Phosphoenolpyruvate Carboxykinase (GTP) Gene Transcription and Hyperglycemia Are Regulated by Glucocorticoids in Genetically Obese db/db Transgenic Mice*

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The molecular mechanisms underlying increased hepatic phosphoenolpyruvate carboxykinase (PEPCK) gene transcription and gluconeogenesis in type II diabetes are largely unknown. To examine the involvement of glucocorticoids and the cis-acting insulin response sequence (IRS, −416/−407) in the genetically obese db/db mouse model, we generated crosses between C57BL/KsJ- db/db mice and transgenic mice that express −460 or −2000 base pairs of the rat PEPCK gene promoter containing an intact or mutated IRS, linked to a reporter gene. Transgenic mice expressing the intact PEPCK(460)-CRP (C-reactive protein) transgene bred to non-obese and the db/db strains were obese, hyperinsulinemic, and developed fasting hyperglycemia (389 ± 26 mg/100 ml) between 4 and 10 weeks of age. Levels of CRP reporter gene expression were increased 2-fold despite severe hyperinsulinemia compared with non-diabetic non-obese transgenic mice. Reporter gene expression was also increased 2-fold in transgenic obese diabetic db/db mice bearing a mutation in the IRS, −2000(IRS)-hGx, compared with non-obese non-diabetic transgenic 2000(IRS)-hGx mice. Treatment of obese diabetic db/db transgenic mice with the glucocorticoid receptor blocker RU 486 decreased plasma glucose by 50% and reduced PEPCK, GLUT2, glucose-6-phosphatase, tyrosine aminotransferase, CRP, and hGx reporter gene expression to levels similar to those of non-obese normoglycemic transgenic mice. Taken together, these results establish that −460 bp of 5′-flanking sequence is sufficient to mediate the induction of PEPCK gene transcription in genetically obese db/db mice during the development of hyperglycemia. The results further demonstrate that the mechanism underlying increased expression of gluconeogenic enzymes in the db/db mouse requires the action of glucocorticoids and occurs independently of factors acting through the PEPCK IRS (−416/−407) promoter binding site.

Non-insulin-dependent diabetes mellitus (NIDDM) is a complex metabolic disease with environmental and genetic components (1, 2). Hyperglycemia develops via mechanisms that are not understood completely; however, a classic defect involves the inability of insulin to inhibit hepatic glucose production. Most isotopic studies in man and rodents suggest that increased gluconeogenesis is a major source of increased glucose production in type II diabetes (NIDDM) (3, 4); however, with rare exceptions, the primary mechanisms for increased gluconeogenesis at the molecular level are unknown (5–7). Gluconeogenesis is controlled from seconds to minutes by the delivery of substrates and by hormone-mediated changes in the activity of several key gluconeogenic enzymes. In the long term, usually involving hours to days, gluconeogenesis is regulated by the synthesis rate of gluconeogenic enzymes at the level of gene expression. In the liver, phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and is considered the major rate-controlling enzyme in the pathway of gluconeogenesis from pyruvate, lactate, and alanine (8). Normally, insulin rapidly and substantially inhibits transcription of the PEPCK gene in liver and in rat hepatoma cells. However, in several animal models of obesity and NIDDM, gluconeogenesis and PEPCK mRNA are increased by 2–3-fold over non-diabetic animals, despite circulating insulin levels that may be 4–10-fold greater than non-diabetic controls (9–11), suggesting a defect(s) in insulin regulation of gene expression.

In addition to abnormalities in insulin receptor signaling, defects (genetic or acquired) in regulatory proteins that control gene expression may play an important role in the pathogenesis and progression of type II diabetes. We found previously that deleting the glucocorticoid response unit (GRU; see Refs. 12 and 13), located between bases −455 and −349 upstream from the transcription start site of the PEPCK gene promoter in transgenic mice, prevented reporter gene induction in mice made diabetic with streptozotocin (14), whereas a mutation in the cAMP regulatory element, located between positions −93 and −86, had no effect on the diabetic response of the PEPCK promoter (21). The GRU (Fig. 1) is ≈100 bp and consists of two glucocorticoid receptor binding sites GR1 and GR2, located between positions −385 and −349, flanking two accessory factor elements termed AF1 and AF2. AF1 (−455 to −431) is a

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* The abbreviations used are: NIDDM, non–insulin-dependent diabetes mellitus; PEPCK, phosphoenolpyruvate carboxykinase; GRU, glucocorticoid regulatory unit; bp, base pair(s); GR, glucocorticoid receptor; AF, accessory factor; IRS, insulin response sequence; kb, kilobase(s); CRP, C-reactive protein; TAT, tyrosine aminotransferase; hGx, human growth hormone; Glu-6-Pase, glucose-6-phosphatase; IRS-1, insulin receptor substrate 1; IR-β, insulin receptor β subunit; PI3-kinase, phosphatidylinositol 3-kinase.

