Antiobesity Effect of Diazoxide in Obese Zucker Rats

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Hyperinsulinism and insulin resistance are characteristic findings in obese subjects. Obesity in both humans and experimental animals is associated with a reduced number of insulin receptors and a decreased insulin-mediated glucose disposal, whereas sensitivity to insulin's antilipolytic action is unaltered. To evaluate the antiobesity effect of diazoxide (DZ), an inhibitor of glucose-stimulated insulin release, 7-week-old Zucker obese and lean rats were studied. Obese and lean rats were grouped into DZ-treated (150 mg/kg/d) and control (C) groups. DZ-treated obese rats consumed similar amounts of calories per kilogram body weight (BW) compared with C obese animals, but gained less weight ($P < .01$). Postabsorptive plasma free fatty acids (FFA), cholesterol, and triglycerides were significantly higher in obese versus lean animals ($P < .01$). DZ treatment reduced plasma triglyceride levels in obese animals ($P < .001$), but had no significant effect on FFA or cholesterol concentrations. Plasma glucose concentrations in the postabsorptive state and during glucose tolerance tests (GTTs) were significantly lower in DZ obese versus C obese rats ($P < .01$) despite a decrease in plasma insulin concentrations in DZ-treated animals ($P < .01$). In contrast, DZ lean rats developed glucose intolerance ($P < .05$). Sensitivity and responsiveness to the antilipolytic effect of insulin in isolated adipocytes were significantly decreased in DZ obese as compared with C obese rats ($P < .01$). Moreover, adipocyte specific insulin receptor binding was increased in both DZ lean and DZ obese animals ($P < .01$). This was accompanied by increased basal and insulin-stimulated glucose transport in both genotypes ($P < .01$). In conclusion, DZ increased insulin receptor binding and glucose transport while decreasing hyperinsulinemia and insulin sensitivity to the antilipolytic action of insulin. This combined effect resulted in improved glucose tolerance and a decrease in weight gain in obese rats, implying that pharmacologic modification of the disturbed insulin metabolism of obesity may be therapeutically beneficial.

INCREASED INSULIN secretion is a characteristic feature of obesity syndromes in humans and animals.[1-3] Enhanced insulin secretion occurs before the emergence of insulin resistance[4-6] and appears to play a major pathogenic role in the development of obesity.[7,8] In young preobese Zucker rats, hyperinsulinemia already exists, corresponding to the view that excessive insulin secretion precedes insulin resistance.[9] In these animals, persistent hyperinsulinemia leads to an enhanced lipogenic state by 15 weeks of age.[10] Moreover, it has been shown that insulin secretion increases as the degree of obesity increases,[11,12] and is accompanied by varying degrees of resistance to insulin-mediated glucose disposal.[13-15] In both humans and experimental animals, diminished sensitivity of skeletal muscle and adipose tissue to insulin-stimulated glucose uptake and metabolism is associated with a decreased number of insulin receptors.[16-19]

It is generally assumed that since fat deposition is so persistent in obese individuals, sensitivity to insulin's antilipolytic action is maintained. Forearm perfusion studies suggest that insulin inhibition of adipocyte free fatty acids (FFA) release is not impaired in obesity.[20] Further, with the euglycemic clamp technique, morbidly obese subjects who demonstrate marked resistance to the glycoregulatory action of insulin show only minimal resistance to the antilipolytic action of insulin.[21] This is supported by observations that adipocytes from lean and obese individuals show little resistance to insulin inhibition of lipolysis, and that the antilipolytic action of insulin occurs at lower insulin concentrations than its glycoregulatory effects.[22] These observations suggest that maintenance of insulin's antilipolytic action is a crucial factor in the maintenance of fat deposition in obese individuals. If this is so, one would expect that by decreasing circulating insulin levels, the tendency for lipogenesis and continued weight gain in obese subjects would be diminished.

We recently demonstrated that attenuation of hyperinsulinemia in obese Zucker rats by diazoxide (DZ) treatment resulted in a decreased rate of weight gain and an increased adipocyte insulin receptor binding.[23] The reduced rate of weight gain was believed to be due to a decrease in insulin-induced lipogenesis. The present study specifically examined the effect of long-term DZ treatment on the rate of weight gain in relationship to serum lipids, rate of lipolysis, glucose transport, and insulin receptor binding in isolated adipocytes from obese and lean Zucker rats.

