EXPERIMENTAL STUDY

Chronic suppression of insulin by diazoxide alters the activities of key enzymes regulating hepatic gluconeogenesis in Zucker rats

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Abstract

Objectives: Chronic attenuation of hyperinsulinemia by diazoxide (DZ), an inhibitor of glucose-mediated insulin secretion, improved insulin sensitivity and glucose tolerance and caused down-regulation of lipid metabolizing enzymes in adipose tissue and decreased the rate of weight gain in mildly hyperglycemic obese Zucker rats. Since the liver plays a central role in glucose homeostasis, we studied the effect of chronic insulin suppression on key insulin-sensitive enzymes regulating hepatic gluconeogenesis.

Methods: DZ (150 mg/kg per day) or vehicle (control) was administered to 7-week-old female obese and lean Zucker rats for a period of 4 weeks.

Results: DZ-treated animals showed lower fasting plasma insulin levels ($P < 0.001$) than their controls. Plasma glucose levels were lower in DZ obese rats than in controls ($P < 0.001$), without a significant change in DZ lean animals. DZ had no effect on glucose transporter 2 protein expression in either strain. DZ treatment resulted in lower hepatic glucokinase ($P < 0.001$) and glucose-6-phosphatase ($P < 0.0001$) and phosphoenolpyruvate carboxykinase (PEPCK) activities only in obese rats compared with controls ($P < 0.001$). However, DZ-treated lean rats demonstrated higher PEPCK activity than controls ($P < 0.002$). DZ-treated animals demonstrated enhanced hepatic glucose-6-phosphate content ($P < 0.01$), glycogen synthase activity ($P < 0.0001$) and glycogen content ($P < 0.02$) compared with their controls despite increased hepatic glycogen phosphorylase activity in these animals ($P < 0.02$).

Conclusions: Chronic suppression of hyperinsulinemia in obese Zucker rats by DZ decreased the activities of key enzymes regulating hepatic gluconeogenesis, implying that attenuation of the hyperinsulinemic state by DZ may be therapeutically beneficial.

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Introduction

Abnormalities in glucose metabolism and insulin resistance are common features of obesity and type 2 diabetes mellitus (DM) in humans and experimental animals but the nature of the mechanisms responsible for these abnormalities remains controversial (1–3). Both impaired glucose utilization by peripheral and hepatic tissue and increased hepatic glucose output are thought to be important contributory factors to these syndromes (4–6). Insulin deficiency in type 1 DM and the insulin resistance in obesity and type 2 DM lead to a decrease in glucose utilization by the liver, muscle, and adipose tissue and to an increase in hepatic glucose production (7, 8).

The liver plays a central role in maintaining glucose homeostasis. Glucose transport and phosphorylation are the first steps in glucose utilization (9). In the non-diabetic state, glucose transporter 2 (Glut-2), a member of the glucose transporter family, provides glucose to hepatocytes at a rate that is 100 times greater than the rate of phosphorylation by glucokinase (GK), a key enzyme in the regulation of glucose utilization (10). Hepatic GK activity is not inhibited by physiological concentrations of glucose-6-phosphate (11) but is regulated mainly due to changes in the transcription of its gene and its regulatory protein (12). Insulin increases and glucagon inhibits GK gene transcription, and these effects are not glucose-dependent (13, 14). The main role of insulin in the activation of glycolytic

genes appears to be the stimulation of GK synthesis leading to increased glucose phosphorylation. Furthermore, increasing glucose concentration increases the amount of free GK, due to translocation of GK from the nucleus to cytoplasm (15). The cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase), which catalyzes the hydrolysis of glucose-6-phosphate (G-6-P) to glucose, are the key enzymes controlling gluconeogenesis and glucose output from the liver (16, 17). The activity of G-6-Pase is markedly regulated by insulin (18) which plays a major role in the long-term suppression of hepatic glucose production in the fed state but appears to have little or no effect on G-6-Pase activity in the fasted state (19). The activity of PEPCK is controlled primarily at the level of transcription, being stimulated by glucagon and inhibited by insulin (17, 20).