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PEPCK Gene Expression in db/db Mice

Animals—Two transgenic mouse lines were used to study the effects of obesity and diabetes on expression from the PEPCK promoter. The first utilized homozygous transgenic C57BL6 SJL mice (a hybrid of C57BLK× SJL) containing sequences from −460 bp to +73 bp of the rat cytosolic PEPCK gene ligated to the rabbit CRP gene, PEPCK(460)-CRP, obtained from previously established transgenic lines (Dr. D. Samols, Department of Biochemistry, Case Western Reserve University). The production and characterization of PEPCK(460)-CRP transgenic mice have been described in detail previously (18). Transgenic expression in this model is directed by the −460 bp of the 5′-regulatory region of the PEPCK promoter and has been found to contain all of the necessary elements for developmental, hormonal (including diabetes), and dietary control in the liver in a manner similar to the endogenous PEPCK gene (19–23)

Two transgenic mouse lines were used to study the effects of obesity and type II diabetes on promoter function, mice homozygous for the autosomal recessive db gene containing either intact or mutated promoter were produced using the breeding procedure outlined previously for outcrossing db/db mice (26). Because the recessive db mutation produces sterility in homozygotes, it was propagated by mating db/+ heterozygotes with homozygous transgenic mice. Based on the average basal level of expression and results from dietary manipulation, a representative transgenic line containing the intact promoter or a mutation in the IRS was chosen for breeding the PEPCK transgene onto the db/db background. Female C57BL/KsJ-m/db/+ mice were obtained from Jackson Laboratories and were kept on a standard diet and dietary control in the liver in a manner similar to the endogenous PEPCK gene (19–23). Table 1 shows the basal reporter gene expression in livers of mice carrying the intact PEPCK promoter compared with mice with a mutation in the IRS. hGx mRNA expression was characterized by Northern blot analysis in all six lines in response to feeding a high carbohydrate diet (decreased expression) or following a high protein diet (increased expression). There were no significant differences in response to dietary carbohydrate or protein in three lines of mice carrying the PEPCK(IRS)-hGx gene compared with induction and suppression of hGx in transgenic mice expressing the intact promoter.

Introduction of PEPCK Transgene into C57BL/KsJ-db/+ Mice—To study the effects of obesity and type II diabetes on promoter function, mice homozygous for the autosomal recessive db gene containing either intact or mutated promoter were produced using the breeding procedure outlined previously for outcrossing db/db mice (26). Because the recessive db mutation produces sterility in homozygotes, it was propagated by mating db/+ heterozygotes with homozygous transgenic mice. Based on the average basal level of expression and results from dietary manipulation, a representative transgenic line containing the intact promoter or a mutation in the IRS was chosen for breeding the PEPCK transgene onto the db/db background. Female C57BL/KsJ-m/db/+ mice were obtained from Jackson Laboratories and were kept on a standard diet and dietary control in the liver in a manner similar to the endogenous PEPCK gene (19–23). Table 1 shows the basal reporter gene expression in livers of mice carrying the intact PEPCK promoter compared with mice with a mutation in the IRS. hGx mRNA expression was characterized by Northern blot analysis in all six lines in response to feeding a high carbohydrate diet (decreased expression) or following a high protein diet (increased expression). There were no significant differences in response to dietary carbohydrate or protein in three lines of mice carrying the PEPCK(IRS)-hGx gene compared with induction and suppression of hGx in transgenic mice expressing the intact promoter.