MATERIALS AND METHODS

Seven-week-old female Zucker obese (fa/fa) rats weighing 190 to 246 g and female Zucker lean (Fa/Fa) rats weighing 123 to 156 g were used in this study. Animals were obtained at 6 weeks of age from Charles River Laboratory (Wilmington, MA). They were phenotyped on the basis of body weight (BW) at 4 weeks of age. The animals were housed in pairs in standard animal cages, and were provided standard RMH 3000 rat chow (Agway, Syracuse, NY) and water ad libitum. Obese and lean rats were divided into two subgroups of six animals per group: DZ-treated and control (C) subgroups. DZ (150 mg/kg/d) was administered in two doses daily by gavage using Proglycem pediatric suspension 50 mg/mL (kindly provided by Baker-Norton Pharmaceuticals, Miami, FL). C groups were treated with an equivalent volume of vehicle suspension twice daily. Studies lasted for a period of 6 weeks. Rats were weighed twice weekly to determine weight gain. Food consumption

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was measured while animals were housed in separate metabolic cages during the last week of treatment.

At the end of the 6-week period, intraperitoneal glucose tolerance tests (IP-GTTs) were performed after an overnight fast (12 hours). Rats were anesthetized with an intramuscular injection of ketamine (65 to 100 mg/kg BW). Blood was drawn into heparinized tubes from the supraorbital sinus. Baseline blood samples were analyzed for glucose, insulin, cholesterol, triglycerides, and FFA levels. Following baseline measurement, animals were administered 1 g glucose/kg BW IP, followed by repeat blood collection at 15, 30, and 60 minutes. One to 2 days following GTT, animals were fasted overnight (12 hours) and killed, and omental fat was harvested for preparation of isolated adipocytes. Animal procedures were reviewed and approved by the University of Tennessee Medical Center Animal Care Committee.

Assays

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

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

Isolation of fat cells. Adipocytes were isolated by the method of Rodbell as modified by Pedersen et al.[24] Omental fat tissue (10 to 20 g) was cut into small pieces (1 to 2 mm) and digested with collagenase (0.5 mg/mL, type I CLS; Worthington, Freehold, NJ) in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 mmol/L glucose for 60 minutes at 37°C and 150 rpm in an orbital water bath. Cells were filtered through polyethylene mesh (230 to 400 μm) and washed with 4% bovine serum albumin (BSA)–Krebs-Ringer bicarbonate buffer.

Fat cell number, size, and viability. A 5-μL sample of cell suspension (10% to 20% lipocrit) was placed on the grid of a Fuchs-Rosenthal cell-counting chamber turned upside down. The diameter of the whole cell population was measured at 200× magnification using an eyepiece micrometer. Cell surface area ($D^2\pi$) was calculated using a previously described method.[25,26] Adipocyte viability was determined by trypan blue exclusion (0.1% wt/vol) and was greater than 95% in all preparations.

Lipolysis in adipocytes. Isolated fat cells were incubated at a final concentration of approximately 200,000/ml at 37°C for 2 hours in 4% BSA–Krebs-Ringer buffer containing 10 mmol/L glucose and insulin (0 to 5.33 pmol/mL) at a final volume of 500 μL. Each insulin concentration was run in triplicate. After incubation, glycerol concentration in cell-free aliqouts of medium was determined using a modified colorimetric assay[27] and enzyme-linked immunosorbent assay plate reader (Boehringer, Mannheim, Germany). The detection limit was 0.3 μmol glycerol/L.

[^{125}I]insulin binding to adipocytes. Four hundred microliters of a freshly isolated adipocytes suspension (~200,000 to 350,000 cells/mL) was incubated for 1 hour at 22°C with [^{125}I]insulin (0.1 pmol/mL) in the absence and presence of varying concentrations of unlabeled insulin (0.02 to 5.33 pmol/mL) in a total volume of 500 μL. Triplicate samples were then centrifuged through silicone oil, and radioactivity of the supernatant cells was determined. Nonspecific binding was measured in the presence of 3.34 nmol/mL unlabeled insulin and was less than 2.0% of the total. Specific binding was defined as the difference between total and nonspecific binding and was normalized to cell surface area.[28] Binding affinity and capacity were determined by two-site Scatchard analysis using the National Institutes of Health Ligand program (Ligand PC version 3.1).[29,30]

Glucose transport in adipocytes. Isolated fat cells (~200,000 cells/mL) were preincubated for 1 hour at 37°C in the absence and presence of varying insulin concentrations (0.02 to 5.33 pmol/mL) in 500 μL 4% BSA–Krebs-Ringer bicarbonate buffer. Adipocyte glucose uptake was then determined in triplicate by adding ^{14}C-deoxyglucose (100 nmol). At 3 minutes, transport was terminated by addition of 400 μL ice-cold 4% BSA–Krebs-Ringer buffer followed by rapid centrifugation through silicone oil. Cell-associated ^{14}C-radioactivity was determined by liquid scintillation counting. Extracellular labeled glucose level was measured at time zero by immediate addition of ice-cold stop buffer. All values were corrected for extracellularly trapped radioactivity, which averaged 3.3% ± 1.6% at a concentration of 2×10^5 cells/mL. Glucose transport was normalized to adipocyte cell surface area at each insulin concentration.

