DISCREPANCIES IN THE REGULATION OF PLASMA ADIPONECTIN AND TNF-α LEVELS AND ADIPOSE TISSUE GENE EXPRESSION IN OBESE AFRICAN AMERICANS WITH GLUCOSE INTOLERANCE: A PILOT STUDY USING ROSIGLITAZONE

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Objectives: We examined the effects of rosiglitazone: 1) on glucose homeostasis, insulin action, beta-cell function, and plasma adiponectin and TNF-alpha (TNF-α) levels; and 2) the expression of adipose tissue TNF-α and adiponectin mRNA in African Americans with parental history of type 2 diabetes and with varying degrees of glucose intolerance.

Subjects and Methods: The study groups comprised 11 African Americans with normal glucose tolerance and six with diabetes and impaired glucose tolerance. The glucose-intolerant subjects received rosiglitazone (4–8 mg/day) every morning for 12 weeks. They underwent oral glucose tolerance test (OGTT) and subcutaneous adipose tissue biopsy (under local anesthesia) before and after 12 weeks of rosiglitazone therapy. Beta cell function and insulin resistance were calculated by using homeostasis model assessment (HOMA). Adipose tissue gene expression (mRNA) was measured by real-time polymerase chain reaction in both groups.

Results: Rosiglitazone monotherapy improved both fasting and two-hour serum glucose levels during OGTT in the glucose-intolerant group. However, mean serum insulin and C-peptide levels did not change when compared with baseline. Rosiglitazone monotherapy improved insulin resistance but not overall beta-cell secretion. Mean adiponectin levels at fasting and two hours after oral glucose ingestion were significantly (50%) lower in the glucose-intolerant group than in the control group. Rosiglitazone monotherapy significantly increased plasma adiponectin levels at fasting and two hours after oral challenge by two-fold in the glucose-intolerant group. Mean plasma TNF-α levels were not significantly different at fasting and after two hours during OGTT. Rosiglitazone had no significant effect on plasma TNF-α levels during OGTT. No significant differences were seen in the expression of adipose tissue TNF-α and adiponectin mRNA in the groups at baseline. Rosiglitazone did not significantly change the adipose tissue adiponectin and TNF-α mRNA. Rosiglitazone was well tolerated, without experiencing weight gain, edema, and liver function test abnormality in the glucose intolerant subjects.

Summary: Rosiglitazone improved glucose homeostasis and insulin resistance in high-risk African Americans. We found that adiponectin was lower in the glucose-intolerant group, while TNF-α was similar. While rosiglitazone increased plasma adiponectin, it had no effect on adipose tissue adiponectin mRNA. In addition, rosiglitazone had no effect on plasma TNF-α and adipose tissue TNF-α mRNA. We conclude that the metabolic effects of rosiglitazone could be mediated by adiponectin but not TNF-α in African Americans with glucose intolerance. Our study demonstrates that: 1) the role of adipocytokines in the etiology of type 2 diabetes in African Americans is complex; and 2) that adiponectin, but not TNF-α, could mediate the metabolic benefits of thiazolidinediones in African Americans with glucose intolerance. (*Ethn Dis*. 2005;15:641–648)

Key Words: Adiponectin, African Americans, Insulin Resistance, Insulin Secretion, mRNA, Rosiglitazone, Tumor Necrosis Factor-Alpha

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INTRODUCTION

Adipocytokines, including tumor necrosis factor-alpha (TNF-α), interukin-6 (IL-6), adiponectin, and resistin, have received tremendous attention as important mediators of in vivo fuel metabolism, glucose homeostasis, insulin sensitivity, and perhaps atherosclerosis in several populations.[1] Adiponectin, a 244-amino acid peptide, solely produced and secreted by adipose tissues, has been reported to improve insulin sensitivity and could prevent type 2 diabetes and atherosclerosis.[2–13]

In addition to adiponectin, tumor necrosis factor-alpha (TNF-α) has also been implicated in the pathogenesis of insulin resistance and type 2 diabetes.[14–22] Several investigators have reported elevated levels of TNF-α in insulin-resistant states.[18–22] Moreover, peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists suppress adipose tissue expression of TNF-α.[25,26] Whether TNF-α plays a significant role in insulin resistance and development of type 2 diabetes and cardiovascular risk factors in African Americans remains to be elucidated.