Experimental Procedures

Materials—ATP, CTP, GTP, yeast tRNA, proteinase K, and restriction enzymes were purchased from Boehringer Mannheim. [α-32P]dCTP (300 Ci/mmol), [α-32P]UTP (300 Ci/mmol), and GeneScreen Plus were purchased from NEN Life Science Products. RNase-free DNase I (1000 units) was obtained from Promega. All other reagents were of the highest purity available. The following segments of DNA were used as hybridization probes. PEBP2 corresponded to a 6.2-kb genomic fragment containing the entire regulatory region was chosen to study regulation of reporter gene expression, binding, and/or phosphorylation of nuclear proteins acting through the IRS might be expected to impair the ability of insulin to suppress PEPCK gene transcription, thereby leading to increased gluconeogenesis and NIDDM.

In an attempt to explore the role of glucocorticoids and the IRS binding site to increased PEBP2 gene transcription in a model of type II diabetes, we generated a cross between transgenic mice expressing 2.0 or 0.46 kb of PEBP2 regulatory sequences containing a mutated or intact IRS into the C57BL/KsJ-db/+ mouse. Our results indicate that increased PEBP2 reporter gene transcription parallels the onset of hyperglycemia in the genetically obese db/db mouse, despite severe hyperinsulinemia. The overexpression of genes coding for gluconeogenic enzymes is driven by glucocorticoids and is independent of the cis-acting IRS sequence of the PEBP2 promoter.

FIG. 1. Schematic representation of the GRU located between −455 and −349 bp upstream of the rat PEBP2 gene promoter (12, 13). The GRU consists of two AF binding sites, AF1 and AF2, necessary for the full response to glucocorticoids, and two adjacent GR binding sites, GR1 and GR2. The AF2 site contains an IRS, −416 to −407, which mediates a negative effect of insulin on PEBP2 gene transcription (15).
treated mice were killed by cervical dislocation at 12:30 p.m., and 0.5 ml of blood was withdrawn immediately from the abdominal aorta for insulin and corticosterone assay. Preliminary experiments showed that a dose of RU 486 at 25 μg/kg of body weight had no effect on food intake overnight in db/db mice and showed a maximal effect on lowering blood glucose (data not shown). The liver was removed and 200 mg frozen in liquid nitrogen for RNA isolation.

Isolation of Total RNA and Northern Blot Analysis—RNA was isolated from frozen mouse liver by the guanidine thiocyanate-phenol method of Chomczynski and Sacchi (27). For Northern blot analysis total RNA samples (20 μg) were size fractionated in 0.9% agarose-formaldehyde gels and transferred to nitrocellulose filters. After washing with 2 × SSC, the filter was dried and baked for 2 h at 80 °C. Hybridization was carried out overnight at 65 °C in Church buffer (1% bovine serum albumin, 1 mmol/liter EDTA, 0.7% SDS, 0.25 mol/liter NaH2PO4, pH 8.0). Membranes were hybridized with probes labeled with [α-32P]dCTP to a specific activity of ~108 cpm/μg DNA using a random primed labeling kit according to the manufacturer’s instructions (Boehringer Mannheim). After hybridization, the filters were washed with 2 × SSC, 0.1% SDS at room temperature; and 0.2 × SSC, 0.1% SDS at 65 °C for 30 min. Membranes were placed in contact with X-AR film (Eastman Kodak), and the image intensity of the autoradiogram was determined using a Scan 5000 laser densitometer (U. S. Biochemical Corp.). Levels of specific transcripts were estimated by quantitating probe-specific signals. Hybridization to β-actin or 18 S RNA was used to correct for differences in RNA content/loading.