Chemicals

Crystalline porcine insulin and bovine albumin (fraction V) radioimmunoassay grade were obtained from Sigma. Mono ^{125}I-(Tyr^{A14}) insulin (100 μCi/μg) and D-[U-^{14}C]glucose (2.4 mCi/mmol) were obtained from New England Nuclear-Dupont (Boston, MA).

Statistical Analysis

The reported values represent the mean ± SEM. Statistical analysis of subgroups was performed by one-way ANOVA, with significant differences between means determined by post-hoc analysis using Dunnett's multiple range test at P less than .05.

RESULTS

BW, Caloric Intake, and Fat Cell Size

C obese rats had a higher initial weight and greater weight gain over the 6-week observation period than lean animals (Table 1). Final BW (Table 1) and average weekly weight gain (Fig 1) among DZ obese animals was decreased as compared with C obese rats ($P < .01$), whereas DZ treatment had no effect on rate of weight gain in lean

Table 1. Clinical Data in Obese and Lean Zucker Rats

Subgroup	No.	Weight (g)		Weight Gain (per 100 g BW)	Daily Calories (per 100 g BW)	Adipocyte Diameter (μm)
		Initial	Final			
DZ obese	6	221 ± 11	297 ± 18*†	25 ± 4*	26 ± 6	96 ± 3*
C obese	6	223 ± 11†	362 ± 14†	39 ± 2†	25 ± 7	127 ± 3†
DZ lean	6	141 ± 6	197 ± 11	28 ± 2	33 ± 4	65 ± 2
C lean	6	146 ± 3	207 ± 4	29 ± 1	37 ± 4	69 ± 2

NOTE. Data are the mean ± SEM and were analyzed by one-way ANOVA.

*Significantly different from strain control (DZ *v* C), $P < .01$.

†Significantly different from C lean (C obese *v* C lean), $P < .001$.

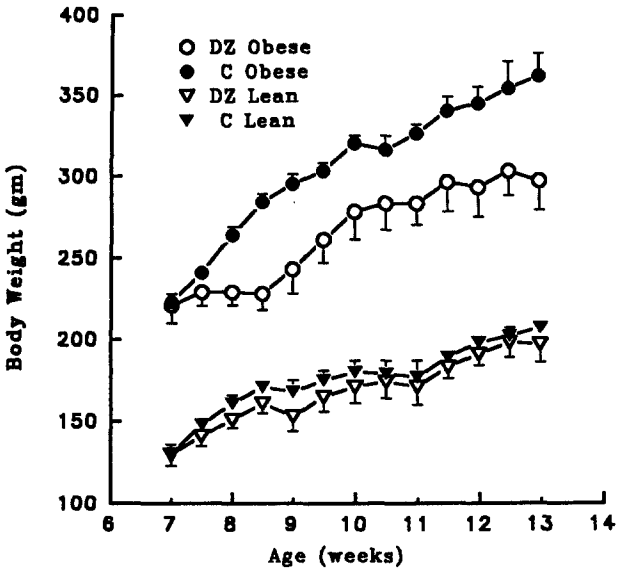

Fig 1. BW (mean ± SEM) in DZ and C obese and lean rats (n = 6 for each subgroup).

animals. When food intake was measured after 6 weeks of treatment, DZ-treated animals consumed similar amount of calories per kilogram BW compared with their respective controls. The diameter of isolated adipocytes in DZ-treated obese rats was lower than in C obese animals ($P < .001$), whereas DZ had no effect on adipocyte diameter in lean animals (Table 1).

Plasma Lipids, Glucose, and Insulin

Table 2 shows postabsorptive plasma levels of FFA, cholesterol, triglycerides, glucose, and insulin following 6 weeks of C or DZ treatment. Postabsorptive plasma glucose and insulin concentrations were significantly higher among C obese animals compared with C lean rats. Postabsorptive plasma levels of triglycerides, glucose, and insulin were significantly decreased in DZ obese ($P < .001$) but not in DZ lean animals. Plasma FFA and cholesterol concentrations were not significantly affected by DZ treatment in either obese or lean animals.

Plasma glucose and insulin responses to IP-GTT in obese and lean animals are shown in Fig 2. DZ obese rats showed significantly lower plasma glucose levels during IP-GTT ($P < .001$) as compared with C obese animals. On the other hand, DZ lean animals displayed elevated plasma glucose indicative of glucose intolerance when compared with lean C rats ($P < .05$). Plasma insulin concentrations were significantly lower in DZ obese and DZ lean animals as compared with their respective controls.