Genetically obese Zucker rats are characterized by hyperinsulinemia and peripheral insulin resistance with normal or slightly elevated fasting blood glucose levels (21). Persistent hyperinsulinemia and insulin resistance in obese Zucker rats is believed to increase simultaneously activities of glycolytic and gluconeogenic enzymes even in the fasted state, leading to enhanced glycolysis and lipogenesis (22). Chronic attenuation of hyperinsulinemia by diazoxide (DZ), an inhibitor of glucose-mediated insulin secretion, has been shown to cause enhanced insulin sensitivity in isolated adipocytes, down-regulation of lipid metabolizing enzymes in adipose tissue, improved glucose tolerance and weight reduction in obese Zucker rats (23–25). To investigate the effect of chronic insulin suppression on hepatic glucose metabolism, we measured the Glut-2 protein expression and the activities of key insulin-sensitive enzymes regulating hepatic gluconeogenesis namely GK, G-6-Pase, active glycogen synthase (GSa), active glycogen phosphorylase (GPa) and PEPCK in diazoxide-treated obese and lean Zucker rats.

Research design and methods

Experimental animals, dietary and diazoxide treatment

Seven-week-old female Zucker obese (*fa/fa*) and lean (*Fa/?*) rats were obtained at 6 weeks of age from Charles River Laboratory (Wilmington, MA, USA). The animals were provided with rat chow, Rodent diet 22/5 (W) 8640 (Teklad Harlan Laboratories, Madison, WI, USA), and water available *ad libitum*. Obese and lean rats were divided into two subgroups: DZ treated and control subgroups. Diazoxide (150 mg/kg per day) was administered in two doses daily by gavage using Proglycem suspension (50 mg/ml, kindly provided by Baker-Norton Pharmaceuticals, Miami, FL, USA). The control groups were treated with an equivalent volume of vehicle suspension twice daily. Studies

lasted for a period of 4 weeks. Rats were weighed twice weekly to determine weight gain. Food consumption was measured while animals were in separate metabolic cages during the last two weeks of treatment. At the end of the 4-week period and after an overnight fast (12 h), rats were anesthetized with an intramuscular injection of ketamine (65 to 100 mg/kg body weight (BW)). Blood was drawn into heparinized tubes by cardiac puncture and plasma was frozen. Hepatic tissue was excised and rapidly frozen in liquid nitrogen. Frozen livers were stored in labeled containers at -80°C pending enzyme studies and metabolic determinations. Animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee.

Assays

Plasma glucose and insulin assays Glucose was measured by the glucose oxidase method (Sigma Chemical, St Louis, MO, USA). Insulin concentration was determined by radioimmunoassay using a double-antibody method (LINCO Research, St Louis, MO, USA).

Plasma cholesterol, triglycerides and free fatty acids (FFA) Cholesterol and triglycerides levels were measured by an enzymatic method (Sigma Diagnostics, St Louis, MO, USA). Plasma FFA were determined by an enzymatic colorimetric method (Wako Chemicals, Richmond, VA, USA).

Glucose transporter-2 immunoblots Several different amounts of liver membrane protein (26) for Glut-2 were tested in immunoblotting to optimize the detection system. Protein samples were run on 12%/0.75 mm SDS-PAGE gel and blotted to a PVDF membrane (Millipore, Bedford, MA, USA) using a Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). The membrane was then blocked with 10% non-fat dry milk for Glut-2. The blot was then probed with Glut-2 antibody (a kind gift from S W Cushman, National Institutes of Health, Bethesda, MD, USA) using the Aurora Western Blot Chemiluminescent Detection System (ICN, Costa Mesa, CA, USA). The secondary antibody was a peroxidase-conjugated affinity pure donkey anti-rabbit IgG (Jackson Immuno-Research). The membrane was then exposed to Biomax ML film (Kodak) from 15 s to 2 min. The intensity of the bands was measured by densitometry.

Enzyme assays and metabolites

Glucokinase assay Hepatic GK activity was determined using a continuous spectrophotometric method as previously described (27). Pieces of liver weighing 1000 mg were minced with scissors and homogenized

in a standard buffer. The homogenate was centrifuged and the supernatant was used for GK assay. The GK activity was calculated as the difference between glucose phosphorylation capacity at 100 and 0.5 mmol/l glucose and hexokinase activity as the glucose phosphorylating capacity at 0.5 mmol/l glucose. Protein content of fractions was estimated using the Lowry method (28).

Glucose-6-phosphatase Two methods were used to assay G-6-Pase. In the first, G-6-Pase cleavage by microsomal fractions incubated at 37 °C and pH 7.5 in Tris-HCl buffer was measured by the amount of P_i liberated (29). In studies using liver homogenates and in work with concentrated microsomal membranes, phosphates from the tissue interfered with the assay of G-6-Pase based on P_i release. In these studies, release of [^{14}C]glucose from [^{14}C]G-6-P was measured as previously described (30). β -Glycerophosphate was added to the buffer to inhibit nonspecific phosphatase activity. The specificity of the assay was confirmed by using [^{14}C]glucose-1-phosphate as substrate.