Western Blot Analysis—Mice were anesthetized with chloralose (40 mg/kg), and the liver was removed, minced coarsely, and homogenized immediately in a 10 × volume of solubilization buffer A (50 mm Heps, pH 7.5, 137 mm NaCl, 1 mm MgCl2, 1 mm CaCl2, 2 mm Na3VO4, 10 mm sodium pyrophosphate, 10 mm sodium fluoride, 2 mm EDTA, 1% Nonidet P-40, 10% glycerol, 2 μg/ml aprotinin, 10 μg/ml antipain, 5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 1.5 mg/ml benzamidine, 34 μg/ml phenylmethylsulfonyl fluoride) with a Polytron PTA 20S generator at maximum speed for 30 s. The homogenate was then centrifuged at 65,000 rpm at 4 °C in a model Ti-70 rotor (Beckman Instruments, Inc.) for 60 min to remove insoluble material, and the supernatant was used for analysis. Protein was measured using the Bradford procedure (Pierce Biochemical). For IR-β, IRS-1, and PI-3 kinase, 40 μg of protein was treated with Laemmli sample buffer containing 100 mM dithiothreitol, heated in a boiling water bath for 4 min, and subjected to SDS-polyacrylamide gel electrophoresis on a 7% Tris-acrylamide gel using a Bio-Rad mini-protein gel apparatus at 100 volts for 1 h. Proteins were electrotransferred from the gel to nitrocellulose at 90 V constant for 1 h using a mini-transfer apparatus. Nonspecific protein binding to the filter was blocked using 5% milk, 10 μl Tris, 150 mM NaCl, and 0.02% Tween 20. The polyvinylidene difluoride filter was incubated with antibodies to IR-β (1.5 μg/ml), IRS-1 (1.5 μg/ml), or PI-3 kinase (0.75 μg/ml) diluted in blocking buffer for 4 h at 22 °C, followed by extensive washing with Tris-buffered saline (150 mM NaCl, 10 mM Tris + Tween 20). At the end of the final wash, the blots were incubated with secondary antibody linked to horseradish peroxidase in 10 ml of blocking buffer for 1 h at 22 °C and washed again before exposing the membranes to enhanced chemiluminescence reagent (Amersham). Autoradiography was carried out using Kodak X-AR x-ray film. The specific band intensities were quantitated by optical densitometry using a Digiscan scanner (U. S. Biochemical Corp.) for integrating the autoradiographic signals.

Plasma Hormones and Metabolites—Blood glucose levels were determined in samples using an Accu-Check II glucose monitor (Boehringer) and a glucose-oxidase assay kit (Sigma). The plasma immunoreactive insulin concentration was determined by radioimmunoassay using a rat insulin radioimmunoassay kit (Linco, Inc., St. Louis) using rat insulin as standard. The circulating level of rabbit CRP in transgenic mice was determined in blood samples collected by retro-orbital bleeding and a radial immunoassay in agarose, as described previously (18), using a goat anti-rabbit CRP antiserum specific for native rabbit CRP. This method is sensitive to levels as low as 1–2 μg/ml and does not detect murine CRP. Plasma levels of corticosterone were assayed using a rat corticosterone radioimmunoassay kit according to the manufacturer’s instructions (ICN Biomedicals, Costa Mesa, CA). Glycerol was measured in deproteinized plasma samples by enzymatic determination using glycerol kinase-coupled with glycerophosphate dehydrogenase according to the conditions supplied by the manufacturer (Boehringer Mannheim).

Presentation of Data and Statistical Analysis—All data are presented as means ± S.E. Specific mRNA levels were calculated by expressing the effects of perturbations as a percentage of readings in paired samples from non-treated or vehicle-treated control samples in the same Northern blot or transcription assay after correction for loading differences with signal from β-actin or ribosomal mRNA. Statistical analyses were performed using analysis of variance between treatments or groups. Differences were considered statistically significant at p < 0.05.

RESULTS

Obesity and Hyperglycemia in db/db Transgenic Mice—We introduced two separate PEPCK promoter-reporter genes from transgenic C57B6/SJL mice into the C57BL/KsJ-db/db strain, the background strain in which the db gene interacts to produce diabetes, obesity, and hyperinsulinemia. Data presented in Table I show the effects of the experimental cross on the development of obesity and diabetes in the PEPCK(460)-CRP, db/db mice. After five successive back-crosses between transgenic db/+ mice with breeder db/+ mice, more than 100 progeny were produced from which a group of 10 male transgenic db/db and 8 male transgenic +/+ mice were obtained for analysis. The transgenic PEPCK(460)-CRP, db/db mice demonstrated a developmental pattern of diabetes similar to that of C57BL/KsJ-db/db mice (9, 28). At four weeks of age, PEPCK(460)-CRP, db/db mice were significantly heavier (p < 0.01) and had a 12-fold greater insulin level than their lean transgenic PEPCK(460)-CRP, +/+ littermates. However, plasma glucose and CRP (from the PEPCK promoter) were similar in transgenic PEPCK(460)-CRP, db/db and PEPCK(460)-CRP, +/+ mice, suggesting that higher plasma insulin levels in PEPCK(460)-CRP, db/db mice did not suppress PEPCK or CRP gene expression but were still capable of preventing hyperglycemia. After 10 weeks of age, hyperinsulinemia increased further by 1.75-fold (p < 0.01) in transgenic PEPCK(460)-CRP, db/db mice compared with 4-week-old PEPCK(460)-CRP, db/db mice. Fasting plasma glucose increased 2.8-fold (p < 0.01) above levels at 4 weeks, and the levels of plasma CRP increased by 1.7-fold (p < 0.01), suggesting a transition to a state of hepatic insulin resistance and a failure of hyperinsulinemia to suppress expression from the PEPCK promoter.