Basal Lipolysis

After an overnight fast, the basal rate of lipolysis was markedly higher in isolated adipocytes from C obese animals versus C lean animals ($P < .001$; Fig 3). DZ treatment reduced basal glycerol release by isolated adipocytes from obese rats (61 ± 3.2 *v* 80 ± 5.0 μmol/10^7 cells/2 h, $P < .001$), but had no effect in lean animals (33 ± 3.0 *v* 35 ± 3.5 μmol/10^7 cells/2 h).

Sensitivity of the Antilipolytic Effect of Insulin

Dose-response curves for insulin-dependent inhibition of glycerol release are shown in Fig 4. Isolated adipocytes demonstrated greater sensitivity to insulin-mediated inhibition of glycerol release in obese versus lean animals (ED_{50}, 0.04 ± 0.01 *v* 0.23 ± 0.03 pmol/mL, $P < .001$). DZ treatment resulted in a right-shifted insulin dose-response curve in obese animals (ED_{50}, 0.04 ± 0.01 to 0.09 ± 0.01 pmol/mL, $P < .02$) while having no effect in lean animals (ED_{50}, 0.28 ± 0.06 to 0.23 ± 0.03 pmol/mL).

Responsiveness of the Antilipolytic Effect of Insulin

As illustrated by the mean dose-response curves in Fig 4, maximum insulin-dependent antilipolysis is greater in C obese animals than in C lean rats ($P < .001$). Furthermore, adipocytes from DZ obese animals showed a significant reduction in maximal responsiveness to insulin inhibition of lipolysis versus those from C obese rats (15.4 ± 2.1 *v* 22.9 ± 1.5 μmol/10^7 cells/2 h, $P < .02$). No difference in maximal responsiveness to insulin inhibition of lipolysis was observed in DZ lean versus C lean animals (10.6 ± 0.77 *v* 11.8 ± 1.9 μmol/10^7 cells/2 h).

Insulin Receptor Binding

Figure 5 shows competition curves and curvilinear Scatchard plots of isolated adipocyte insulin binding from obese and lean animals. Obese rats showed significantly lower specific insulin receptor binding than lean rats, consistent with receptor downregulation by hyperinsulinemia (Table 2). Insulin receptor binding in both DZ obese and DZ lean animals was higher than in their respective C rats. When insulin binding data were analyzed using a two-site model, C lean rats demonstrated higher-affinity constants for both high- and low-affinity receptors as

Table 2. Biochemical Data in Obese and Lean Zucker Rats

Subgroup	No.	Postabsorptive Plasma FFA (mEq/L)	Postabsorptive Plasma Cholesterol (mmol/L)	Postabsorptive Plasma Triglycerides (mmol/L)	Postabsorptive Plasma Glucose (mmol/L)	Postabsorptive Plasma Insulin (pmol/mL)
DZ obese	6	0.98 ± 0.19	2.61 ± 0.22	4.30 ± 0.59*	6.83 ± 0.22*	1.20 ± 0.3*
C obese	6	1.31 ± 0.23†	2.64 ± 0.17†	9.26 ± 1.33†	9.72 ± 0.72†	3.19 ± 0.31†
DZ lean	6	0.60 ± 0.09	1.75 ± 0.12	1.48 ± 0.27	6.50 ± 0.36	0.21 ± 0.02*
C lean	6	0.57 ± 0.08	1.74 ± 0.11	1.41 ± 0.14	6.27 ± 0.51	0.36 ± 0.04

NOTE. Data are the mean ± SEM and were analyzed by one-way ANOVA.

*Significantly different from strain control (DZ *v* C), $P < .001$.

†Significantly different from lean animals (C obese *v* C lean), $P < .001$.