Phosphoenolpyruvate carboxykinase assay The cytosolic fraction from liver homogenates was separated by ultracentrifugation for PEPCK assay. Enzymatic activity was assayed in the reverse direction (carboxylation of phosphoenolpyruvate to form oxaloacetic acid (OAA)) in the presence of deoxyguanosine diphosphate as previously described (31). PEPCK activity is expressed as nmol OAA/min/g liver.

Glycogen synthase assay The GS assay procedure was a modification of a method previously described by Thorburn *et al.* (32). The GS activity was calculated using the method described by Nuttall and Gannon (33). Frozen liver samples were homogenized and enzyme activity was measured by the incorporation of [U- ^{14}C]uridine diphosphate glucose (UDPG) (New England Nuclear-Dupont, Boston, MA, USA) into glycogen in the presence of a low substrate concentration of UDPG for active GS and a high concentration for determination of total GS activity.

Glycogen phosphorylase a assay The GP α activity was assayed in the direction of glycogen synthesis as described by Nuttall *et al.* (34). The assay was conducted at 30 °C and initiated by adding a reaction mixture containing 2[N-morpholino]ethanesulfonic acid (pH 6.3), glucose-1-phosphate, caffeine, glycogen and ^{14}C -labeled glucose-1-phosphate diluted hepatic tissue extract. The reaction was terminated at 20 min by placing aliquots of reaction mixture on the anion-exchange columns, washing the columns with water and counting the eluate. The hepatic GP α enzyme activity is expressed in μ mol of substrate transformed per min per g tissue.

Glucose-6-phosphate, liver glycogen and xylulose-5-phosphate (X-5-P) determinations The hepatic G-6-P content was measured using a previously described method and was expressed as nmol/g liver (35). The glycogen content was measured using the α -amylglucosidase method (36). The values were expressed as mg glycogen/g liver. The hepatic X-5-P was determined as previously described by Casazza and Veech (37). The values were expressed as nmol/g liver.

Statistical analysis

The reported values represent the means $\pm$ s.e. Statistical comparisons between subgroups was assessed by two-way analysis of variance (ANOVA) and Dunnett's and Tukey's tests. A *P* value less than 0.05 was considered statistically significant.

Results

Effect of diazoxide on food intake and body weight

Table 1 shows weight and food intake in obese and lean Zucker rats. Control obese rats exhibited higher initial weight and greater weight gain over the 4-week observation period than lean animals (*P* < 0.001). Final body weight and average weight gain among DZ

Table 1 Weight and food intake data in obese and lean Zucker rats. Data are means $\pm$ s.e.

Subgroup	<i>n</i>	Initial weight (g)	Final weight (g)	Weight gain (per 100 g BW)	Cal/day (per 100 g BW)
DZ obese	8	203 $\pm$ 4	274 $\pm$ 6	26 $\pm$ 3 ^a	28 $\pm$ 3 ^a
C obese	8	210 $\pm$ 5	335 $\pm$ 6	38 $\pm$ 2	47 $\pm$ 2
DZ lean	8	144 $\pm$ 1	182 $\pm$ 2	21 $\pm$ 1	31 $\pm$ 2
C lean	8	148 $\pm$ 2	196 $\pm$ 3	24 $\pm$ 1	34 $\pm$ 2
<i>P</i> value					
DZ vs C		NS	< 0.001	< 0.001	< 0.001
Obese vs lean		< 0.001	< 0.001	< 0.001	< 0.001

Data were analyzed by two-way ANOVA.

^aDZ obese vs C obese.

NS, not significant.

obese animals was reduced compared with control obese rats ($P < 0.001$), whereas DZ treatment had no effect on the rate of weight gain in lean animals. When food intake was measured during the treatment period, control obese rats consumed larger amounts of food than control lean animals ($P < 0.01$). DZ obese rats demonstrated a significant reduction in food intake compared with control obese rats ($P < 0.01$) while DZ had little effect on food intake in lean animals.

Effect of diazoxide on plasma glucose, lipids and insulin

Table 2 shows fasting plasma levels of glucose, insulin, FFA, cholesterol and triglycerides, following 4 weeks of DZ or vehicle treatment. Fasting plasma glucose and insulin concentrations were significantly higher among control obese animals compared with lean rats ($P < 0.001$). Fasting plasma levels of glucose, insulin, FFA and triglycerides were significantly decreased in DZ obese animals compared with controls ($P < 0.001$). In lean animals, however, only plasma FFA ($P < 0.001$) and insulin ($P < 0.001$) were significantly decreased by DZ treatment.

