2, we evaluated the expression of the α subunit of the Na+/K+-ATPase. Fig. 8 shows that at the maximal dexamethasone concentration tested, expression of this protein was not decreased but rather increased by dexamethasone treatment, suggesting that the effect of dexamethasone was not due to a general effect on membrane proteins.

**FIG. 3.** Effect of dexamethasone on GLUT2 protein expression. Isolated pancreatic islets were kept in culture for 48 h in the presence of different concentrations of dexamethasone. After cell lysis, equal amount of proteins were separated by gel electrophoresis and analyzed by Western blotting. A, Western blot of GLUT2; B, quantitation of GLUT2 expression relative to control at 30 mM glucose. Results are expressed as mean ± S.E. for n = 5.

**FIG. 4.** Time course of dexamethasone effect on GLUT2 protein expression. Isolated pancreatic islets were maintained in the presence (+) or absence (−) of 1 μM dexamethasone for the indicated periods of time, and GLUT2 expression was analyzed as described in the legend to Fig. 3. A, Western blot; B, quantitation of GLUT2 expression relative to control at 30 mM glucose. Results are expressed as mean ± S.E. for n = 4. The maximal decrease in GLUT2 expression is reached at 24 h.

**FIG. 5.** Dexamethasone-induced decrease in GLUT2 expression is prevented by the glucocorticoid receptor antagonist RU-486. Isolated pancreatic islets were maintained in the presence (+) or absence (−) of 0.1 μM dexamethasone for 48 h and in the presence of the indicated concentrations of RU-486. GLUT2 was then analyzed by Western blot analysis. The inhibitory effect of dexamethasone could be completely reversed by 1 μM of RU-486.

**FIG. 6.** The effect of dexamethasone is glucose-dependent. Pancreatic islets were kept in culture for 48 h with the indicated concentrations of glucose and in the presence (+) or absence (−) of dexamethasone. The reduction in GLUT2 expression relative to incubation in the presence of high glucose concentrations. Results are expressed as mean ± S.E. for n ≥ 6.

Combined Effect of Palmitic Acid and Dexamethasone on GLUT2 mRNA and Protein—The combined effect of dexam-
Dexamethasone and palmitic acid was tested in 48-h incubations of islets with 0.6 mM palmitate plus increasing dexamethasone concentrations. At the mRNA level, the presence of dexamethasone at 0.1 μM increased the inhibitory effect of palmitic acid, leading to a decrease in GLUT2 mRNA levels from 65.6 ± 2.8% of control levels (n = 5), in the presence of palmitic acid alone, to 41.5 ± 8.1% of control levels (n = 5), in the presence of palmitic acid and dexamethasone (p < 0.01) (Fig. 9, A and B). Combination of palmitic acid and dexamethasone led to a decrease in GLUT2 protein down to 24.6 ± 5.3% of the control at 1 μM dexamethasone (n = 4), which was not significantly different from the effect of dexamethasone alone (see Fig. 3).

**Insulin Secretion, Islet Insulin Content, and Insulin mRNA**—The effect of dexamethasone and/or palmitic acid on insulin secretion was assessed in islet perifusion experiments. Islets treated for 48 h with 0.6 mM palmitic acid and/or 1 μM dexamethasone showed no change in basal insulin secretion compared with control islets. However, a 73.1 ± 10.0% (n = 2) inhibition of the glucose-induced insulin secretion was observed following dexamethasone treatment, a 72.1 ± 7.7% (n = 2) decrease following palmitic acid treatment, and an 81.8 ± 2.8% (n = 2) decrease when both treatments were combined (Fig. 10, A–C).

The insulin content of islets treated for 48 h with 0.6 mM palmitic acid and/or 1 μM dexamethasone was then measured. Palmitic acid, with or without dexamethasone, induced a decrease in insulin content which reached 46.7 ± 2.8% for palmitic acid and 41.5 ± 8.1% of control levels (n = 5), in the presence of palmitic acid alone, to 41.5 ± 8.1% of control levels (n = 5), in the presence of palmitic acid and dexamethasone (p < 0.01) (Fig. 9, A and B). Combination of palmitic acid and dexamethasone led to a decrease in GLUT2 protein down to 24.6 ± 5.3% of the control at 1 μM dexamethasone (n = 4), which was not significantly different from the effect of dexamethasone alone (see Fig. 3).

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FIG. 10. Palmitic acid and dexamethasone alone or in combination suppress glucose-stimulated insulin secretion. Pancreatic islets were maintained in culture in the presence or absence of palmitic acid (0.6 mM), dexamethasone (1 μM), or a combination of both for 48 h. 10 islets were then picked for analysis of glucose-stimulated insulin secretion in a perifusion chamber. Perifusion were performed in parallel for control and treated islets. A, palmitic acid-treated islets; B, dexamethasone-treated islets; C, palmitic acid and dexamethasone-treated islets.

Palmitic acid and dexamethasone treatment increased insulin content to 177.2 ± 17.0% (n = 4) of the control islets. Islet insulin mRNA levels following palmitic acid and/or dexamethasone treatment were measured by Northern blot analysis. Dexamethasone, present at concentrations from 1 nM to 1 μM, did not induce any change in insulin mRNA, except possibly at 1 μM, where a small increase was observed: 118.4 ± 5.0% of the control (n = 3) (p < 0.05 versus control). On the opposite, islets exposed to palmitic acid exhibited a decrease in insulin mRNA down to 66.4 ± 12.9% of control at 0.6 mM palmitic acid (n = 6). Addition of different concentrations of dexamethasone did not alter palmitic acid effect.