The source of higher levels of plasma CRP noted in 10-week-old transgenic PEPCK(460)-CRP, db/db mice was identified by...
Northern blot analysis of RNA from the livers of the transgenic mice at 12 weeks of age (Fig. 2). The expression of both PEPCK and CRP mRNA was increased significantly by 2-fold \((p < 0.01)\) in PEPCK(460)-CRP, \(db/db\) compared with PEPCK(460)-CRP, \(+/+\) mice, thus confirming that the increased plasma levels of CRP most probably resulted from increased activity from the PEPCK gene promoter during the postweaning period, uninhibited by the excessive plasma insulin levels.

**Effects of GR Antagonist RU 486 on Serum Parameters**—Plasma corticosterone levels (Table II) in non-transgenic \(db/db\) mice were increased by 427\% compared with \(db/+\) mice \((p < 0.01)\). To determine whether hypercorticism in the \(db/db\) mouse might underlie the mechanism for insulin resistance and increased PEPCK and CRP mRNA expression, we administered the synthetic glucocorticoid antagonist RU 486 to \(db/db\) and \(db/+\) mice and later to PEPCK(460)-CRP, \(db/db\) mice. RU 486 was tested at doses 0.1, 0.25, and 0.5 mg/kg (data not shown) and found to have its maximal effect on blood glucose at a dose of 25 mg/kg of body weight. RU 486 treatment significantly increased the serum corticosterone levels in \(db/db\) and \(db/+\) mice 2–11-fold over vehicle-treated controls, respectively \((p < 0.01)\), indicative of GR blockade. RU 486 or vehicle treatment had no significant effects on plasma glucose in \(db/+\) mice.

**Table II**

| Treatment     | Glucose (mg/dl) | Insulin (ng/ml) | Corticosterone (µg/ml) | Glycerol (mM) |
|---------------|-----------------|-----------------|------------------------|--------------|
| \(db/+\) Vehicle | 173 ± 7 (8)    | 0.83 ± 0.23 (8) | 4.08 ± 1.39 (5)     | 0.152 ± 0.016 (4) |
| \(db/+\) RU 486     | 150 ± 17 (8)   | 0.79 ± 0.29 (7) | 46.96 ± 4.23 (5)    | 0.178 ± 0.012 (5) |
| \(db/db\) Vehicle   | 443 ± 12 (10)  | 9.88 ± 2.36 (13)| 17.46 ± 1.81 (7)    | 0.228 ± 0.026 (6) |
| \(db/db\) RU 486    | 229 ± 23* (8)  | 7.84 ± 1.64 (5) | 34.01 ± 6.14* (5)   | 0.212 ± 0.022 (5) |

*Significantly different from \(db/db\) vehicle-treated, \(p < 0.01\).

*Significantly greater than \(db/+\) vehicle-treated, \(p < 0.01\).
in Fig. 4 and show that PEPCK mRNA concentration in the liver of db/db mice was increased 220 ± 25% relative to db/+ control animals (p < 0.01), whereas RU 486 administration reduced the concentration of PEPCK mRNA in db/db to 125 ± 22% of db/+ control mice. RU 486 had no effect on PEPCK gene expression in db/+ mice. The level of Glu-6-Pase mRNA expression in db/db mice was increased by 175 ± 26% over db/+ controls, whereas RU 486 reduced the concentration of Glu-6-Pase mRNA to 89 ± 23% of controls (p < 0.05). The level of GLUT2 mRNA in the liver of db/db mice was increased by 370 ± 37% relative to db/+ controls (p < 0.05), and RU 486 reduced the concentration of GLUT2 mRNA expression to 120 ± 17% of controls (p < 0.05). TAT mRNA concentration was increased in livers of db/db by 150 ± 29% (p < 0.05) and was reduced to 110 ± 18% of controls after RU 486 treatment.