Effect of diazoxide treatment on Glut-2

Figure 1 shows Western blot analysis of hepatic Glut-2 in obese and lean Zucker animals. There was no significant difference in Glut-2 protein between obese and lean controls. DZ treatment appeared to have no significant effect on Glut-2 protein levels in either obese or lean rats compared with their controls.

Effect of diazoxide treatment on hepatic GK, G-6-Pase, PEPCK and X-5-P

Figure 2 illustrates activities of key glycolytic and gluconeogenic enzymes and hepatic X-5-P in fasted obese and lean animals. Control obese rats had higher GK activity than control lean animals ($P < 0.0001$). Similarly, control obese animals demonstrated higher G-6-Pase activity than control lean rats ($P <$

Figure 1 Hepatic Glut-2 from plasma membrane preparations in the obese (Ob) (DZ, diazoxide; C, control) and lean (Ln) (DZ, diazoxide; C, control) Zucker rats. (A) A representative immunoblot containing samples from each of the four treatment groups. (B) Illustration of relative intensity of the Glut-2 band in each lane normalized to that of the standard sample. Data are means $\pm$ S.E.

0.01). DZ treatment lowered GK and G-6-Pase activities in obese animals compared with their controls ($P < 0.001$) without a significant effect in lean rats.

Obese animals had higher PEPCK activity than lean rats ($P < 0.001$). While DZ treatment resulted in significant reduction of PEPCK activity in obese rats compared with controls ($P < 0.001$), it increased PEPCK activity in lean animals ($P < 0.002$).

Obese animals demonstrated significantly higher liver X-5-P content than lean animals ($P < 0.001$) in the fasting state. However, DZ-treated animals did not demonstrate any significant change in their hepatic X-5-P content compared with their respective controls.

Table 2 Biochemical data in obese and lean Zucker rats. Data are means $\pm$ S.E.

Subgroup	n	Plasma glucose (mmol/liter)	Plasma insulin (pmol/ml)	Plasma FFA (meq/liter)	Plasma cholesterol (mmol/liter)	Plasma triglycerides (mmol/liter)
DZ obese	8	6.43 $\pm$ 0.11 ^a	0.33 $\pm$ 0.02	0.79 $\pm$ 0.12	2.45 $\pm$ 0.20	4.50 $\pm$ 0.53 ^a
C obese	8	8.91 $\pm$ 0.22	0.69 $\pm$ 0.04	1.51 $\pm$ 0.13	2.55 $\pm$ 0.14	9.23 $\pm$ 1.29
DZ lean	8	5.83 $\pm$ 0.17	0.13 $\pm$ 0.03	0.21 $\pm$ 0.05	1.69 $\pm$ 0.13	1.61 $\pm$ 0.33
C lean	8	6.49 $\pm$ 0.33	0.21 $\pm$ 0.02	0.46 $\pm$ 0.06	1.75 $\pm$ 0.11	1.54 $\pm$ 0.27
P value						
DZ vs C		<0.001	<0.001	<0.001	NS	<0.001
Obese vs lean		<0.001	<0.001	<0.001	<0.01	<0.001

Data were analyzed by two-way ANOVA.

^aDz obese vs C obese.

NS, not significant.

Effect of diazoxide treatment on hepatic GS, GP_a, G-6-P and glycogen content

Figure 3 illustrates the activity of GS and GP_a and hepatic G-6-P and glycogen contents. Obese animals exhibited higher GS (active, a) ($P < 0.0001$) activity, liver G-6-P ($P < 0.001$) and glycogen contents ($P < 0.0001$) than control lean rats. Similarly, hepatic GP_a activity was higher in control obese than in lean rats ($P < 0.0001$) in the fasting state. DZ treatment increased GS_a activity ($P < 0.0001$), hepatic G-6-P ($P < 0.01$) and glycogen contents ($P < 0.02$) in obese and lean animals compared with their respective controls despite a significant increase in GP_a activity in obese ($P < 0.02$) and lean ($P < 0.001$) rats.

