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Diazoxide enhances basal metabolic rate and fat oxidation in obese Zucker rats

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Abstract

Persistent suppression of hyperinsulinemia in genetically obese (*fa/fa*) Zucker rats by diazoxide (DZ) reduces food intake and weight gain; improves insulin sensitivity, glycemic control, and lipid profile; and enhances β_3-adrenergic function and lipolysis in adipose tissue. The aim of this study was to elucidate the effects of DZ on basal metabolic rate (BMR), fat oxidation, and adrenergic function of lean and obese Zucker rats. Diazoxide (150 mg/kg/d) or vehicle (control) was administered for 4 weeks in 7-week–old obese and lean Zucker rats (n = 8-9 per subgroup). Animals underwent indirect calorimetry, body composition analysis, and determination of uncoupling proteins (UCPs) messenger RNA (mRNA) in brown and white adipose tissues (BAT and WAT) and skeletal muscle (SM), β_3-adrenergic receptor (AR) mRNA in BAT and WAT, β_2-AR in SM as well as WAT, and SM adenylate cyclase (AC) activity at the completion of study. Diazoxide treatment decreased food intake, weight gain, and body fat in obese rats ($P < .01$). Although DZ treatment lowered fasting plasma glucose, insulin, leptin, adiponectin, and lipids in obese rats ($P < .01$), it increased adiponectin-leptin ratio ($P < .01$). Plasma adiponectin-leptin ratio was inversely correlated with fat mass in obese and lean rats ($r = -0.86$, $P < .0001$). Diazoxide treatment resulted in higher BMR and fat oxidation rate in obese compared with control animals ($P < .01$), without any effect in lean animals. Furthermore, plasma adiponectin was inversely correlated with BMR (−0.56, $P < .001$) and lipid oxidation rate (−0.61, $P < .0005$) and was positively correlated with nonprotein respiratory quotient ($r = 0.41$, $P < .01$) in obese and lean rats. This was associated with increased β_3-AR mRNA expression in BAT and WAT ($P < .01$), UCP-1 and UCP-3 in BAT and AC activity in WAT ($P < .02$), and AC activity in SM of DZ obese rats compared with controls ($P < .01$), without significant change in SM β_2-AR mRNA expressions. Diazoxide attenuation of hyperinsulinemia decreased the rate of weight gain but enhanced insulin sensitivity, BMR, and fat oxidation in obese rats. This was associated with increased receptor- and non–receptor-mediated adrenergic function in adipose and muscle tissues in obese rats, respectively. These metabolic changes in obese Zucker rats suggest that antiobesity effects of DZ appear to be not only through its anorectic effect, modification of disturbed insulin metabolism, and inhibition of lipogenesis, but also due to augmentation of adrenergic function, energy expenditure, and fat utilization.

1. Introduction

Obese Zucker rat (*fa/fa*), a genetic model of obesity, converts large amounts of dietary carbohydrate and fat into stored lipid [1,2]. High lipid turnover and the enormous accumulation of fat in obese Zucker rats are consistent with their known hyperphagia and lack of a fully functional thermogenic system [3]. Consequently, obese rats derive a high amount of body heat from the conversion of carbohydrate into fat, which helps maintain their body temperature and thermal homeostasis [4]. Thus, obese Zucker rats tend to have a higher absolute basal metabolic rate (BMR) than lean animals [5], but a lower BMR than lean animals when it is corrected to their respective body weights (BWs). Moreover, obese rats rely less on lipid oxidation with resultant higher respiratory quotient (RQ) than lean animals [6]. Indeed, lower lipid oxidation in *fa/fa* rats compared with lean (*Fa/Fa*)

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animals has been suggested to be a mechanism that contributes to the development of obesity in these animals [7].

The loss of β_3-adrenergic receptor (AR) function in adipose tissue is assumed to play a role in diet-induced obesity in rodents [8] and in monogenic models of obesity [9,10]. Although the molecular basis for blunted β_3-AR function is not clear, hyperinsulinemia is believed to play a role in both diet-induced and genetic models of obesity [11,12]. Indeed, suppression of insulin by diazoxide (DZ), an inhibitor of glucose-mediated insulin release, reversed blunted adipocyte β_3-AR function in a diet-induced rodent model of obesity [13].

We have previously shown that persistent suppression of hyperinsulinemia in obese Zucker rat by DZ reduces food intake and weight gain and improves insulin sensitivity, glycemic control, and lipid profile [14]. This was accompanied by enhanced adipose tissue β_3-AR function and lipolysis. Moreover, DZ treatment of prediabetic Zucker diabetic fatty rats not only resulted in decreased rate of weight gain and prevented β-cell dysfunction, but also enhanced β_3-adrenoreceptor messenger RNA (mRNA) and adenylate cyclase (AC) activity in adipose tissue [15]. Therefore, we hypothesized that DZ increases BMR and fat oxidation in obese Zucker rats. To determine the metabolic effects of DZ in obese Zucker rats and its potential therapeutic role in the management of obesity, we determined BMR and substrate utilization; body composition and uncoupling protein (UCP) mRNA expression in brown and white adipose tissues (BAT and WAT); and adrenergic function in BAT, WAT, and skeletal muscle (SM) of obese and lean Zucker rats.

2. Materials and methods

2.1. Animals

Seven-week–old female obese (*fa/fa*) rats weighing 177 to 240 g and female Zucker lean (*Fa/?*) rats weighing 110 to 150 g were used in this study. Animals were obtained at 6 weeks of age from Charles River Laboratory (Wilmington, MA) and housed in pairs in standard animal cages. They were provided with rat chow, Teklad 22/5 (W) 8640 (Harlan Teklad, Madison, WI), and water ad libitum. Animals were divided into 2 subgroups of 8 to 9 animals per group: DZ-treated and control (C) subgroups. Diazoxide (150 mg/kg/d) was administered in 2 doses daily by gavage using Proglycem pediatric suspension 50 mg/mL (Baker-Norton Pharmaceuticals, Miami, FL). The control group was treated with an equivalent volume of vehicle suspension. Rats were weighed twice weekly, and food consumption was measured while animals were in separate metabolic cages during the last week of treatment. Studies lasted for a period of 4 weeks.

At the end of the 4-week period, animals underwent calorimetric studies. After an overnight fast (12 hours), blood samples were obtained for glucose, insulin, leptin, adiponectin, cholesterol, free fatty acids (FFA), and triglycerides (TG). The animals were anesthetized with an intramuscular injection of ketamine (65 to 100 mg/kg BW) and were then euthanized by a terminal cardiac puncture and exsanguinations. The animal procedures were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee.