We employed a potent PPAR-γ agonist (rosiglitazone, GSK Pharmaceutical Inc, Philadelphia, Pa) to explore the regulation of adiponectin and TNF-α in African Americans who were genetically predisposed to type 2 diabetes. We examined: the effects of rosiglitazone on 1) plasma adiponectin, TNF-α, as well as glucose homeostasis, insulin action, and insulin secretion;

We examined the effects of rosiglitazone on: 1) plasma adiponectin . . . and 2) gene expression of adipose tissue adiponectin and TNF-α mRNA in high-risk African Americans with and without impaired glucose tolerance and type 2 diabetes.

and 2) gene expression of adipose tissue adiponectin and TNF-α mRNA in high-risk African Americans with and without impaired glucose tolerance and type 2 diabetes.

SUBJECTS, MATERIALS, AND METHODS

Populations

The study consisted of 17 first-degree relatives of African Americans with type 2 diabetes. Six patients with glucose intolerance had an impaired glucose tolerance test result (n=3) or were newly diagnosed, drug-naïve patients with type 2 diabetes (n=3). Eleven African Americans with family history of diabetes but with a normal glucose tolerance test result served as healthy controls. The subjects were unselected and were recruited during screening for diabetes in first-degree relatives (offspring and siblings) of African-American patients with type 2 diabetes. Informed written consent—approved by the institutional review board for human biomedical research at The Ohio State University, Columbus, Ohio—was obtained from each subject after the risks entailed in the study had been thoroughly explained.

Study Protocol

All subjects had serum glucose measured after a 10- to 12-hour fast in order to qualify for the study. After at least 10 minutes bed rest, two blood pressure readings were obtained by trained nurses using zero-centered sphygmomanometer at 10-minute intervals. Each subject was weighed to the nearest gram, and height was measured to the nearest centimeter. Body composition was measured by using dual energy x-ray absorptiometer (DEXA-Lunar, Wisc). The clinical characteristics of our African Americans with varying degrees of glucose tolerance are shown in Table 1. We excluded patients with symptoms of hyperglycemia such as polyuria, polydipsia, polyphagia, excessive thirst, recent weight loss, blurred vision, etc during screening. The following subjects were also excluded: 1) those taking medications known to influence glucose and insulin metabolism; 2) those with liver, heart, lung and kidney diseases; 3) those with established diabetes on antidiabetic medications; and 4) those who participated in endurance exercise or indulged in regular competitive sport.

Metabolic Studies

All subjects were admitted to the Endocrine/Diabetes Clinical Research Unit of The Ohio State University, Columbus, Ohio after 10–12 hours of overnight fasting. With the subject in the sitting position, an intravenous needle was inserted into a forearm vein. Blood samples were drawn for serum glucose, insulin, and C-peptide, and plasma TNF-α and adiponectin levels. Fasting lipids and lipoproteins and HbA1C as well as routine kidney and liver function tests were obtained.

Oral Glucose Tolerance Test (OGTT)

After overnight fast and drawing basal blood samples, the subjects ingested 75 g in 250 mL of oral glucose load (Glucola, Baltimore, Md) over a two-minute period. Blood samples were obtained 120 minutes after oral glucose load for serum glucose, insulin, and C-peptide and plasma adiponectin and TNF-α levels. Categories of glucose tolerance status of the subjects were defined according to the new American Diabetes Association recommendation.[31] Each of the glucose-intolerant subjects answered dietary and physical activity questionnaires at baseline and every four weeks for three months.

Adipose Tissue Biopsy

The subcutaneous biopsy of the lateral aspect of the thigh was performed by our surgeon (CC). Healthy controls had a single adipose tissue biopsy. The glucose-intolerant group had 2 biopsies, one at baseline and the other 3 months after rosiglitazone therapy. The skin was anesthetized by using lidocaine 1% without epinephrine. We froze 100–200 mg tissues in triplicates in three separate tubes in liquid nitrogen and stored at −80°C until analyzed. The adipose tissue adipocytokines mRNA were measured at The Human Biomarker Center, Presbyterian Hospital, Philadelphia, Pa.