Discussion

Consistent with our previous studies, DZ treatment of hyperinsulinemic obese Zucker rats decreased insulin secretion, food intake and rate of weight gain and this was accompanied by decreased plasma concentrations of glucose, FFA and triglycerides (23–25). DZ treatment resulted in suppression of the activities of glycolytic and gluconeogenic enzymes in hyperinsulinemic obese rats. The activities of GK, G-6-Pase, and PEPCK were suppressed which led to increased hepatic glycogen synthesis. *In vitro* and *in vivo* studies have shown that increasing concentrations of insulin not only alters insulin receptor affinity but also down-regulates the number of insulin receptors as the chronic hyperinsulinemic state develops (38–41). The liver of obese Zucker rats exhibits an abnormal regulation of glucose production, together with a glycolysis that is continuously overstimulated by hyperinsulinemia and never becomes insulin-resistant (42). This results in a significant increase in hepatic lipid synthesis and increase in very low-density lipoprotein (VLDL) secretion in obese animals (43). As adipose tissue lipoprotein lipase activity always remains higher than normal in obese rats (44), increased VLDL is accompanied by increased triglyceride accumulation within adipocytes leading to an unabated lipogenic state and obesity (43). While DZ lowers the rate of weight gain, in part by its anorectic effect in obese animals, it is also believed to exert its anti-obesity effect by down-regulating fatty acid synthase activity and lipoprotein lipase gene expression in adipose tissue (45), the key enzymes regulating lipogenesis. The DZ effect on fatty acid synthase activity is

Figure 2 Activities of hepatic GK, G-6-Pase, and PEPCK and hepatic X-5-P content in the obese (Ob) (DZ, diazoxide; C, control) and lean (Ln, L) (DZ, diazoxide; C, control) Zucker rats. Data are expressed as means $\pm$ S.E. * $P < 0.001$ or * $P < 0.0001$ (DZ obese vs C obese); ** $P < 0.002$ (DZ lean vs C lean); + $P < 0.01$, + $P < 0.001$, or + $P < 0.0001$ (C obese vs C lean).

Figure 3 Activities of hepatic GSα and GPα along with hepatic G-6-P and glycogen contents in the obese (Ob) (DZ, diazoxide; C, control) and lean (Ln, L) (DZ, diazoxide; C, control) Zucker rats. Data are expressed as means±S.E. *P<0.02, *P<0.01, *P<0.001 or *P<0.0001 (DZ obese vs C obese); **P<0.02, **P<0.01, **P<0.001 or **P<0.0001 (DZ lean vs C lean); +P<0.001 or +P<0.0001 (C obese vs C lean).

believed to be, in part, due to its direct modulation of intracellular Ca^{2+} through K^{+} -ATP channels (45).

Decreased insulin-stimulated glucose uptake is a characteristic of insulin resistance in obesity and diabetes (46) and defects in the glucose transport system could represent a significant contribution to the mechanisms of insulin resistance in liver and muscle (47, 48). The obese Zucker rat does not normally develop overt diabetes and is a better model of impaired glucose tolerance than diabetes (21). In agreement with Sliker *et al.*, we did not observe any differences in hepatic Glut-2 protein levels between lean and obese animals (49). DZ treatment did not significantly affect hepatic Glut-2 levels in obese or lean animals.

Similar to previous studies, obese rats demonstrated higher GK activity and hepatic G-6-P than lean animals, presumably due to their prevailing hyperinsulinemia (42, 50). On the other hand, insulin suppression of hepatic G-6-Pase is believed to be less efficient in the insulin-resistant state which results in increased substrate flux through this enzyme constituting a key feature of type 2 DM (51). In our study, mildly hyperglycemic obese animals demonstrated higher G-6-Pase activity than the lean rats in the fasted state which was a consequence of enhanced enzyme induction by higher circulating glucose concentrations (52, 53). DZ suppression of circulating insulin in obese rats was associated with decreased GK activity due to reduced induction by insulin. However, the reduction in G-6-Pase activity in DZ obese animals was probably due to enhanced insulin sensitivity and decreased glycemia resulting in attenuation of G-6-Pase induction. Recently, Aoki *et al.* demonstrated that treatment of hyperinsulinemic and insulin-resistant diabetic db/db mice and their heterozygote (db/db/+m) littermates with troglitazone, an insulin sensitizer, resulted in decreased plasma insulin and glucose levels and hepatic G-6-Pase activity (54). This was associated with increased hepatic G-6-P and glycogen content and GSα activity suggesting increased hepatic glucose uptake and glycogen synthesis. These findings support a tight correlation between hepatic glycogen content and G-6-Pase activity at any given time as previously shown (55) and are consistent with our observations in DZ obese rats. On the other hand, the lack of a DZ effect on GK and G-6-Pase activities in lean rats, despite the increase in GS activity and glycogen content, is probably due to opposing effects of enhanced hepatic glucose uptake and mild hyperglycemia (23, 24) on these enzymes.