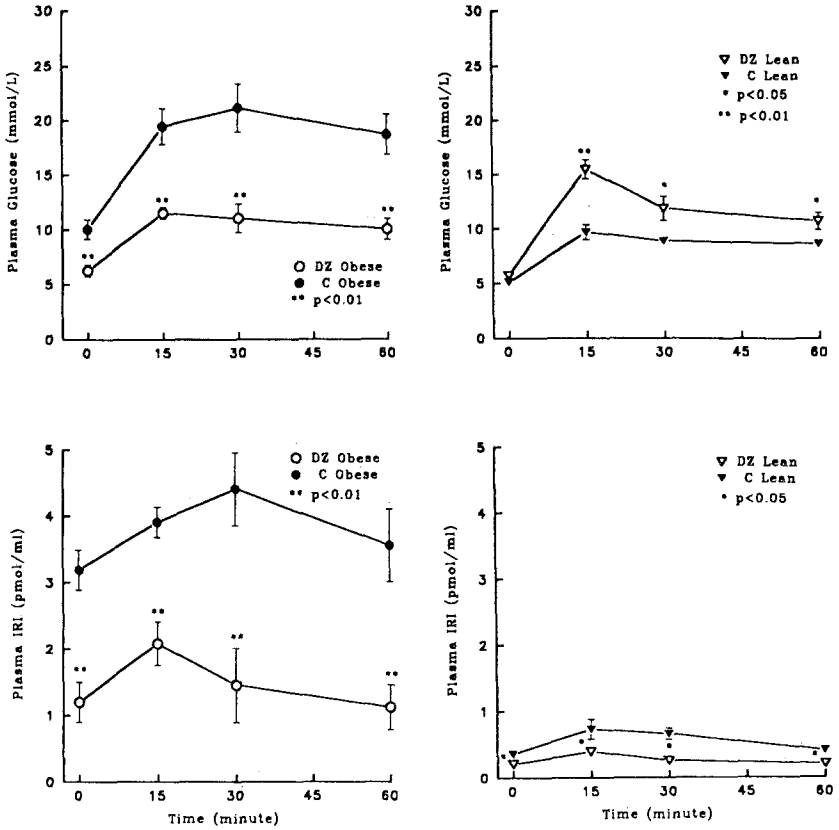

Fig 2. Plasma glucose and insulin (IRI) responses (mean ± SEM) to IP-GTT (1 g/kg) in DZ and C obese rats and lean rats after an overnight fast (n = 6 for each subgroup).

compared with obese C rats (Table 3). DZ treatment modestly increased the affinity constants of high-affinity receptors (K_1) in DZ obese rats versus the C obese subgroup ($P < .01$), and doubled the affinity constant in DZ lean animals as compared with their C subgroup ($P < .005$). DZ treatment also increased low-affinity (K_2) binding constants in obese but not in lean animals. Lean animals showed higher binding capacity of the high-affinity population of receptors (R_1) as compared with C obese rats. DZ obese and DZ lean animals demonstrated greater high-affinity binding capacity (R_1) than their respective controls ($P < .001$). Similarly, binding capacity of low-affinity receptor sites (R_2) was significantly increased in DZ-treated obese and lean animals.

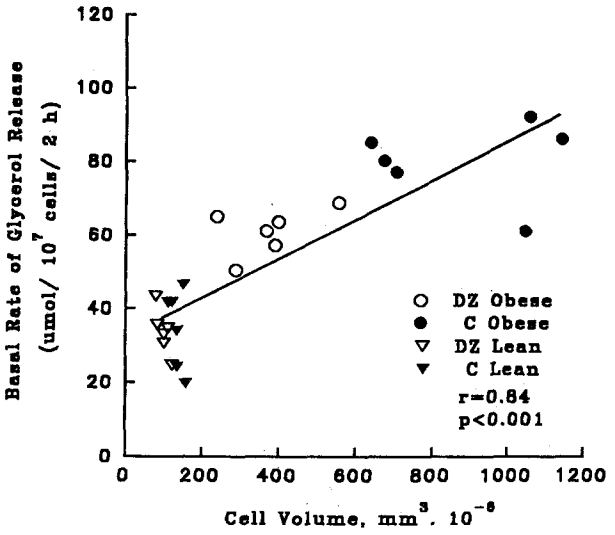

Fig 3. Relationship between basal rate of glycerol release and cell volume of isolated adipocytes from obese and lean rats (DZ and C).

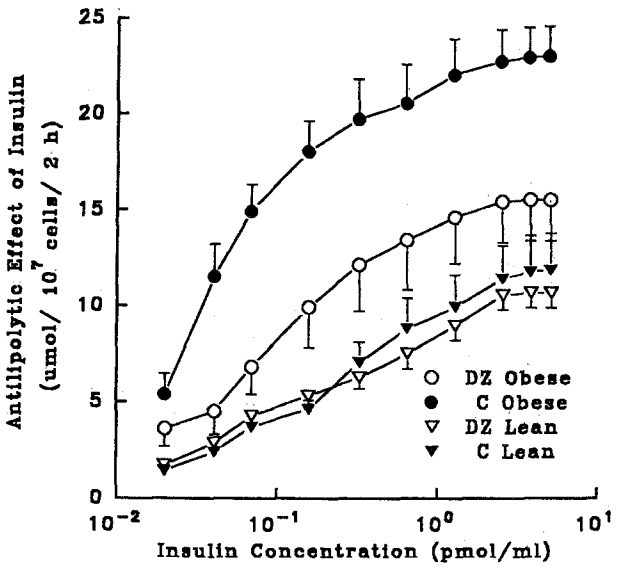

Fig 4. Dose-response curves for insulin-dependent inhibition of lipolysis in isolated adipocytes of obese (DZ *v* C, $P < .02$) and lean (DZ *v* C, p = NS) rats.