Longitudinal Study

The patients with glucose intolerance received rosiglitazone monotherapy (4 mg/day) for the initial four weeks in the mornings before breakfast. The dose was then increased to 8 mg/day from weeks 5 through 12. The subjects were seen at the outpatient clinical research unit at four weekly intervals following a 10- to 12-hour overnight fast. Each subject was instructed to perform a self-glucose monitoring with a portable glucose meter. Patients had a history and physical examination performed at each visit. During each visit the subjects were also weighed, and feet were examined to detect peripheral edema. Fasting blood samples were drawn for fasting glucose, HbA1C, lipids and lipoproteins, and routine liver and renal function tests at baseline and after 3 months of rosigli-

Table 1. Clinical and biochemical characteristics of African-American subjects with normal glucose tolerance and African Americans with glucose intolerance(GIT) treated with rosiglitazone

	NGT(n=10)	GIT (n=6)		*P* value*	*P* value for GIT
Parameter	**Controls**	**Baseline**	**Rosiglitazone**	**GIT vs NGT**	**Pre- vs Post-treatment**
Age(years)	43 ± 7.7	45 ± 12	—	0.680	—
Sex(f/m)	11	6	—	—	—
Ht(m)	1.64 ± 0.05	1.66 ± 0.08	—	0.497	—
B.Weight(kg)	85.5 ± 13.2	99.6 ± 10.79	99.5 ± 8.9	0.041	0.986
BMI(kg/m^2)	32.2 ± 5.1	35.78 ± 3.83	35.9 ± 3.6	0.153	0.964
BFM(%)	47.45 ± 4.45	48.68 ± 3.59	46.4 ± 5.3	0.505	0.278
SBP	129.7 ± 12.7	127.3 ± 11.5	127.8 ± 15.3	0.765	0.980
DBP	82.6 ± 4.7	76.0 ± 5.5	72.0 ± 12.8	0.020	0.482
Biochemistry					
Glucose(mg/dL)					
Fasting	82 ± 12	118 ± 13	92 ± 18	0.001	0.020
2 hour	82 ± 17	188 ± 55	124 ± 55	0.001	0.070
Insulin (μU/mL)					
Fasting	16.4 ± 15.7	16.7 ± 4.7	15.1 ± 4.7	0.885	0.504
2 hour	60 ± 28	102 ± 76	81 ± 53	0.112	0.591
C-peptide(ng/mL)					
Fasting	3.18 ± 1.24	4.03 ± 0.78	4.25 ± 1.33	0.151	0.730
2 hour	9.73 ± 3.25	11.32 ± 3.52	12.16 ± 4.37	0.363	0.760
HOMA-IR	3.21 ± 2.63	4.86 ± 1.31	3.53 ± 1.62	0.095	0.149
HOMA-%B	176 ± 125	115 ± 43	154 ± 88	0.270	0.305
HbA1C (%)	5.34 ± 0.42	6.93 ± 0.69	6.12 ± 0.91	0.001	0.68
Lipids and lipoproteins (mg/dL)					
Total cholesterol	179 ± 20	243 ± 46	196 ± 38	0.001	0.0831
Triglycerides	71 ± 20	135 ± 47	111 ± 44	0.001	0.383
HDL-C	52.5 ± 15.1	43.0 ± 13.0	47.5 ± 12.1	0.214	0.597
LDL-C	112 ± 19	138 ± 35	126 ± 25	0.063	0.476

Values are mean +/− SD; * GIT at baseline

tazone monotherapy. The OGTT and adipose tissue biopsies were repeated at 3 months of rosiglitazone treatment in the glucose-intolerant group.

Analytical Methods

Serum glucose concentrations were measured by the glucose oxidase method with a glucose autoanalyzer (Yellow Spring Instruments, Yellow Spring, Ohio). Serum insulin and C-peptide levels were determined by a standard double antibody radioimmunoassay technique at The Core Laboratories of The Ohio State University Hospitals, Columbus, Ohio. The sensitivity of the insulin assay was 2.5 μU/mL. The intra- and inter-assay coefficients of variation (CV) were 6% and 10%, respectively. The lower limit of the C-peptide assay was 0.47 ng/mL, and the intra- and inter-assay CV were 7% and 13%, respectively. The HbA1C level was measured by the immuno-based method (DCA 2000, Bayer Corporation, Indianapolis, Ind). The normal reference range was 3.6%–6.1%. Serum cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured with enzymatic methods. The intra-assay coefficient of variation for all the lipids and lipoproteins is <5% in our laboratory. Routine serum electrolytes, liver function tests, hematocrit, and hemoglobin were measured with readily available commercial tests.