2.2. Indirect calorimetry

The rats were placed in the metabolic chambers for determination of BMR after an overnight 12-hour fast. Indirect calorimetry was determined by measuring the airflow rate through the metabolic chambers (1.6 L/min) and by analyzing carbon dioxide and oxygen gas concentrations in room air and in the mixture of room air and expired breath by using CD-3A carbon dioxide and S-3A oxygen analyzers (Ametek Thermox Instruments, Pittsburgh, PA). Oxygen consumption and carbon dioxide production were determined every 30 minutes for 2 hours after achieving steady state. Total energy expenditure was calculated according to the Weir [16] equation. In each animal, 24-hour urine was collected for determination of nitrogen excretion.

The following calculations were used for determination of substrate utilization in each animal: (1) $RQ = V_{CO_2}/V_{O_2}$. (2) Nonprotein RQ (NPRQ) = NPV_{CO_2}/NPV_{O_2}, where NPV_{CO_2} is the nonprotein V_{CO_2} (in liters per minute) and NPV_{O_2} is the nonprotein V_{O_2} (in liters per minute). (3) $NPV_{CO_2} = V_{CO_2} - PV_{CO_2}$, where PV_{CO_2} is the protein V_{CO_2} (in liters per minute); $PV_{CO_2} = N \times 0.25 \times 0.774$, where $N \times 6.25$ is the grams of protein oxidized per minute, because proteins contain 16% nitrogen. (4) $NPV_{O_2} = V_{O_2} - PV_{O_2}$, where PV_{O_2} is the protein V_{O_2} (in liters per minute). (5) The fraction of NPV_{O_2} as a result of glucose oxidation is $F_{glu} = (NPRQ - 0.705)/(1 - 0.705)$, and the fraction as a result of fat oxidation is $F_{fat} = 1 - F_{glu}$. (6) The rates of glucose and fat oxidation (in grams per minute) were calculated from the following equations: glucose oxidation = $F_{glu} \times (NP\ V_{O_2}/2.03)$ and fat oxidation = $F_{fat} \times (NP\ V_{O_2}/2.03)$.

2.3. Body composition and tissue collection

Body composition was measured by dissecting and weighing the fresh weight of organs and tissues of the body. In each animal, heart, liver, kidneys, brain, fat pads (retroperitoneal, mesenteric, parametrial, abdominal, and subcutaneous), and tail were removed and weighed to the nearest 0.01 g. Other internal organs such as lungs, genitals, and intestines were also removed and weighed. The empty body (muscle mass and skeleton, excluding the tail) was weighed and classified as carcass after dissection. *Fat mass* (FM) was defined as the sum of the WAT pads that were dissected. *Residual body mass* (RBM) was defined as BW minus the weights of carcass and FM, whereas *fat-free mass* (FFM) was defined as BW minus FM.

Mesenteric WAT, interscapular BAT, and gastrocnemius muscle were removed, trimmed, and weighed. Fat pads (WAT and BAT) and SM tissue were flash-frozen in liquid

nitrogen and stored at −80°C for later determination of β_3-AR (BAT, WAT, and SM), β_2-AR (SM), UCP-1 (BAT), UCP-2 (BAT, WAT, and SM), and UCP-3 (BAT, WAT, and SM), and AC activity in WAT and SM.

2.4. Assays

2.4.1. Plasma glucose, insulin, leptin, and adiponectin/ACRP30

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

2.4.2. Plasma cholesterol, TG, and FFA

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

2.4.3. Urinary nitrogen

Twenty-four–hour urinary nitrogen was analyzed by using an Antek gas-phase pyrochemiluminescent nitrogen system (models 720, 771, and 735; Antek Instruments, Houston, TX) [17].

2.4.4. Extraction of total RNA and multiple real-time reverse transcriptase polymerase chain reaction quantitation

Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD) from BAT, WAT, and SM. Ribonucleic acid was treated with Rnase-free Dnase (Promega, Madison, WI) and stored in Ambion (Austin, TX) RNA storage solution in −80°C. First-strand complementary DNA (cDNA) was generated from 1 μg RNA in a 10-μL solution containing 200 U Superscript II (Invitrogen Life Technologies), 1 mmol/L of deoxyribonucleotide triphosphate (dNTP) mix, 2 U of RNasin (Promega), 2.5 μmol/L random hexamers (Gibco BRL, Gaithersburg, MD), 5× first-strand buffer (Invitrogen), and diethylpyrocarbonate (DEPC) water. After incubation for 1 hour at 42°C, the reaction mixture was heated to 94°C for 2 minutes. The cDNA was then stored at −20°C.

Polymerase chain reaction (PCR) amplification and analysis were performed using a Smartcycler II instrument and software version 1.2 (Cepheid, Sunnyvale, CA) [18]. The final reaction mix of 25 μL consisted of 2 μL cDNA, 0.5 μmol/L of each primer, 12.5 μL QuantiTect SYBR Green Master Mix (Qiagen, Valencia, CA), and 8.0 μL H_2O. For all primer pairs except 18S RNA (internal control), the cDNA was denatured by incubation at 95°C for 10 minutes. The template was amplified for 55 cycles of 30 seconds at 95°C, 40 seconds at 60°C, and 30 seconds at 72°C. The 18S RNA primer pair was incubated at 95°C for 10 minutes; the template was then amplified for 65 cycles of 30 seconds at 95°C, 40 seconds at 55°C, and 30 seconds at 72°C. Fluorescent data were acquired during each extension phase. A standard curve was created for each primer set using a serial dilution series of 1:5, 1:25, 1:125, and 1:625 using control sample cDNA. All samples and standard curve dilutions were run in duplicate. After all cycles were complete, a melting curve was generated by cooling samples to 60°C and slowly heating the samples at 0.2°C/s to 95°C. *Cycle threshold* was defined as the first cycle in which there is significant increase in fluorescence above the background correlating to the log-linear phase of PCR amplification. Cycle threshold values were used to calculate relative amount values in samples.

Table 1 summarizes the sequences of the primers—β_2-AR, β_3-AR, UCP-1, UCP-2, and UCP-3)—that were used to amplify first-strand cDNA for gene products. The products were run on 1% agarose gel for confirmation. 18S ribosomal RNA (QuantumRNA kit, Ambion) was used as an internal control; and levels of UCP-1, UCP-2, UCP-3, β_2-AR, and β_3-AR were expressed as the ratio of gene mRNA relative to 18S mRNA. All primers were obtained from Integrated DNA Technologies (Coralville, IA).

2.4.5. AC activity in WAT and SM

Adipose tissues and SM from each animal were minced, and plasma membranes were prepared as previously described [9]. The enzyme activity was assayed using a bioluminescent assay for AC activity, which was expressed as cyclic adenosine monophosphate (cAMP) (in picomoles per minute per milligram of protein) [19]. Protein content of islet and adipose tissue fractions was estimated using the method of Lowry et al [20].