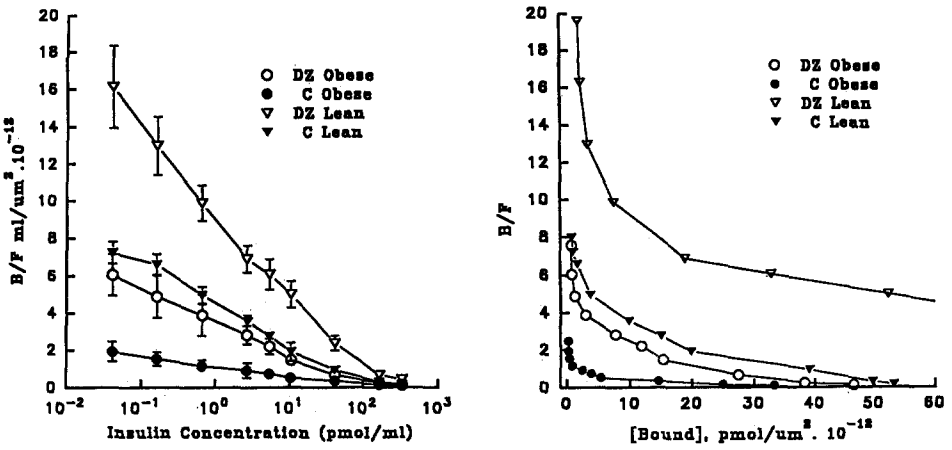

Fig 5. Specific insulin binding (expressed as fat cell surface area) to isolated adipocytes of obese and lean Zucker rats. Binding data are presented as competition plots (left) and scatchard plots (right). B/F, bound/free ratio. Data are the mean ± SEM for competition plots only. DZ *v* C obese, $P < .001$; DZ *v* C lean, $P < .01$.

Glucose Transport

Both basal (0.11 ± 0.01 *v* 0.19 ± 0.02 nmol/$\mu m^2 \cdot 10^{-12}$/min, $P < .01$) and maximal insulin-stimulated (0.25 ± 0.03 *v* 0.43 ± 0.02, $P < .01$) glucose transport rates in adipocytes were significantly lower in obese versus lean rats, respectively. In obese animals, DZ treatment increased basal (from 0.11 ± 0.01 to 0.16 ± 0.01 nmol/$\mu m^2 \cdot 10^{-12}$/min, $P < .01$) and insulin-stimulated (from 0.25 ± 0.03 to 0.41 ± 0.03 nmol/$\mu m^2 \cdot 10^{-12}$/min, $P < .01$) glucose transport. Similarly, DZ treatment increased basal (from 0.19 ± 0.02 to 0.34 ± 0.03 nmol/$\mu m^2 \cdot 10^{-12}$/min, $P < .01$) and insulin-stimulated (from 0.43 ± 0.02 to 0.58 ± 0.05 nmol/$\mu m^2 \cdot 10^{-12}$/min, $P < .01$) values in lean animals. Furthermore, the insulin ED_{50} for glucose transport rate was shifted to the left in DZ-treated versus C obese (0.12 ± 0.02 *v* 0.28 ± 0.03 pmol/mL, $P < .002$) and lean (0.063 ± 0.01 *v* 0.11 ± 0.01 pmol/mL, $P < .005$) animals (Fig 6).

Since insulin binding and glucose transport are primarily membrane processes, insulin receptor binding and glucose transport were expressed per cell surface area. On the other hand, while an increasing basal rate of lipolysis reflects the increasing cellular surface area, as well as surface area of the cell's triglyceride storage droplet, we expressed our data per 10^7 cells, because lipolysis and its regulation by insulin are a cellular phenomenon and are not restricted to the fat cell plasma membrane.

DISCUSSION

The present study demonstrated that DZ treatment led to decreased adipocyte sensitivity to the antilipolytic effect of insulin in obese rats, which was accompanied by decreased plasma triglycerides and weight gain in obese animals. In these same animals, increased adipocyte insulin receptor binding and increased adipocyte glucose transport rate were observed in both DZ-treated obese and lean Zucker rats. Consistent with our previous study, the effect of DZ on blood glucose tolerance differed significantly in obese and lean rats,[23] causing an improvement in glucose tolerance in obese animals and a diabetic response in lean rats.