Measurement of Plasma Adiponectin and TNF-α Levels

Adiponectin was measured by enzyme-linked immunosorbent assay (ELISA) in EDTA plasma by using a commercial assay kit (B-Bridge International Inc, Minneapolis, Minn). The inter-assay variability was 5.78%, and the intra-assay variability was 3.65%. TNF-α was measured by ELISA. The intra-assay variability was 12.4%, and the inter-assay variability was 15.3% for plasma samples.

Measurement of Adipose Tissue Adiponectin and TNF-α mRNA Content

RNA Isolation from Adipose Tissue. We used the RNeasy Lipid Tissue mini-kit from Qiagen to process adipose tissue RNA extraction. The tissue was put directly into QIAzol lysis liquid reagent provided by the kit and homogenized. After addition of chloroform, the homogenate was separated into aqueous and organic phases by centrifugation. The upper, aqueous phase was extracted, and ethanol was added to provide appropriate binding conditions.

The sample was then applied to the RNeasy Mini Spin Column, and high-quality RNA was eluted. This sample was then used for RNA quantitation by 260/280 optical density (OD) and for cDNA processing.

cDNA Synthesis. The cDNA was generated from each study sample by using Invitrogen's Superscript First-Strand Synthesis System for real time, reverse transcription-polymerase chain reaction (RT-PCR). The RT-PCR reactions were performed by using 0.5–1 mg of total RNA, depending on the yield of the sample.

TaqMan primers and probes were designed for adiponectin, TNF-α and β-actin. The copy number for each data point was normalized to its corresponding β-actin copy number to give a unitless value.

The sequences of the primers and probes used are shown below:

Adiponectin Forward Sequence Detection Primer: 5′-GCTCTGTGCTC-CTGCATCTG-3′

Adiponectin Reverse Sequence Detection Primer: 5′-ACGCTCTCCTT-CCCCATACA- 3′

Adiponectin TaqMan Probe: 5′-FAM-AGGTGGGCGACCAAGTCTGG-CTC-TAMRA-3′

β-Actin Forward Sequence Detection Primer: 5′-GAGCTACGAGCTG-CCTGACG-3′

β-ActinReverseSequenceDetectionPrimer: 5′-GTAGTTTCGTGGATG-CCACAGGACT-3′

β-Actin TaqMan Probe: 5′-FAM-CAT-CACCATTGGCAATGAGCGGT-TCC-TAMRA-3′

TNF-α Forward Sequence Detection Primer: 5′-GCCCTGGTATGAG-CCCATCT-3′

TNF-α Reverse Sequence Detection Primer: 5′-GCCGATTGATCTCA-GCGCT-3′

TNF-α TaqMan Probe: 5′-FAM-AGTCGGTCACCCTTCTCCAGC-TGGA-TAMRA-3′ (anti-sense based on mRNA[coding region] of the gene)

Calculations and Statistical Analyses

Results are expressed as mean ± standard deviation (SD), unless stated otherwise. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Obesity was defined as BMI >30 kg/m^2 for both females and males. Insulin resistance and beta-cell function were calculated by using homeostasis model assessment (HOMA).[24] Insulin resistance index (HOMA-IR) was calculated by using the homeostasis model assessment as follows: fasting insulin (μU/mL) × fasting plasma glucose (mmol/mL)/22.5. HOMA-derived, beta cell function (HOMA-%B) was calculated by the formula: 20 × fasting insulin (μU/mL)/fasting glucose (mmol/mL) −3.5.[24]

Statistical analyses were performed by using Student *t* test (paired) within the group analyses and unpaired *t* test between the groups and ANOVA with repeated measures, where appropriate. Bonferroni method was used for post-hoc testing. The nonparametric data were analyzed with chi-square. The relationships of adiponectin, TNF-α, insulin resistance, beta-cell function, fasting insulin, and body composition variables, as well as lipids and lipoproteins, were calculated by using least-square method as well as stepwise linear regression. For comparison of the mean data with unequal variance, Neuman-Keuls Multiple *t* test was used. Probability (P) value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

As shown in Table 1, the mean body weight and BMI were significantly higher in the glucose-intolerant (impaired glucose tolerance + type 2 diabetes) group than in the control (normal glucose tolerance) group. During rosiglitazone therapy, the mean body weight and BMI were not significantly changed in the glucose-intolerance group. Rosiglitazone treatment was well tolerated without any weight gain, pitting edema, or liver function or hematologic abnormalities in the glucose-intolerant group.