2.5. Statistical analysis

The reported values represent the mean ± SD. Statistical comparisons between subgroups were assessed by 2-way analysis of variance (ANOVA). Spearman correlation analyses were used to evaluate the relationship between plasma adiponectin and BMR, fat oxidation rate, and NPRQ and the relationship between plasma adiponectin-leptin ratio and FM in obese and lean rats. P less than .05 was considered statistically significant.

Table 1
Sequences of PCR primers

Gene	Sense	Antisense	Size (base pairs)	GenBank accession no.
β_2-AR	GACGTTAGGCATCATCATGG	TTGACGACACACTTCTGGAGG	168	X17607
β_3-AR	ACCTTG GCGCTGACTGG	ATG GGC GCAAAC GACAC	233	S56152
UCP-1	ACCCTGGCCAAGACAGAAG	CAATCCTGAGGGAAGCAAAG	211	X12925
UCP-2	TTGCCCGAATGCCATTG	GCAAGGGAGGTCGTCTGTCA	92	AF039033
UCP-3	ATTCATGCCCTCCTTTCTGCGTCT	TTCCCGCAGTACCTGGACTTTCAT	103	AF035943

3. Results

3.1. Effect of DZ on weight gain and body composition

Table 2 shows BW, rate of weight gain, and body composition data in obese and lean Zucker rats. Control obese rats exhibited higher initial weight and greater weight gain over the 4-week period than C lean animals ($P < .001$). Diazoxide obese rats had lower final BW and weight gain than C obese animals ($P < .01$). Diazoxide treatment did not affect BW in lean rats.

Body composition analysis revealed higher FM, RBM, and FFM ($P < .001$) but lower carcass weight ($P < .05$) in C obese as compared with C lean animals. Similarly, C obese rats exhibited higher organ weights including liver, heart, and kidney ($P < .001$). Diazoxide-treated obese animals had lower FM ($P < .001$) and liver weight ($P < .02$) than C obese rats, without any effect on absolute carcass weight, RBM, and FFM. Diazoxide treatment did not affect FM, RBM, FFM, and organ weights in lean animals. Although DZ obese rats had lower percentage of FM ($P < .0005$), they had higher percentage of carcass weight ($P < .04$), RBM ($P < .01$), and FFM ($P < .0005$) than C obese rats. Moreover, C obese rats showed higher percentage of subcutaneous fat than C lean animals ($P < .0001$), although they exhibited lower percentage of internal fat pads as compared with lean animals ($P < .0001$). Diazoxide treatment reduced percentage of subcutaneous fat ($P < .01$) and internal fat pad (mesenteric and parametrial but not retroperitoneal) ($P < .01$) weights in obese rats, without any effect in lean animals.

3.2. Effect of DZ on energy consumption and indirect calorimetry

Table 3 summarizes the energy consumption, BMR, and substrate utilization data in obese and lean Zucker rats. Control obese rats had higher intake of energy and macronutrients than lean animals ($P < .0001$). Although DZ treatment resulted in decreased energy intake in obese rats ($P < .001$), it had no effect in lean animals. On the other hand, control obese rats had higher excretion of urinary nitrogen than lean animals ($P < .001$); and nitrogen excretion was only decreased in DZ-treated obese rats as compared with C obese animals ($P < .01$).

Although control obese animals had higher daily absolute BMR values than lean animals ($P < .01$), BMR expressed in relationship to FFM was lower in C obese animals as compared with C lean rats ($P < .01$). Control obese rats had higher overall percentage of glucose oxidation ($P < .03$) but lower percentage of fat oxidation ($P < .03$) than C lean rats. In addition, C obese animals exhibited higher RQ ($P < .05$) and NPRQ ($P < .01$) values than lean animals, corresponding to lower fat oxidation in obese as compared with lean rats ($P < .01$). Diazoxide obese rats displayed significantly higher absolute BMR values ($P < .05$) and BMR expressed relative to FFM ($P < .01$) than C obese rats. Diazoxide-treated obese rats demonstrated lower overall glucose oxidation ($P < .001$) and higher fat oxidation rate than C obese rats ($P < .001$). This was accompanied by higher fat oxidation in DZ obese compared

Table 2
Body weight and composition data in obese and lean Zucker rats

Parameters	DZ obese (n = 8)	C obese (n = 9)	DZ lean (n = 9)	C lean (n = 9)	*P* (ANOVA) (strain)	*P* (ANOVA) (strain × DZ)
Initial BW (g)	202.4 ± 23.3	204.8 ± 21.8	122.2 ± 12.3	121.1 ± 13.7	<.001	NS
Final BW (g)	275.5 ± 21.8[a]	310.6 ± 25.7	167.6 ± 17.3	161.2 ± 17.6	<.001	<.01
BW gain (g)	73.1 ± 13.9[a]	105.8 ± 16.9	45.3 ± 7.8	40.1 ± 11.7	<.001	<.001
Tissues and organs (g)						
Carcass	89.9 ± 6.9[a]	95.7 ± 7.4	104.2 ± 7.5	104.0 ± 8.7	<.05	<.01
FM	57.4 ± 5.5[a]	79.2 ± 8.3	9.8 ± 3.0	9.9 ± 2.2	<.0001	<.001
RBM	128.1 ± 13.1	135.7 ± 13.0	53.5 ± 8.5	49.9 ± 8.8	<.001	NS
FFM	218.1 ± 19.0	231.3 ± 19.0	157.7 ± 14.8	153.9 ± 16.1	<.001	NS
Liver	8.3 ± 0.7	9.3 ± 0.8	6.4 ± 0.5	5.3 ± 0.6	<.0001	<.01
Heart	0.90 ± 0.14	1.05 ± 0.18	0.75 ± 0.15	0.68 ± 0.15	<.001	NS
Kidney	1.36 ± 0.11	1.41 ± 0.13	1.10 ± 0.06	1.03 ± 0.09	<.001	NS
Brain	1.00± 0.14	0.98 ± 0.08	1.05 ± 0.08	1.01 ± 0.04	NS	NS
(Percentage of final BW)						
FM	20.9 ± 1.6[a]	25.4 ± 1.4	5.8 ± 1.4	6.0 ± 0.9	<.0001	<.0005
Carcass	32.7 ± 0.8[a]	30.9 ± 1.6	62.4 ± 3.3	63.7 ± 2.7	<.0001	<.04
RBM	46.4 ± 1.9[a]	43.7 ± 1.1	31.8 ± 2.3	30.3 ± 2.7	<.0001	<.01
FFM	79.1 ± 1.6[a]	74.5 ± 1.4	94.2 ± 1.4	94.0 ± 0.9	<.0001	<.0005
Fat pads (% of BW)						
Subcutaneous	55.4 ± 0.6[a]	52.1 ± 1.8	33.2 ± 1.9	34.5 ± 1.0	<.0001	<.01
Mesenteric	14.8 ± 0.7[a]	16.4 ± 0.5	20.1 ± 1.5	19.9 ± 0.8	<.0001	<.01
Parametrial	15.9 ± 0.5[a]	17.6 ± 0.7	24.2 ± 1.2	24.6 ± 1.0	<.0001	<.01
Retroperitoneal	13.8 ± 1.0	13.9 ± 2.3	22.5 ± 1.6	21.1 ± 1.7	<.0001	NS
Internal fat[b]	44.6 ± 0.62[a]	47.9 ± 1.8	66.8 ± 1.9	65.5 ± 1.0	<.0001	<.01