In young obese Zucker rats, the development of hyperinsulinemia leads to an enhanced lipogenic state by 15 weeks of age.[10] It has long been debated that the initiating factor in obesity may not be hyperinsulinemia, but rather the hyperphagia that is already present at the time of weaning.[31] However, it has recently been observed that the earliest stages of excess fat deposition in fatty Zucker rat pups are not associated with increased food intake.[32] Moreover, Tenenbaum et al[33] recently demonstrated that hyperinsulinemia is present in at least 80% of 5-week-old preobese Zucker rat pups. In their study, about half of the preobese Zucker rat pups demonstrated altered insulin secretion profiles as early as day 21 of gestation, and altered insulin secretion developed in most of the remaining fa/fa pups over the ensuing 5 weeks. In our study, total caloric intake at the end of the experiments was significantly greater in obese animals than in lean rats, although when normalized for body weight, strain differences were not apparent. Interestingly, we did not observe the DZ-induced hyperphagia shown in previous studies using smaller drug doses.[23] However, there may have been differences in food intake between DZ and C groups earlier in the experiment when BW differences were emerging.

Table 3. Receptor Binding Data in Obese and Lean Zucker Rats

Subgroup	No.	K_1 (× 10^9 mol/L)	K_2 (× 10^9 mol/L)	R_1 (× 10^{-12} mol/L)	R_2 (× 10^{-12} mol/L)
DZ obese	6	1.28 ± 0.05*	0.023 ± 0.002*	4.69 ± 0.30*	128 ± 4*
C obese	6	1.04 ± 0.03†	0.011 ± 0.003†	1.43 ± 0.17†	97 ± 7†
DZ lean	6	2.65 ± 0.14*	0.020 ± 0.002	10.6 ± 0.95*	263 ± 19*
C lean	6	1.30 ± 0.04	0.022 ± 0.003	5.25 ± 0.56	118 ± 6

NOTE. Data are the mean ± SEM and were analyzed by ANOVA. Binding capacity data were normalized for cell surface area.

*Significantly different from strain control (DZ *v* C), $P < .01$.

†Significantly different from lean (C obese *v* C lean), $P < .01$.

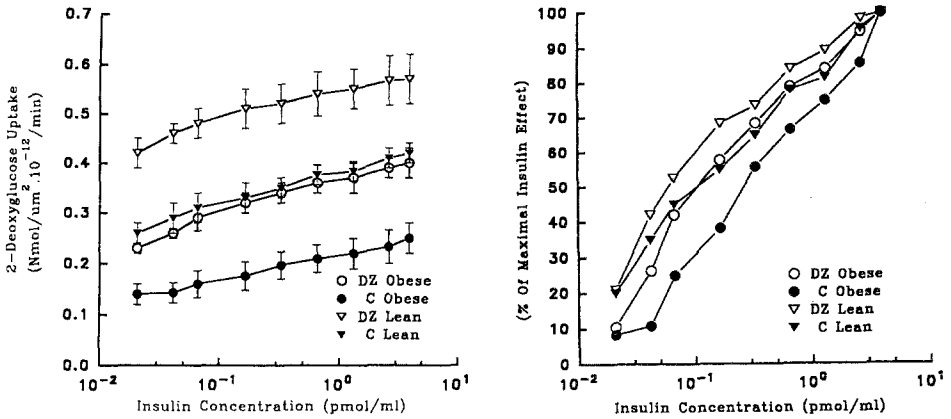

Fig 6. Basal and insulin-stimulated ^{14}C-deoxyglucose transport rate (expressed as fat cell surface area/min) of obese and lean Zucker rats (left). Transport data are also presented as a percentage of the maximal difference in the absence and presence of insulin (right). Data are the mean ± SEM. DZ obese *v* C obese, $P < .01$; DZ lean *v* C lean, $P < .005$.