Effects of Rosiglitazone

As shown in Table 1, the glucose-intolerant group had significantly higher fasting and postprandial serum glucose levels when compared with the control group at baseline. Both fasting and two-hour serum glucose levels during OGTT were significantly decreased by rosiglitazone treatment in the glucose-intolerant group when compared to baseline (Table 1). Furthermore, HbA1C was higher in the glucose-intolerant group when compared to controls at baseline. Rosiglitazone treatment insignificantly decreased HbA1C by 13% when compared with the baseline values in the glucose-intolerant group (Table 1), perhaps because of the short duration or small sample size.

As shown in Table 1, mean fasting and two-hour serum insulin during OGTT were not different between the groups. Fasting serum insulin was not changed by rosiglitazone treatment (15.1 ± 4.7 vs 16.7 ± 4.7 uU/ml, $P>0.05$). Mean two-hour serum insulin during OGTT slightly decreased (81.1 ± 53.1 vs 102 ± 76 uU/ml, $P>0.05$), but differences were not statistically significant. The serum C-peptide levels followed a trend similar to those of serum insulin responses in the glucose-intolerant group (Table 1). Rosiglitazone treatment had no significant effect on beta-cell function. Insulin resistance was 30% higher in the glucose-intolerant than in the control group at baseline. Rosiglitazone significantly reduced insulin resistance by 27% at three months when compared with baseline.

Effects of Rosiglitazone on Serum Lipids and Lipoproteins

Fasting serum cholesterol, low-density lipoprotein cholesterol (LDL-C),

Table 2. Clinical and biochemical characteristics of African-American subjects with normal glucose tolerance(NGT) and those treated with rosiglitazone

	NGT	GIT		P value*	P value for GIT
Parameter	Healthy Controls	Baseline	3 months	GIT vs NGT	Pre- vs Post-treatment
Plasma					
TNF-α (ng/mL)					
0 mins	2.09 ± 0.36	3.18 ± 2.07	2.58 ± 1.23	0.101	0.555
120 mins	2.13 ± 0.37	2.98 ± 1.87	2.41 ± 1.09	0.121	0.533
Adiponectin (μg/mL)					
0 mins	13.15 ± 7.57	7.35 ± 2.68	13.37 ± 4.81	0.053	0.025
120 mins	12.88 ± 7.21	6.94 ± 2.54	12.65 ± 4.40	0.057	0.020

Values are mean ± SD; GIT=IGT+DM. * GIT at baseline

and triglycerides were significantly higher in the glucose-intolerant group. Following rosiglitazone monotherapy, serum cholesterol and triglycerides were decreased, but the differences did not reach statistical significance when compared to the baseline. Mean serum HDL-C and LDL-C levels were not significantly different between groups. Rosiglitazone had no significant effect on both lipoprotein parameters when compared to baseline.

Effects of Rosiglitazone on Plasma Adiponectin

Mean plasma adiponectin levels at fasting and two-hour level during OGTT were significantly lower in the glucose-intolerant group at baseline than in the control group (7.35 ± 2.68 vs 13.15 ± 4.81 μg/mL, $P<.05$). As shown in Table 2, rosiglitazone treatment significantly increased plasma adiponectin levels by approximately two-fold from 7.35 ± 2.68 to 13.37 ± 4.81 μg/mL at fasting and from 6.94 ± 2.54 to12.65 ± 4.40 μg/mL at two hours after OGTT in the glucose-intolerant patients ($P<.01$) at three months.

Effects of Rosiglitazone on Adipose Tissue Adiponectin mRNA

The mean adipose tissue adiponectin mRNA expression was not different between groups at baseline (3.87 ± 2.14 vs 3.15 ± 3.36). Following 12 weeks of rosiglitazone, mean adiponectin mRNA expression tended to be higher in glucose-intolerant patients, but the difference did not reach statistical significance when compared to baseline values (5.05 ± 1.87 vs 3.87 ± 2.14, $P=.333$). The fold change for adiponectin mRNA did not change after rosiglitazone therapy in the glucose-intolerant group (Table 3).

Effects of Rosiglitazone on Plasma TNF-α

Mean fasting plasma TNF-α was not significantly different between groups at baseline (0 minutes: 3.18 ± 2.07 vs 2.09 ± 0.36 ng/mL, 120 minutes: 2.98 ± 1.87 vs 2.13 ± 0.37 ng/mL, P=ns). Following 12 weeks of rosiglitazone, mean TNF-α did not significantly change in the glucose-intolerant subjects ($P=.15$) when compared to baseline values (Table 2).