Data are mean ± SD. RBM = BW − carcass − FM. FFM = BW − FM. NS indicates not significant.

[a] DZ obese vs C obese rats only.

[b] Calculated as the sum of parametrial, mesenteric, and retroperitoneal fad pads.

Table 3
Energy consumption and BMR in obese and lean Zucker rats

Parameters	DZ obese (n = 8)	C obese (n = 9)	DZ lean (n = 9)	C lean (n = 9)	*P* (ANOVA) (strain)	*P* (ANOVA) (strain × DZ)
Energy intake (kJ/d)	293.9 ± 48.2[a]	498.9 ± 28.7	202.3 ± 47.2	227.4 ± 29.8	<.0001	<.001
Carbohydrate intake (kJ/d)	212.7 ± 33.8[a]	361.1 ± 20.8	146.5 ± 34.2	164.6 ± 21.6	<.0001	<.001
Protein intake (kJ/d)	65.3 ± 10.4[a]	110.7 ± 6.4	44.9 ± 10.5	50.5 ± 6.6	<.0001	<.001
Fat intake (kJ/d)	15.9 ± 2.5[a]	27.1 ± 2.4	10.9 ± 2.3	12.3 ± 1.6	<.0001	<.001
Urinary nitrogen (mg/d)	207.5 ± 19.3[a]	250.1 ± 20.2	147.5 ± 24.1	141.9 ± 30.3	<.001	<.01
BMR (kJ/d)	81.5 ± 12.8[a]	70.8 ± 5.2	57.1 ± 9.9	56.8 ± 8.4	<.01	<.05
BMR (kJ/[FFM d])	372.4 ± 37.0[a]	307.3 ± 23.2	363.4 ± 60.9	370.1 ± 52.7	<.01	<.01
RQ	0.83 ± 0.03	0.86 ± 0.02	0.85 ± 0.01	0.84 ± 0.02	<.05	<.01
NPRQ	0.83 ± 0.04[a]	0.90 ± 0.03	0.87 ± 0.01	0.86 ± 0.03	<.01	<.001
Glucose oxidation (kJ/[FFM d])	101.8 ± 25.2	115.2 ± 25.6	139.3 ± 32.5	124.3 ± 24.2	NS	NS
Fat oxidation (kJ/[FFM d])	135.9 ± 40.7[a]	64.6 ± 22.8	113.7 ± 27.2	111.1 ± 31.8	<.01	<.001
$F_{glucose}$ oxidation (%)	43.4 ± 11.6[a]	64.1 ± 9.5	54.9 ± 3.9	53.1 ± 9.8	<.03	<.0001
F_{fat} oxidation (%)	56.6 ± 11.6[a]	35.9 ± 9.5	45.1 ± 3.9	46.9 ± 9.8	<.03	<.0001

Data are mean ± SD.
[a] DZ obese vs C obese rats only.

with C obese rats ($P < .001$), with no significant change in glucose oxidation rate, corresponding to lower RQ ($P < .01$) and NPRQ ($P < .001$) in DZ obese as compared with control obese rats. Diazoxide treatment did not affect BMR and substrate utilization in lean animals.

3.3. Effect of DZ on plasma glucose, insulin, leptin, adiponectin, and lipid profile

Control obese had higher fasting plasma glucose, insulin, leptin, adiponectin, cholesterol, TG, and FFA than lean animals ($P < .02$) (Table 4). Diazoxide treatment lowered plasma concentrations of glucose, insulin, leptin, adiponectin, TG, and FFA in obese rats ($P < .01$), without any effects on plasma cholesterol concentrations. However, plasma glucose, leptin, adiponectin, and lipids levels were not affected in DZ lean animals despite a significant reduction in fasting plasma insulin concentration ($P < .0001$). Moreover, C obese rats demonstrated significantly lower plasma adiponectin-leptin ratio ($P < .0001$) than C lean animals. Diazoxide treatment increased plasma adiponectin-leptin ratio ($P < .01$) and was inversely correlated with FM in obese and lean rats (-0.86, $P < .0001$).

3.4. Relationship between plasma adiponectin and BMR and fat oxidation

Fig. 1 illustrates the relation between plasma adiponectin and BMR, fat oxidation, and NPRQ in obese and lean rats. Although plasma adiponectin concentration was inversely correlated with BMR ($r = -0.56$, $P < .001$) and fat oxidation rate ($r = -0.61$, $P < .0005$), it was positively correlated with NPRQ ($r = 0.41$, $P < .01$) in obese and lean animals.

3.5. Effect of DZ on BAT and WAT β_3-AR and SM β_2-AR expressions

Fig. 2 illustrates basal β_3-AR mRNA levels in adipose tissues (BAT and WAT) and β_2-AR mRNA levels in SM of obese and lean rats. Control obese animals demonstrated higher β_3-AR mRNA levels in BAT ($P < .0001$) and WAT ($P < .001$) than lean animals. Diazoxide-treated obese rats showed higher β_3-AR expression in both BAT ($P < .01$) and WAT ($P < .01$) as compared with their controls. Diazoxide treatment did not affect β_3-AR mRNA levels in adipose tissues of lean rats, although there was a tendency for BAT β_3-AR expression to increase in DZ lean animals. Moreover,