Hyperinsulinemia and insulin resistance are thought to induce preferential shunting of substrates toward adipose tissue, leading to adipocyte hypertrophy, hyperplasia, and conversion of preadipocytes to adipocytes. These are changes that contribute to a persistent lipogenic state and obesity.[34] In the present study, the reduced rate of weight gain in DZ obese rats is associated with decreased insulin-induced lipogenesis. This was suggested by the reduction in adipocyte size (Fig 3) and decreased plasma triglyceride concentrations without significant improvement in FFA levels in obese animals. The latter may be due to a greater rate of FFA release from intracellular sites as a result of decreased antilipolysis and/or adipocyte size in DZ obese animals. However, caution must be exercised in extrapolating the present in vitro findings to circulating fatty acids, since plasma FFA levels reflect the sum of lipolysis and utilization by fat and other tissues. Isolated adipocytes from DZ-treated obese animals were less sensitive to insulin-mediated inhibition of glycerol release, which may reflect the improved cellular insulin sensitivity as demonstrated by increased glucose transport. Receptor binding studies confirmed a dramatic increase in high-affinity receptor number with a significant increase in hormone affinity. We also noted that DZ treatment reduced the magnitude of insulin-mediated inhibition of glycerol release. Our data regarding the antilipolytic action of insulin are in agreement with those of Arner et al,[35] who showed enhanced sensitivity and responsiveness of the antilipolytic effect of insulin in obese versus normal-weight human subjects. However, additional studies are needed to determine whether this effect is due to modulation of insulin action or an indirect effect of endogenous agents such as adenosine.[36] In adult obese Zucker rats, hyperinsulinemia and decreased sensitivity to insulin action in liver, muscle, and adipose tissue coexist.[11] In vitro studies have shown that increasing concentrations of insulin not only alter insulin receptor affinity, but also downregulate the number of insulin receptors as the chronic hyperinsulinemic state develops.[37-39] In obese (ob/ob) mice, hyperinsulinism is associated with the development of obesity at weaning,[13] leading to glucose intolerance, fasting hyperglycemia, and insulin resistance.[40,41] In the present study, DZ suppression of insulin concentration in obese rats was associated with improved glucose tolerance, increased adipocyte insulin receptor number, and enhanced adipocyte glucose transport rate. Surprisingly, DZ lean rats displayed a decrease in glucose tolerance to glucose challenge despite an increase in insulin sensitivity and adipocyte glucose transport. This strain difference in the glucose response to GTT may be attributed to persistent differences in initial and subsequent plasma insulin concentrations. Although DZ obese animals had lower insulin concentrations than C obese, they remained hyperinsulinemic compared with lean animals. In contrast, plasma insulin was further reduced to the hypoinsulinemic range by DZ treatment of lean animals. As a result, we believe that hyperinsulinemic DZ obese rats were able to normalize their glycemic state with enhanced insulin sensitivity, whereas hypoinsulinemic DZ lean animals had insufficient circulating insulin to adequately use the enhanced insulin sensitivity, with resultant hyperglycemia during IP-GTT despite enhanced insulin sensitivity.

As we had previously shown, DZ treatment led to increased insulin receptor number in adipocytes of both lean and obese Zucker rats,[23] an effect likely due to receptor upregulation secondary to declining plasma insulin concentration.[42,43] This was also accompanied by an enhanced binding constant of high-affinity receptors (K_1) of adipocytes in both genotypes, although the effect was of greater magnitude in lean animals. A similar receptor upregulation and affinity change has been observed in Zucker rats treated with lower doses of DZ.[23] The functional consequence of reduced adipocyte insulin affinity and number would be a decrease in insulin sensitivity and responsiveness, respectively. Indeed, a clear rightward shift was shown in the insulin-stimulated glucose transport dose-response curve in the C obese group as compared with C lean animals. The magnitude of this shift had a reasonable correspondence to the decrease in insulin binding per cell surface area. Furthermore, DZ treatment resulted in an enhanced glucose transport rate at basal and maximally insulin-stimulated states in adipocytes from both genotypes. This observation is of great significance, since it provides evidence for the presence of underlying receptor and postreceptor changes in the chronic hyperinsulinemic state. However, the enhancement of glucose transport in DZ lean rats cannot be explained at this time. It is not clear whether DZ has a target effect on the glucose transport system that is independent of its insulin-lowering action. An

increased binding constant for low-affinity receptor sites was only observed in DZ obese animals compared with their controls. The significance of the latter observation is not clear, since most investigators consider the high-affinity binding site the physiologically relevant receptor.[44,45]

For the most part, basal and insulin-stimulated glucose utilization parallel basal and insulin-stimulated glucose transport activity in cells of both 6-week-old lean and obese rats.[46] However, at 20 weeks of age, the metabolic response to insulin is decreased much more than the transport response in obese animals. In our study, the glucose transport rate in both lean and obese rats was determined at 13 weeks of age, before a significant dissociation of glucose utilization and transport is usually observed in both lean and obese animals.[46]

It is well recognized that adipose cellular enlargement is associated with a decreasing maximum capacity for total glucose utilization,[47] while glucose carbons are increasingly diverted to triglyceride glycerol synthesis at the expense of fatty acid synthesis and glucose oxidation through the hexose-monophosphate shunt.[48] These alterations in relative activities of the pathways for glucose metabolism are probably mediated by an observed increase in intracellular FFA with increasing adipocyte size.[48,49] In DZ-treated obese animals, plasma triglyceride levels decreased significantly; this reduction was accompanied by enhanced glucose transport into adipocytes. One can postulate that partial normalization of insulin levels by DZ resulted in a reversal of an increased triglyceride glycerol synthesis, with an enhanced rate of glucose oxidation in fatty Zucker rats. This combined effect resulted in decreased weight gain and improved glucose tolerance. Therefore, we can speculate that pharmacologic modification of the disturbed insulin metabolism of obesity by DZ may be of potential clinical value for weight reduction without inducing hyperglycemia.

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