Effect of a Mutation in the PEPCK IRC on Reporter Gene Expression in Transgenic Obese Diabetic db/db Mice—In light of the increase in expression from the PEPCK promoter in PEPCK(460)-CRP db/db mice and reduction in transcription with RU 486 (Figs. 2 and 3), we hypothesized that increased PEPCK gene transcription in db/db might be caused by glucocorticoid-dependent interactions that stimulate transcription from the PEPCK promoter through factors binding at the IRS. The PEPCK(IRS)-hGx × db/+ back-cross produced 86 offspring, of which 34 expressed the transgene, and 9 developed severe obesity and fasting hyperglycemia. At 12 weeks of age, the transgenic PEPCK(IRS)-hGx, db/db mice had 3.3-fold higher fasting glucose levels (p < 0.01) and were significantly heavier than their PEPCK(IRS)-hGx, +/+ littermates, and they exhibited similar fed and fasting hyperglycemia compared with db/db mice. In obese hyperglycemic transgenic PEPCK(IRS)-hGx, db/db mice treated with RU 486, blood glucose decreased by 53% from 342 to 159 mg/dl (p < 0.01). In the livers of obese diabetic transgenic PEPCK(IRS)-hGx, db/db mice, the level of hGx reporter gene expression increased 2-fold over normoglycemic PEPCK(IRS)-hGx, +/+ mice (Fig. 5). PhosphorImager quantitation of the relative levels of PEPCK or reporter gene expression in obese hyperglycemic transgenic mice expressing either CRP or hGx is presented in Fig. 6; the relative increase in PEPCK or reporter gene expression is very similar in both lines of hyperglycemic transgenic mice and is reduced to levels similar to +/+ mice after RU 486 treatment. Levels of CRP mRNA in obese diabetic mice were increased 225 ± 26% above normoglycemic transgenic PEPCK(460)-CRP, +/+ mice. CRP mRNA in RU 486-treated mice was significantly reduced to 135 ± 15% above normoglycemic transgenic mice (p < 0.05). The levels of expression of hGx mRNA in obese diabetic transgenic mice were increased 201 ± 18% above control PEPCK(IRS)-hGx, +/+ mice (p < 0.05), and they were reduced to values similar to those of control non-obese non-diabetic mice after RU 486 treatment.

Effect of RU 486 on Levels of IR-β, IRS-1, and PI3-Kinase (p85) Expression—To determine whether blocking the action of the GR affects the levels of insulin-signaling intermediates IR-β, IRS-1, and the 85-kDa regulatory subunit of PI3-kinase, Western blots were performed with liver cell extracts from vehicle-treated and RU 486-treated db/+ and db/db mice (Fig. 7). Compared with db/+ mice, the levels of IR-β, IRS-1, and p85 in db/db mice were reduced by 42, 48, and 61, respectively (p < 0.01). Upon treatment with RU 486, there were no significant changes in the pattern of expression of any of these signaling proteins in either db/+ or db/db mice, suggesting that blocking...
the action of the GR did not correct the decrease in expression of insulin signaling proteins in db/db mice.

**DISCUSSION**

The present results suggest that elements located in the proximal -460 bp of the PEPCK promoter are of major importance in up-regulating PEPCK gene expression in the hyperinsulinemic obese diabetic db/db mouse. The elevated plasma CRP levels observed during the onset of hyperglycemia in transgenic PEPCK(460)-CRP, db/db mice and the fact that the half-life for clearance of circulating rabbit CRP in transgenic mice is 45 min (18) suggest a shift in hepatic metabolism during the postweaning stage and increases in transcription from the PEPCK gene promoter. Our studies suggest that interactions between the homozygous mutant leptin receptor db gene and transcriptional modifier gene(s) or protein(s) expressed in C57BL/KsJ background stimulate PEPCK gene transcription and increase hepatic gluconeogenesis and diabetes between 4 and 10 weeks of age. The genetic basis for susceptibility to NIDDM in the db/db mouse model bearing a mutation in the leptin receptor has been related to strain-specific differences in glucose metabolism and pancreatic β cell function (29). However, it is difficult to distinguish between the pleiotropic effects of the mutation in the leptin receptor and the secondary effects of increased adiposity. To distinguish effects resulting from direct interactions between the NIDDM susceptibility genes in C57BL/6/KsJ background and the db mutation from those resulting from obesity per se will require the identification of quantitative trait loci, i.e. genetic modifiers for NIDDM in this strain and subsequent outbreeding into other rodent models of genetic or diet-induced obesity.