DISCUSSION

In the present study, we demonstrated that free fatty acids and the synthetic glucocorticoid dexamethasone down-regulate GLUT2 expression in isolated pancreatic islets. Whereas palmitic acid induced a decrease in GLUT2 mRNA levels, it did not induce consistent changes in GLUT2 protein expression. In contrast, dexamethasone induced a strong decrease in GLUT2 protein levels but no change in mRNA levels. Both substances, however, displayed a very strong inhibitory action on glucose-induced insulin secretion.

Free fatty acids are elevated in both type I and type II diabetes and have been shown to have a number of negative effects on insulin sensitivity of peripheral tissues and function of pancreatic β cells. The present experiments were undertaken to determine whether palmitic acid could have a role in the control of GLUT2 expression in addition to its inhibitory effect on GSIS. The effect of palmitic acid was detectable only on the regulation of transporter mRNA levels, and no consistent changes could be observed at the protein level. This indicates that although free fatty acids are able to induce glucose unresponsiveness in β cells, in agreement with previously published work (12–14), they are certainly not the only factor inducing the dysfunction of these cells in diabetes, since GLUT2 levels are unaffected.

Dexamethasone effect on islet function as observed in the present experiment is at least 2-fold: a strong reduction in GLUT2 protein expression and a severe inhibition of GSIS. The decrease in GLUT2 protein expression is relatively rapid, occurring within 24 h of exposure to dexamethasone. Strikingly, there is no parallel decrease in mRNA levels. This, therefore, indicates that the regulation of transporter expression is at the translational or posttranslational level. Our pulse-labeling experiments demonstrated only a minimal decrease in the rate of transporter translation, suggesting that the regulation was at a posttranslational level. This was indeed directly demonstrated in pulse-chase experiments, which showed a 50% decrease in GLUT2 half-life, induced by glucocorticoid treatment. Since the effect of dexamethasone could be inhibited by the glucocorticoid receptor antagonist RU-486, transcriptional activation of a gene or a set of genes is required to increase the rate of GLUT2 degradation. Although we do not know which gene products are responsible for stimulating transporter degradation, possible candidates include components of the ubiquitin-proteasome degradation system. Indeed, in muscle, dexamethasone has been shown to activate the energy-dependent protein degradative system and the expression of ubiquitin (37). Although in this report degradation of myofibrillar proteins was assessed, it is known that membrane proteins such as CFTR can also be degraded by the proteasome-ubiquitin system (38, 39). Whatever degradative system is induced, it must display a selectivity for the transporter, since another membrane protein, the α subunit of the Na+/K+ -ATPase, was not decreased in the presence of dexamethasone. Another interesting observation is that degradation of GLUT2 induced by dexamethasone was more pronounced in the presence of high glucose concentrations. The effect of dexamethasone cannot simply be explained as an inhibition of the glucose effect, since it has been demonstrated that the increase in GLUT2 expression induced by glucose is due to transcriptional activation of its gene (35, 36), whereas the effect of dexamethasone on
GLUT2 is at the posttranslational level. Activation of the degradative system is thus both glucose- and dexamethasone-dependent.

When added to islets in the presence of palmitic acid, dexamethasone increased the inhibitory action of fatty acids on GLUT2 mRNA. It might then be postulated that the effect of fatty acids is increased by dexamethasone by a mechanism involving interaction at the GLUT2 promoter of glucocorticoid receptors and fatty acid-activated transcription factors such as the peroxisome proliferator-activated receptors or stimulation of peroxisome proliferator-activated receptors expression by dexamethasone (40).

**Insulin Secretion, Islets Insulin Content, and Insulin mRNA—Dexamethasone and fatty acids, alone or in combination, had a negative impact on the first and second phases of glucose-induced insulin secretion. This has been demonstrated previously for fatty acids (13, 14, 18). Here, however, we did not observe the increase in basal secretory activity reported in these preceding studies. We, however, observed a decrease in proinsulin mRNA levels and in total insulin content. The inhibitory effect of dexamethasone correlated with an increase in total insulin content and in the absence of a reduction in proinsulin mRNA. The mechanism by which fatty acids and dexamethasone exert their inhibitory effect on GSIS is not known. For fatty acids, a decrease in pyruvate dehydrogenase and increase in pyruvate dehydrogenase kinase has been reported which could result in impaired glucose signaling (15). For dexamethasone, an increase in glucose-6-phosphatase, which increases glucose cycling in pancreatic β cells, may reduce the glucose signaling pathway (41, 42). Dexamethasone has also been demonstrated to increase islets neuropeptide Y content and secretion (43). This peptide, by binding to specific Gi-coupled receptors present on β cells, has been shown to have an inhibitory action on insulin secretion (44). In addition, the activation of a proteolytic activity, as demonstrated in the present study, may also lead to the degradation of essential components of the insulin granules exocytotic machinery.

Together, our data show that in addition to fatty acids, dexamethasone has profound effects on the function of isolated pancreatic β cells. The observed decreased expression of GLUT2 is due to the induction of a protein degradative system, which is better induced in hyperglycemic conditions and which shows apparent specificity for the transporter when compared with the α subunit of the Na+/K+-ATPase. The strong inhibitory action on insulin secretion may be due to a combination of different causes, including alterations in glucose metabolism, increased secretion of neuropeptide Y, or degradation of key components of the exocytic machinery. High glucocorticoid levels in the presence of hyperglycemia may therefore have inhibitory effects on β cells functions that are different from those reported in dexamethasone-induced insulin resistance when normoglycemia is prevailing. These effects may explain the decrease in GLUT2 expression observed for instance in db/db mice (6) which have high circulating levels of glucocorticoids (45).