Table 4
Biochemical data in obese and lean Zucker rats

Parameters	DZ obese (n = 8)	C obese (n = 9)	DZ lean (n = 9)	C lean (n = 9)	*P* (ANOVA) (strain)	*P* (ANOVA) (strain × DZ)
Plasma glucose (mmol/L)	4.9 ± 0.4[a]	6.7 ± 0.2	4.7 ± 0.4	4.6 ± 0.3	<.0001	<.0001
Plasma insulin (pmol/mL)	0.87 ± 0.12	1.73 ± 0.23	0.20 ± 0.02	0.31 ± 0.04	<.0001	<.0001
Leptin (ng/mL)	11.4 ± 2.5[a]	17.7 ± 2.3	1.6 ± 0.3	1.9 ± 0.4	<.0001	<.002
Adiponectin (μg/mL)	5.3 ± 0.5[a]	6.6 ± 0.9	4.1 ± 0.8	4.7 ± 0.7	<.001	<.01
Adiponectin-leptin ratio	0.49 ± 0.11[a]	0.37 ± 0.09	2.60 ± 0.72	2.63 ± 0.70	<.0001	<.01
Plasma cholesterol (mmol/L)	2.53 ± 0.47	2.64 ± 0.67	1.83 ± 0.26	1.78 ± 0.33	<.02	NS
Plasma TG (mmol/L)	2.94 ± 0.71[a]	4.62 ± 0.82	1.30 ± 0.17	1.26 ± 0.18	<.0001	<.01
Plasma FFA (mEq/L)	0.84 ± 0.24[a]	1.88 ± 0.56	0.48 ± 0.10	0.45 ± 0.12	<.0005	<.01

Data are mean ± SD.
[a] DZ obese vs C obese rats only.

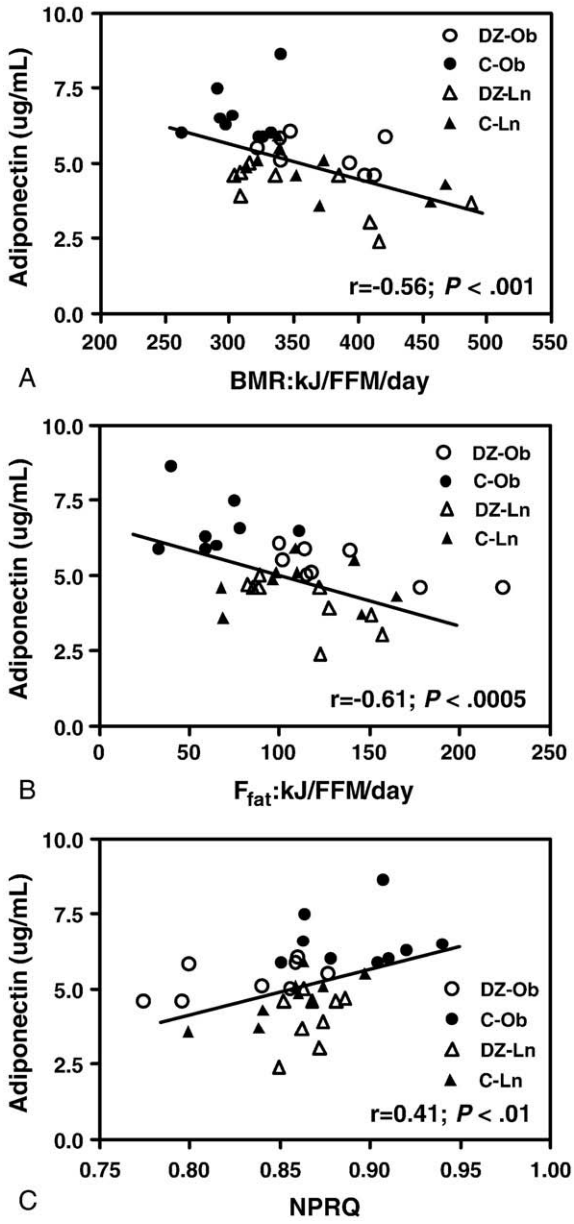

Fig. 1. Relationship between plasma adiponectin and BMR, fat oxidation, and NPRQ in obese and lean Zucker rats.

control obese rats had lower SM β_2-AR mRNA levels than lean rats ($P < .01$). However, DZ did not affect β_2-AR mRNA expression in SM of either strain.

3.6. Effect of DZ on BAT and WAT UCP-1 expressions

Fig. 3 illustrates UCP-1 mRNA expressions in BAT and WAT of obese and lean rats. Control obese rats had lower UCP-1 mRNA content in BAT than lean animals ($P < .001$). Diazoxide treatment increased UCP-1 mRNA expression in BAT of obese rats ($P < .02$) but not lean animals. However, UCP-1 mRNA content of WAT was similar and very low in both strains. Diazoxide treatment did not affect WAT UCP-1 mRNA content in either strain.

3.7. Effect of DZ on BAT, WAT, and SM UCP-2 expressions

Fig. 4 illustrates UCP-2 mRNA expression in BAT, WAT, and SM of obese and lean rats. Conytol obese rats demonstrated lower UCP-2 mRNA levels in BAT ($P < .01$), WAT ($P < .01$), and SM ($P < .01$) than lean rats. However, UCP-2 mRNA contents of BAT, WAT, and SM were not affected by DZ treatment in either strain.

3.8. Effect of DZ on BAT, WAT, and SM UCP-3 expressions

Fig. 5 illustrates UCP-3 expression in BAT, WAT, and SM. Control obese rats had lower UCP-3 mRNA content in BAT than lean animals ($P < .0001$). Diazoxide treatment significantly increased UCP-3 mRNA expression in BAT only in obese rats. White adipose tissues of C obese rats