It is well established that the net glycogen accumulation is the result of the balance between its synthesis by GS and its degradation by GPα (56). In our study, the hepatic GPα activity was higher in obese than in lean animals as previously shown by Aiston *et al.* (57). It has been suggested that enhanced glycogenolysis resulting from elevated GPα levels might be a consequence of hepatic insulin resistance or of dysregulation

of insulin counter-regulatory hormones (42). Nevertheless, the net glycogen accumulation is believed to be increased due to enhanced insulin-induced phosphorylase phosphatase activity which results in inactivation of GP_a in obese rats (58). DZ suppression of insulin in obese and lean animals not only augmented hepatic GS activity and glycogen content but also increased GP_a activity which indicates an appropriate metabolic response to enhanced glycogen synthesis and reduced circulating insulin levels in these animals. These metabolic effects of DZ on enzymes regulating glycogen metabolism are believed to be mediated through changes in circulating insulin levels and insulin sensitivity. On the other hand, a direct effect of DZ on hepatic glucose metabolism, although possible, does not appear likely. For instance, it has been shown that high hepatic GP_a activity may be due to persistently active phosphorylase kinase which may be maintained by high concentrations of intracellular Ca²⁺ in the obese state (59, 60). Since DZ is a K⁺-ATP channel agonist it decreases cellular Ca²⁺ influx (61), and therefore it is expected to result in an opposite effect by decreasing instead of increasing hepatic GP_a activity.

The ability of liver cells to sense glucose and to coordinately modify the gene expression of several key enzymes may represent an important feedback control system in response to sustained changes in the rate of glucose flux and/or metabolism. Indeed, the expression of glycolytic and lipogenic enzymes is increased whereas that of a pivotal gluconeogenic enzyme, PEPCK, is decreased by glucose. While in the non-diabetic state PEPCK expression is suppressed with increasing glucose concentration, elevated PEPCK activity is observed in the insulin-resistant state (type 2 DM) (62). This glucose-sensing mechanism appears to be impaired in obese Zucker rats which demonstrate higher PEPCK activity and hepatic glucose production than lean rats (21). However, Perez *et al.* demonstrated that GK mRNA of obese rats remained elevated after 24 h of food deprivation and this was associated with a compensatory increase in hepatic PEPCK mRNA (22). They suggested starvation-induced changes in regulatory enzymes might be due to the hyperinsulinemic state which can lead to persistent stimulation of glycolysis. Although DZ treatment is shown to have an anorectic effect only in obese rats, DZ suppression of hyperinsulinemia in obese animals decreased PEPCK activity probably due to an enhanced inhibitory effect of insulin. On the other hand, DZ-induced hypoinsulinemia in lean rats increased PEPCK activity. Massillon *et al.* have suggested that PEPCK gene expression may also be regulated by intrahepatic glucose metabolism at or beyond G-6-P and by a pentose entering the nonoxidative branch of the pentose phosphate pathway (63). However, we found that higher hepatic G-6-P and pentose, X-5-P, in obese rats appeared to have no apparent inhibitory effect on PEPCK activity compared with lean animals. While

DZ treatment increased hepatic G-6-P content, it had no significant effect on hepatic X-5-P content and it resulted in diverse effects on hepatic PEPCK activity in these animals. These findings suggest that circulating insulin levels probably play a greater role in the regulation of PEPCK than hepatic G-6-P content and products of the nonoxidative branch of the pentose pathway. In support of our observations in DZ obese rats, Carr *et al.* recently demonstrated that DZ suppression of hyperinsulinemia in obese Zucker rats enhanced insulin sensitivity and reduced hepatic glucose production in DZ-treated obese Zucker rats by utilizing a hyperinsulinemic euglycemic clamp (64).

In conclusion, attenuation of hyperinsulinemia by DZ in obese Zucker rats resulted in reduced food intake, decreased rate of weight gain and improved glucose tolerance. These effects were accompanied by suppression of hepatic gluconeogenic activity and enhancement of glycogen synthesis, implying that modulation of hyperinsulinemia and insulin resistance by DZ may be therapeutically beneficial in obesity. However, further studies are indicated to assess the effect of short-term DZ treatment on hepatic glucose metabolism during fasting and refeeding in obese and lean Zucker rats.

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