Adrenalectomy has been shown to lessen or reverse many of the metabolic abnormalities found in genetic models of obesity and insulin resistance, including hyperglycemia in the C57BL/KsJ-db/db mouse (30). We found that the steady-state levels of the mRNA coding for PEPCK, GLUT-2, Glut-Pase, and TAT in the db/db mouse were elevated at 12 weeks of age and were reduced significantly after treatment with RU 486. Previous studies have shown that RU 486 administered chronically to genetically obese Zucker fatty fa/fa rats reduces hyperphagia and weight gain (31, 32), whereas in rats made insulin-resist-

T. Ishizuka and J. E. Friedman, unpublished observation.
and reduce gluconeogenesis.

In most genetic models of obesity and type II diabetes, excess corticosterone is required to achieve hyperglycemia and increased gluconeogenesis; however Valera and Bosch (40) showed that transgenic mice overexpressing a PEPCK minigene driven by the PEPCK promoter developed hyperinsulinemia and chronic fasting hyperglycemia, suggesting that solely increasing PEPCK activity in the liver can increase gluconeogenesis, which results in hyperglycemia and a failure of hyperinsulinemia to down-regulate PEPCK gene expression in experimental animals. Although no evidence exists to suggest that the PEPCK promoter regulatory region is mutated in families with diabetes (41), it is possible that acquired or genetic defects in insulin or other signal transduction pathways could lead to increased PEPCK gene expression and gluconeogenesis because of a failure of insulin to modify one or more DNA-binding factors that regulate transcription from the PEPCK gene promoter. It is important to recognize, however, that gluconeogenesis is a coordinated pathway and that sites other than PEPCK may also contribute to stimulating increased hepatic glucose production, such as increased substrate delivery. However, inasmuch as the PEPCK gene is a major regulatory control point for gluconeogenesis and is highly resistant in hyperinsulinemic models of NIDDM, it represents a prime candidate for understanding the mechanisms whereby type II diabetes induces aberrant trans-regulation of otherwise normal genes coding for key enzymes of carbohydrate metabolism.