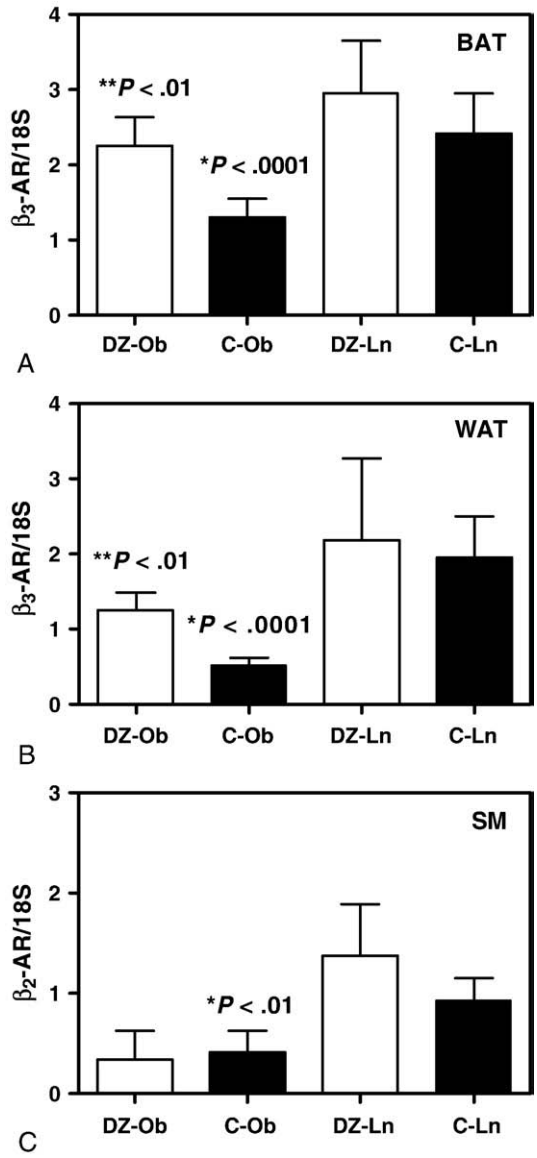

Fig. 2. β_3-Adrenergic receptor mRNA levels in adipose tissues (BAT and WAT) and β_2-AR mRNA levels in SM of obese and lean rats. *$P < .01$ to $P < .0001$, control obese vs lean rats; **$P < .01$, DZ obese vs control obese rats.

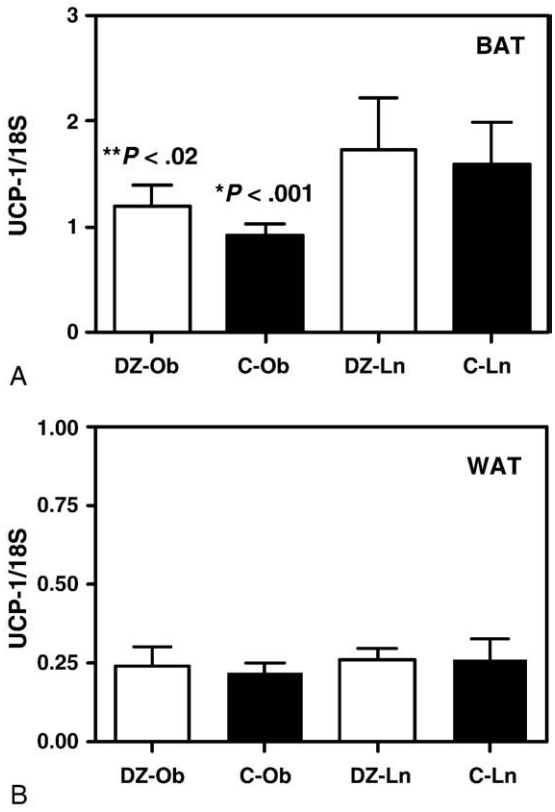

Fig. 3. Brown adipose tissue and WAT UCP-1 mRNA expressions in obese and lean rats. *P< .001, control obese vs lean rats; **P < .02, DZ obese vs control obese rats.

demonstrated lower UCP-3 mRNA levels than those of lean rats (P < .001). Diazoxide treatment did not affect UCP-3 expression in WAT of obese or lean rats. Furthermore, SM UCP-3 mRNA levels were lower in C obese than C lean rats (P < .0001). However, SM UCP-3 was not affected by DZ treatment in either strain.

3.9. Effect of DZ on AC activities in WAT and SM tissues

Fig. 6 illustrates adipose tissue and muscle AC activities in obese and lean rats. Control obese animals demonstrated lower AC activity in WAT (P < .001) than lean rats. Diazoxide-treated obese rats exhibited higher adipose tissue AC activity (P < .02) than obese controls. Diazoxide treatment did not affect adipose tissue AC activity in lean animals.

On the other hand, control obese animals demonstrated higher muscle AC activity (P < .001) than lean rats. Diazoxide-treated obese rats exhibited higher muscle AC activity (P < .01) than obese controls. Diazoxide treatment did not affect muscle AC activities in lean rats.

4. Discussion

Diazoxide attenuation of hyperinsulinemia in obese rats lowered fasting plasma glucose and lipids concentrations, but increased adiponectin-leptin ratio consistent with increased insulin sensitivity. This was accompanied by decreased food consumption, rate of weight gain, and body fat, with a significant increase in percentage of FFM in obese animals. Increased BMR and fat oxidation accompanied these changes in body composition without altering carbohydrate oxidation in obese animals. In addition, DZ treatment restored adipose tissue and muscle adrenergic activity in obese rats.

Insulin is a potent anorexigenic hormone, and insulin receptors are extensively distributed throughout the central nervous system [21]. Indeed, it has been hypothesized that the obesity state and hyperphagia in Zucker rats are, in part, due to insulin resistance in the brain, characterized by reduced capillary insulin binding, which is believed to mediate the transport of insulin into the brain [22,23]. In obese hyperphagic Zucker rats, DZ treatment has considerable anorectic and antiobesity effects [14] that are, in part, attributed to augmentation of central nervous system insulin sensitivity and uptake [24] and reduction of

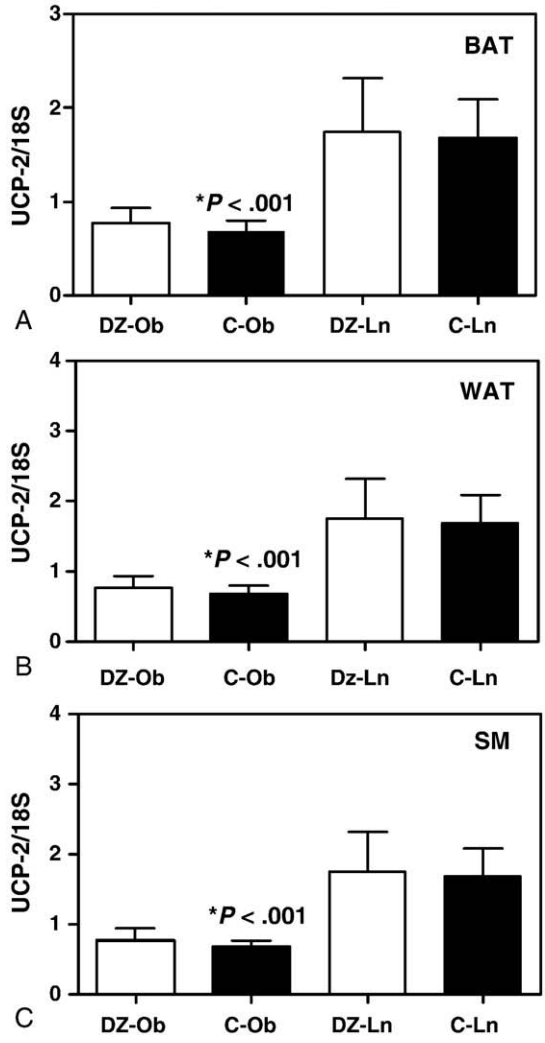

Fig. 4. Brown adipose tissue, WAT, and SM UCP-2 mRNA expressions in obese and lean rats. *P < .01, control obese vs lean rats.