Effects of Rosiglitazone on Adipose Tissue TNF-α mRNA

The mean adipose tissue TNF-α mRNA expression was not significantly different between groups at baseline (6.00 ± 2.84 vs 3.53 ± 3.88). Rosiglitazone had no significant effect on mean adipose tissue TNF-α mRNA in the glucose-intolerant group when compared to baseline values (7.12 ± 0.96 vs 6.00 ± 2.84, $P=.382$). The fold change for TNF-α mRNA did not change after rosiglitazone therapy in the glucose-intolerant patients (Table 3).

Relationships of Adiponectin and TNF-α Levels and Metabolic and Obesity Parameters

Plasma adiponectin correlated with insulin resistance ($r=-0.502$, $P=.048$)

Table 3. Adipose Tissue m RNA Expression in healthy controls with NGT and in GIT subjects treated with rosiglitazone

	NGT	IGT +DM		*P* value*	*P* value for GIT
Parameter	Healthy Controls	Baseline	3 months	GIT vs. NGT	Pre- vs. Pre-ROSI
Adipose Tissue mRNA×10^3					
TNF-a	3.53 ± 2.88	6.00 ± 2.84	7.12 ± 0.96	0.110	0.382
Adiponectin	3.15 ± 3.36	3.87 ± 2.14	5.05 ± 1.87	0.644	0.333
B-Actin	1.58 ± 1.69	2.65 ± 1.50	3.73 ± 6.04	0.215	0.680

Values are mean +/− SD. * GIT at baseline

and beta-cell function ($r=-0.498$, $P=.042$) but not percentage body fat ($r=-0.368$, $P<.068$), BMI ($r=-.068$, $P=.79$), HbA1C, insulin, C-peptide, or age. Plasma TNF-α did not significantly correlate with BMI, insulin resistance, and beta-cell function. We found a negative relationship between plasma adiponectin and TNF-α.

DISCUSSION

Health disparities in Blacks and Whites can not be fully explained by the current conventional risk factors, eg, lipoproteins, obesity, etc. Thus, examining both conventional and nonconventional risk factors for insulin resistance could explain the dissociation between conventional markers for insulin resistance and body composition. Understanding of these dichotomies could also help develop prevention and therapeutic strategies for African-American patients. To this end, our study was performed in subjects who have genetic predisposition to type 2 diabetes (by family history) but manifested varying degrees of glucose tolerance and insulin resistance.

We focused on two adipocytokines, adiponectin and TNF-α, with actions that appear to be diametrically opposite on cardiovascular risk factors, cardiovascular disease (CVD), and type 2 diabetes. Adiponectin is associated with reduced prevalence of type 2 diabetes and CVD, while TNF-α is associated with higher rates of type 2 diabetes and CVD.

In the present study, rosiglitazone monotherapy improved overall glycemic control as assessed by fasting and two-hour postprandial glucose and HbA1C mostly in the glucose-intolerant group.

Effects of Rosiglitazone on Glucose, Insulin and C-peptide and Insulin Resistance

In the present study, rosiglitazone monotherapy improved overall glycemic control as assessed by fasting and two-hour postprandial glucose and HbA1C mostly in the glucose-intolerant group. The mean fasting and post-glucose beta-cell secretion (absolute and incremental serum insulin and C-peptide levels) were, however, not significantly changed by rosiglitazone therapy in the glucose-intolerant African-American patients. In addition, beta-cell function did not significantly change after three months of rosiglitazone therapy. This finding is consistent with several previous studies that have confirmed the efficacy of thiazolidinediones in patients with type 2 diabetes.[23,25–27] Rosiglitazone treatment was associated with lower fasting and/or two-hour serum glucose during OGTT. However, it could be inferred that rosiglitazone enhanced beta-cell responsiveness to glucose stimulation by undefined mechanisms. This finding was confirmed by our insulin resistance data. Indeed, insulin resistance was significantly reduced by 30% during rosiglitazone therapy in the glucose-intolerant group at three months.