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REFERENCES

1. Kahn, C. R. (1994) Diabetes 43, 1066–1084
2. Bogardus, C. (1993) Diabetes Care 16, 228–231
3. Consoli, A., Nurjhan, N., Capani, F., and Gerich, J. (1991) Diabetes 38, 550–557
4. Consoli, A., Nurjhan, N., Reilly, J. J., Bier, D. M., and Gerich, J. (1990) J. Clin. Invest. 86, 2038–2045
5. Olefsky, J. M. (1980) Diabetes 30, 148–162
6. Taylor, S. I. (1992) Diabetes 41, 1473–1490
7. Gidh-Jain, M., Takada, J., Xu, L. Z., Lage, A. J., Viannet, N., Stoffel, M., Freguel, P., Vello, G., Sun, F., and Cohen, D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1932–1936
8. Hanson, R. W., and Garber, A. J. (1972) Am. J. Clin. Nutr. 25, 1010–1021
9. Shafrir, E. (1988) in Frontiers in Diabetes Research: Lessons Learned from Animal Diabetes (Shafrir, E., and Renold, A. E., eds) 2nd Ed., pp. 304–315, John Libbey and Co.
10. Noguchi, T., Matsuda, T., Tomari, Y., Yamada, K., Imai, E., Wang, Z., Ikeda, H., and Tanaka, R. (1993) FEBS Lett. 328, 145–148
11. Hofmann, C. A., Edwards, C. W., Hillman, R. M., and Colca, J. R. (1992) Endocrinology 130, 735–740
12. Imai, E., Stromstedt, P. E., Quinn, P. G., Carletted-Duke, J., Gustafsson, J. A., and Granner, D. K. (1990) Mol. Cell. Biol. 10, 4712–4719
13. Mitchell, J., Noisin, E., Hall, R., O’Brien, R. M., Imai, E., and Granner, D. K. (1994) Mol. Endocrinol. 8, 585–594
14. Friedman, J. E., Yun, J. S., Patel, Y. M., McGregor, M. G., and Hanson, R. W. J. Biol. Chem. 268, 12952–12957, 1993
15. O’Brien, R. M., Lucas, P. C., Forest, C. D., Magnuson, M. A., and Granner, D. K. (1990) Science 249, 553–557
16. Lucas, P. C., O’Brien, R. M., Mitchell, J. A., Davis, C. M., Imai, E., Forman, B. M., Samuels, H. H., and Granner, D. K. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2184–2188
17. O’Brien, R. M., Lucas, P. C., Yamasaki, T., Noisin, E. L., and Granner, D. K. (1994) J. Biol. Chem. 269, 30419–30428
18. Lin, C. S., Xia, D., Yun, J. S. Wagner, T., Magnuson, T., Mold, C., and Samols, D. (1995) Immunol. Cell Biol. 73, 521–531
19. McGregor, M. D., de Vente, J., Yun, J., Bloom, J., Park, E., Wynshaw-Boris, A., Wagner, T., Rottman, F. M., and Hanson, R. W. (1995) J. Biol. Chem. 263, 11443–11451
20. Patel, Y. M., Yun, J. S., Liu, J., McGregor, M. G., and Hanson, R. W. (1994) J. Biol. Chem. 269, 5619–5628
21. McGregor, M. G., Yun, J. S., Moorman, A. F., Lamers, W. H., Hendrick, G. K., Arafah, B. M., Park, E. A., Wagner, T. E., and Hanson, R. W. (1990) J. Biol. Chem. 265, 22371–22379
22. Short, M. K., Cloutier, D. E., Schaefer, I. M., Hammer, R. E., Magnuson, M. A., and Beale, E. G. (1992) Mol. Cell. Biol. 12, 1007–1020
23. Eisenberger, C. L., Neckushthian, H., Cohen, H., Shani, M., and Reshef, L. (1992) Mol. Cell. Biol. 12, 1396–1403
24. Iizuka, R., Behbringer, R. R., Theisen, M., Huggenvik, J. I., McKnight, G. S., and Binster, R. L. (1989) Mol. Cell. Biol. 9, 5154–5162
25. Laird, P. W., Ziderveld, A., Liners, K., Rudnicki, M. A., Jaenisch, R., and Berns, A. (1991) Nucleic Acids Res. 19, 4293–4294
26. Leiter, E. H., Prochazka, M., and Schultz, L. D. (1987) J. Immunol. 10, 3224–3229
27. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
28. Roesler, W. J., and Khandelwal, R. L. (1985) Mol. Cell. Biochem. 92, 99–106
29. Coleman, D. L. (1982) Diabetes 31, 1–6
30. Shimonura, Y., Bray, G. A., and Lee, M. (1987) Horm. Metab. Res. 19, 285–299
31. Okada, S., York, D. A., and Bray, G. A. (1992) Am. J. Physiol. 262, R1106–R1110
32. Langley, S. C., and York, D. A. (1990) Am. J. Physiol. 259, R539–R544
33. Kawasaki, M., Conney, G. J., Harra, T., and Storlien, L. H. (1995) Diabetes 44, 718–720
34. Reshef, L., and Shapiro, B. (1960) Metab. Clin. Exp. 9, 551–555
35. Williamson, J. R., Krebs, G. A., and Felts, P. W. (1966) Proc. Natl. Acad. Sci. U. S. A. 6, 247–254
36. Yang, S., and Dickson, A. J. (1995) Biochem. J. 310, 375–378
37. Gabbay, R. A., Sutherland, C., Guedi, L., Kahn, B. B., O’Brien, R. M., Granner, D. K., and Flier, J. S. (1996) J. Biol. Chem. 271, 1890–1897
38. Saad, M. J., Arai, K., Miraflips, M., Rothenberg, P. L., White, M. F., and Kahn, C. R. (1992) J. Clin. Invest. 90, 1839–1849
39. Saad, M. J., Folli, F., Kahn, J. A., and Kahn, C. R. (1993) J. Clin. Invest. 92, 2063–2072
40. Valera, A., and Bosch, F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9151–9154
41. Ludwig, D. S., Vidal-Puig, A., O’Brien, R. M., Printz, R. L., Granner, D. K., Moller, D. E., and Flier, J. S. (1996) J. Clin. Endocrinol. Metab. 81, 593–596