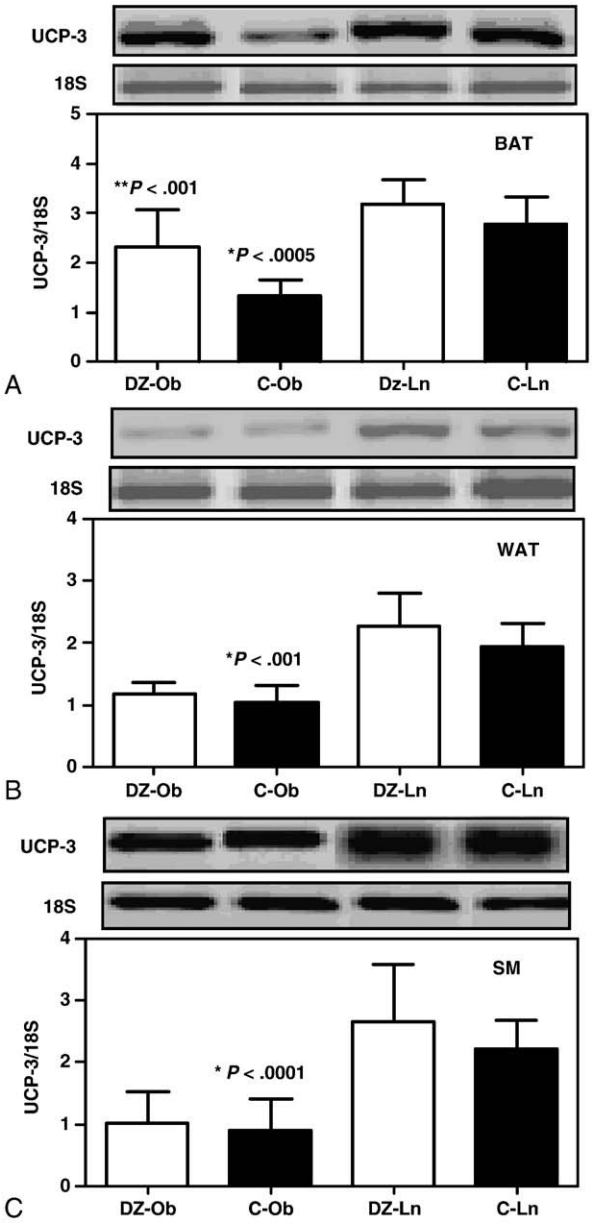

Fig. 5. Brown adipose tissue, WAT, and SM PCR blots and UCP-3 mRNA expressions in obese and lean rats. *$P < .001$ to $P < .0001$, control obese vs lean rats; **$P < .001$, DZ obese vs control obese rats.

lipogenesis, leading to reduction in food intake and rate of weight gain. Consistent with our previous studies, DZ-treated obese rats had a lower rate of weight gain than control obese animals, suggesting that the antiobesity effect of DZ is not only due to decreased food intake but also attenuation of lipogenesis [25].

Insulin stimulates the synthesis of leptin, an obese gene product, in adipose tissue [26]; and in turn, leptin regulates energy metabolism and feeding behavior through leptin-sensitive centers in the hypothalamus [27-29]. Indeed, leptin plays a major role in inhibiting the agouti-related protein/neuropeptide Y, a key orexigenic peptide in hypothalamus [30]. There is also evidence for direct leptin action on adipocytes [31] and other tissues [32]. However, because obese Zucker rat has an underlying mutation in the leptin receptor [33], its ability to regulate feeding and energy expenditure and balance is *permanently* impaired [34]. Moreover, we have previously shown that DZ treatment of obese Zucker rats not only lowers plasma leptin levels [24] but also increases hypothalamic neuropeptide Y content [35], suggesting that anorexic effect of DZ is not influenced by changes in brain leptin and is likely due to direct effect of increased insulin uptake in the brain [21,24]. On the other hand, it has been observed that leptin resistance in obese rats is accompanied by paradoxical increase in plasma adiponectin [36], a humoral mediator of glucose and lipid metabolism in adipocytes, which can modify insulin sensitivity and energy balance [37,38]. In our study, plasma leptin and adiponectin levels were higher in obese rats than lean animals, which was accompanied by lower adiponectin-leptin ratio in obese compared with lean rats. Indeed, it has been shown that adiponectin release from BAT and WAT in obese rats is significantly higher than that in lean rats, which contributes to the high plasma adiponectin in obese animals [36]. Furthermore, Oana et al [36] showed that high plasma adiponectin levels in obese rats were accompanied by decreased adiponectin receptor–1 mRNA expression in the adipose tissues of these animals, suggesting decreased adiponectin receptor–1 sensitivity. Diazoxide treatment led to significant suppression of plasma leptin and adiponectin levels in obese rats, which corresponded to FM loss but

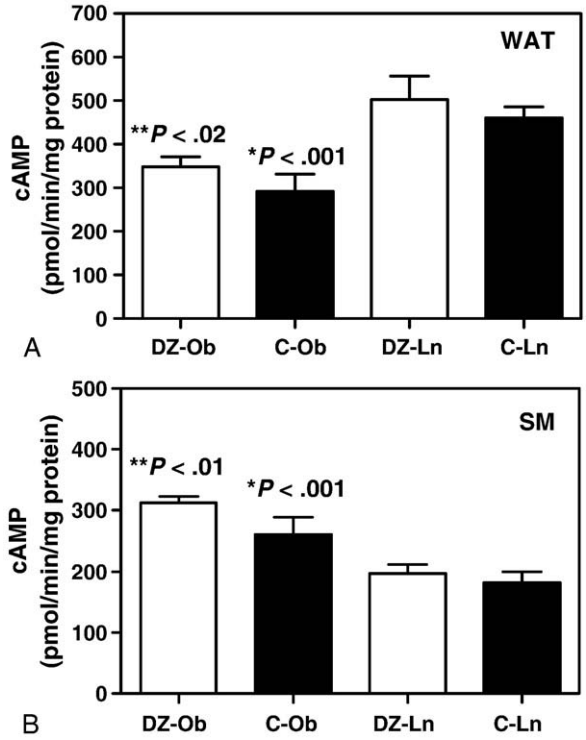

Fig. 6. White adipose tissue and SM AC activities in obese and lean rats. *$P < .001$, control obese vs lean rats; **$P < .02$ to $P < .01$, DZ obese vs control obese rats.

higher adiponectin-leptin ratio and insulin sensitivity [39]. Indeed, adiponectin-leptin ratio was inversely correlated with FM in obese and lean rats [39,40].