Effects of Rosiglitazone on Adiponectin Levels

Plasma adiponectin has been associated with increased insulin sensitivity and could potentially prevent impaired glucose tolerance, type 2 diabetes, and coronary artery disease.[7,8,12,13] Adiponectin level is reported to be reduced in patients with obesity and insulin resistance and hence could predict future development of type 2 diabetes.[2,3] We and others have shown that thiazolidinediones (troglitazone, rosiglitazone, and pioglitazone) increase adiponectin secretion.[4,5,10] In addition, adiponectin has direct insulin-sensitizing effects, ie, suppression of hepatic glucose production and could be responsible for some of the thiazolidinediones' pharmacologic properties.[11]

The most important finding in our present study was that plasma adiponectin levels were significantly lower in the obese, first-degree relatives of glucose-intolerant African-American patients when compared to the normal glucose tolerant group. Thus, similar to our previous studies,[10] we have extended the previous findings in non-Black populations that showed that adiponectin levels were lower in patients with impaired glucose tolerance and type 2 diabetes to African Americans. Rosiglitazone increased the adiponectin levels by two-fold at fasting and two hours after oral glucose challenge in the GIT group. We found a negative relationship between adiponectin and insulin resistance but not with serum glucose, insulin, C-peptide, and HbA1C in our population. We postulate that adiponectin could mediate the tissue insulin sensitization during rosiglitazone therapy in glucose-intolerant African Americans.[4,5] In addition, we speculate that adiponectin could be important in the etiology of insulin resistance in African Americans predisposed to type 2 diabetes.

Effects of Rosiglitazone on Plasma TNF-α

TNF-α levels are elevated in obese subjects and are reduced following weight loss, chronic exercise, and thiazolidinedione therapy in obese and nonobese patients.[14–17] In the present study, we found that plasma TNF-α was not significantly different in the glucose-intolerant group when compared to the control group at baseline. Furthermore, rosiglitazone therapy was not associated with significant decreases in plasma TNF-α in the glucose-intolerant group. The reasons for the lack of suppression of TNF-α by rosiglita-

zone in African Americans with impaired glucose tolerance and type 2 diabetes is unclear. This issue is important since some previous in vitro studies have demonstrated that rosiglitazone suppresses TNF-α gene expression or its actions, but this finding remains controversial. Indeed, Moore et al[23] have reported differential regulation of various adipocytokines by rosiglitazone in db/db obese mice.

Effects of Rosiglitazone on Adipose Tissue Adiponectin and TNF-α mRNA

Recent studies have found that modulation of adiponectin and TNF-α mRNA content in various tissues are partly under the influence of PPAR-ε agonists.[14–22] We examined the role of adipose tissue adiponectin and TNF-α gene regulation in African Americans with and without glucose intolerance. In addition, we also employed thiazolidinediones in our study since previous studies have shown that these agents increase adipose tissue adiponectin mRNA[14–17] and reduce TNF-α and interleukin-6 mRNA in the other[19–22] in several populations. Rosiglitazone increased plasma adiponectin levels but had no significant effects on adipose tissue mRNA in African Americans at high risk for type 2 diabetes. Thus, the increasing plasma adiponectin levels could be due to posttranscriptional and posttranslational effects of rosiglitazone. Most importantly, rosiglitazone treatment had no significant effects on either plasma TNF-α levels or adipose tissue TNF-α mRNA in glucose-intolerant African Americans. Adiponectin may have suppressed the adipose tissue TNF-α gene expression as mRNA. The extent of adipocytokine-on-adipocytokine regulation in African Americans remains to be investigated. We are tempted to conclude that TNF-α is unlikely to be involved in the development of type 2 diabetes in African Americans. In addition, we have also inferred that the metabolic effects of rosiglitazone are unlikely to be mediated by TNF-α in African Americans. Whether TNF-α was suppressed by adiponectin was not tested in our present study. Nevertheless, we found that adiponectin negatively correlated with TNF-α in our study.

In summary, we report the first systematic study on the regulation of plasma and adipose tissue adiponectin and TNF-α in high-risk African Americans with and without glucose intolerance. Our study revealed diverse effects of rosiglitazone on adiponectin and TNF-α. Based on our findings, we conclude that metabolic effects of rosiglitazone could be partly mediated by increases in adiponectin, but not the concurrent suppression of TNF-α, in African Americans with impaired glucose tolerance and/or type 2 diabetes. The lack of effects of rosiglitazone on adiponectin and TNF-α gene expression in glucose-intolerant African Americans were surprising and deserve further investigation. Whether our findings are unique to African Americans deserves further studies in larger populations of African Americans and other different ethnic groups.

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