Body weight is protected by adjustments in the rate of whole-body energy expenditure [41]. When the food intake is limited and weight loss ensues, the reduction in BMR is considerably in excess of that anticipated from the loss of metabolically active FFM. The weight loss resulting from decreased food intake can be blunted because of a sharp reduction in BMR consistent with a metabolically adaptive response to reduction in caloric intake [42]. However, in our study, DZ attenuation of food intake in obese rats not only was accompanied by relative preservation of FFM, but also increased absolute BMR and BMR relative to FFM. This was also associated with enhanced fat oxidation without a significant alteration in glucose oxidation rate in these animals. On the other hand, plasma adiponectin was inversely correlated with BMR and fat oxidation in obese and lean rats, implying that reduction in plasma adiponectin concentrations in DZ obese rats had humoral mediation effect on energy metabolism [43,44].

Adipose cellular enlargement is associated with a diminishing maximum capacity for total glucose utilization in the obesity state [45], whereas glucose carbons are progressively diverted to triacylglycerol synthesis at the expense of fatty acid synthesis and glucose oxidation through the hexose-monophosphate shunt [46]. These alterations in relative activities of the pathway for glucose metabolism are likely mediated by an observed increase in intracellular FFA with increasing adipocytes cell size [46,47]. Diazoxide treatment of obese Zucker rats reduces plasma FFA and TG and enhances glucose transport into adipocytes [14,48], suggesting reversal of increased triacylglycerol synthesis and enhanced glucose oxidation. Indeed, reduction in the rate of weight gain and lower plasma glucose concentrations in DZ-treated obese rats are believed to result in lower plasma lipids. Nevertheless, in our study, we observed increased lipid oxidation rate without a significant change in glucose oxidation rate in DZ-treated obese rats, suggesting that increased glucose uptake in these animals is likely associated with enhanced glycogen synthesis [49,50].

The sympathetic nervous system via β_3-adrenoreceptors plays an essential role in controlling energy expenditure and body composition [51]. Stimulation of β_3-AR increases WAT TG hydrolysis, and thermogenesis in BAT and SM [51,52]. The mechanism of brown fat thermogenesis involves fatty acid oxidation and uncoupling of oxidative phosphorylation through UCP-1 [53,54]. Although UCP-1–dependent BAT thermogenesis plays a central role in the overall metabolic response, it has been shown that persistent β_3-AR activation induces metabolic adaptation in WAT that contributes to β_3-AR–mediated thermogenesis, which is accompanied by mitochondrial biogenesis and the induction of genes involved in lipid oxidation [55]. Similarly, UCP-2 and UCP-3 in adipose tissue and muscle have been associated with thermogenesis and mitochondrial proton gradient regulation [52,56,57]. Impairment of β-adrenergic receptor (β_3-AR) function in adipose tissue of diet-induced and genetic models of obesity is associated with hyperinsulinemia and altered carbohydrate and lipid metabolism [11,12,34]. In our study, DZ treatment enhanced basal β_3-AR, UCP-1, and UCP-3 expressions in BAT of obese rats, with a parallel increase in AC activity. Furthermore, DZ-treated animals demonstrated increased basal β_3-AR in WAT, with a corresponding increase in AC activity. However, UCP-1, UCP-2, and UCP-3 were not altered by DZ in WAT. The absence of a DZ effect on BAT basal β_3-AR, UCP-1, and AC as well as WAT β_3-AR and AC in lean animals may be due to unaltered ambient glucose level during the fasting state as compared with their controls. Indeed, these findings are consistent with the effects of β_3-agonists on insulin sensitivity, adiposity, and lipid metabolism [58,59].

Intracellular concentration of cAMP is established by the balance between its synthesis by insulin-regulated AC and degradation by phosphodiesterases [60]. In obese Zucker rats, abnormal β-adrenergic responsiveness results in inhibition of lipolysis activity and lipolysis but an enhanced insulin sensitivity and antilipolytic effect of insulin in adipose tissues [34,61]. It is also likely that the persistent insulin-resistant state in the obese rats resulted in decreased phosphodiesterase activity, leading to decreased degradation of cAMP in the SMs of these animals [62]. On the other hand, it has been shown that β_3-adrenergic agonists do not stimulate cAMP accumulation in SMs and that β-agonist–induced increases in AC activity and cAMP levels are mainly mediated by β_2-adrenoceptors [63]. However, both β_2- and β_3-adrenergic agonists have been shown to restore SM insulin responsiveness in rats [64,65]. In our study, DZ treatment of obese rats significantly increased SM AC activity without enhancement of β_2-AR mRNA expressions, implying that increased adrenergic activity in the SM of these animals is primarily through non–receptor-mediated mechanism [66].

There were some limitations to our study. First, we did not include pair-fed subgroups in obese and lean groups. However, we have previously shown that DZ effect on weight and adrenergic function is independent of its anorectic effect using pair-fed subgroups in obese and leans animals [15,67]. Second, there were no measures of spontaneous motor activity among animals especially because increased BMR in DZ obese rats may have been due to their higher level of spontaneous activity. However, it has been previously shown that the use of β-agonists does not affect spontaneous physical activity in rats [68]. Finally, because rats were dosed with DZ based on their BWs, it is likely that obese rats received more drugs relative to their organs than lean rats, which might account for the lack of metabolic effect in the latter group. Nevertheless, DZ lean rats demonstrated significant reduction in fasting plasma insulin compared with their controls, suggesting that the β-adrenergic effect of DZ is secondary to the modification of hyperinsulinemia in these animals [13] as previously

shown. Finally, we compared basal cAMP activity with β_3-AR expression in adipose tissue without comparing basal with β_3-AR–stimulated cAMP, which may limit the interpretation of our data in the adipose tissue. However, we should point out that Surwit et al [13] evaluated β_3-AR stimulation in WAT of DZ-treated C57BL/6J obese mice and showed a significant dose-dependent increase in cAMP response compared with control groups. Therefore, we anticipate that DZ-treated obese Zucker rats will also have a significant increase in cAMP in response to β_3-AR stimulation in adipose tissue.

In conclusion, DZ attenuation of hyperinsulinemia decreased the rate of weight gain but enhanced insulin sensitivity, BMR, and fat oxidation in obese rats. This was associated with increased receptor- and non–receptor-mediated adrenergic function in adipose and muscle tissues in obese rats, respectively. These metabolic changes in obese Zucker rats suggest that antiobesity effects of DZ appear to be not only through its anorectic effect, modification of disturbed insulin metabolism, and inhibition of lipogenesis, but also due to augmentation of adrenergic function, energy expenditure, and fat utilization.

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