CURRENT THERAPEUTIC RESEARCH®
VOLUME 65, NO. 1, JANUARY/FEBRUARY 2004

Effects of Rosiglitazone Treatment on the Pentose Phosphate Pathway and Glutathione-Dependent Enzymes in Liver and Kidney of Rats Fed a High-Fat Diet

Esen Akbay, MD,[1] Nuriye Nuray Ulusu, PhD,[2] Füsun Törüner, MD,[3] Göksun Ayvaz, MD,[3] Ferit Taneri, MD,[4] Müjde Aktürk, MD,[3] Metin Arslan, MD,[3] and Çimen Karasu, PhD[5]

[1]Department of Endocrinology, Faculty of Medicine, Mersin University, Mersin, Turkey, [2]Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey, and Departments of [3]Endocrinology-Metabolism, [4]Surgery, and [5]Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey

ABSTRACT

Background: Animals fed high-fat diets have been shown to develop hyperglycemia, insulin resistance, hyperlipidemia, and moderate obesity, which resemble the human metabolic syndrome. Obesity, the metabolic syndrome, and some thiazolidinediones, which act as insulin sensitizers, may increase oxidative stress, and/or influence the levels of cellular reducing equivalents and homeostasis.

Objective: This study investigated the effects of a high-fat diet, rosiglitazone, or a high-fat diet plus rosiglitazone on metabolic syndrome parameters and crucial liver and kidney enzyme activities in rats.

Methods: Male Wistar rats were assigned to 4 groups (n = 6 per group): (1) the fat (F) group was fed a rodent diet comprising 45 kcal% fat, (2) the rosiglitazone (R) group was fed a standard rat chow comprising 4.97 kcal% fat plus rosiglitazone (3 mg/kg·d), (3) the fat + rosiglitazone (FR) group was fed a rodent diet comprising 45 kcal% fat (as lard, product D12451) plus rosiglitazone (3 mg/kg·d), and (4) the control (C) group was fed a standard rat chow comprising 4.97 kcal% fat. Animals were housed for 4 weeks, at which time the liver and kidney were isolated for spectrophotometric determination of enzyme activities. Body weight was measured before treatment (baseline) and then weekly throughout the study. Adiposity was measured at the end of the 4 weeks.

Results: The activities of glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione reductase (GR), and glutathione-*S*-transferase (GST) were significantly reduced in the livers of groups F, R, and FR compared with group C (all $P < 0.05$). Kidney G6PD, 6-PGD,

Accepted for publication December 8, 2003.

0011-393X/04/$19.00

and GR were found to be significantly lower in group R compared with the other groups (all $P < 0.05$). Kidney GST was similar in all groups. Plasma glucose, triglyceride, and insulin concentrations were significantly higher than in group F versus the other groups (all $P < 0.05$). Adiposity was increased in groups F and FR compared with groups C and R (all $P < 0.05$). Serum cholesterol concentrations were similar in all groups.

Conclusions: In this study, high-fat diet in rats decreased the enzyme activities responsible for pentose phosphate pathway and glutathione-dependent metabolism in liver but not in kidney. Similarly, these enzyme activities were inhibited with rosiglitazone treatment alone in both organs. (*Curr Ther Res Clin Exp.* 2004;65:79–89)

Key words: rosiglitazone, pentose phosphate pathway, glutathione-dependent metabolism, metabolic syndrome, high-fat diet, rat, obesity.

INTRODUCTION

Abnormalities in tissue oxidative status and antioxidant defense may be associated with a number of diseases.[1] Glutathione (reduced thiol form) (GSH) and GSH-related enzymes—glutathione peroxidase, glutathione-*S*-reductase (GR), and transferase (GST)—play a crucial role in the cellular defense against free-radical attacks.[2–4] GSH is a substrate for GST, and the regeneration of GSH from oxidized glutathione (GSSG) depends on the activity of nicotinamide adenine dinucleotide phosphate (NADPH)–dependent GR.[5] The principal enzymes involved in the pentose phosphate pathway are glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6-PGD),[6] which produce NADPH, a cofactor for GR and other NADPH-dependent oxidases. G6PD and 6-PGD are also considered lipogenic enzymes because NADPH is used as an electron donor for the synthesis of fatty acids.

Laboratory animals fed a high-fat diet have demonstrated hyperglycemia, insulin resistance, hyperlipidemia, and moderate obesity, which resemble the human metabolic syndrome.[7] Obesity or metabolic syndrome has been shown to increase oxidative stress, resulting in decreased levels of reducing equivalents of cells.[8,9] Thiazolidinediones induce peroxisome proliferator–activated receptor-γ (PPAR-γ), decrease insulin resistance, improve insulin sensitivity, and reduce blood glucose levels without stimulating insulin secretion. Troglitazone was the first thiazolidinedione drug to be approved by the US Food and Drug Administration (1999) for the treatment of type 2 diabetes mellitus,[10–12] but it was removed from the US market in March 2000 due to liver failure and death in some patients. Troglitazone and some peroxisome proliferators have been shown to affect tissue oxidative and antioxidant status, and may cause oxidative stress through redox cycling processes.[13–15] However, according to a MEDLINE search (key terms: *rosiglitazone, oxidative stress, antioxidant defense, antioxidant enzymes, NADPH, pentose phosphate pathway, glucose-6-phosphate*

dehydrogenase, and *glutathione*; years: no limits), previous studies have demonstrated conflicting results for the effects of rosiglitazone, the second drug in this class to be approved, on oxidative stress and antioxidant defense, and evidence of NADPH-producing metabolic pathways for rosiglitazone is unavailable.[15,16]

The aim of this study was to investigate the effects of rosiglitazone treatment, alone or in combination with a high-fat diet, on liver and kidney enzyme activity related to the pentose phosphate pathway and the elimination of reactive oxygen species, and on some metabolic syndrome parameters. We developed a metabolic syndrome model in Wistar rats fed a high-fat diet.

MATERIALS AND METHODS

This 4-week, randomized, controlled study was performed at the Department of Endocrinology, Faculty of Medicine, Gazi University (Ankara, Turkey). Approval for the study protocol was provided by the Animal Ethics Committee of the Gazi University.

Male Wistar rats aged 10 to 11 weeks were used in the study. The rats were adapted to the laboratory housing conditions for 1 week before the experiment was started. After 1 week, based on a computer-generated randomization list, 24 rats were randomly assigned to 1 of 4 groups (n = 6 per group): (1) the fat (F) group was fed a rodent diet comprising 45 kcal% fat (as lard, product D12451, Research Diets, Inc., New Brunswick, New Jersey), (2) the rosiglitazone (R) group was fed a standard rat chow comprising 4.97 kcal% fat plus rosiglitazone (3 mg/kg·d), (3) the fat + rosiglitazone (FR) group was fed a rodent diet comprising 45 kcal% fat (as lard, product D12451, Research Diets, Inc.) plus rosiglitazone (3 mg/kg·d), and (4) the control (C) group was fed a standard rat chow comprising 4.97 kcal% fat.

Animals were housed for 4 weeks, and diets and water were provided ad libitum during the study period. Rosiglitazone was prepared by dissolution in 0.5% carboxymethyl cellulose solution and administered by intraoral gavage. Control animals were given an equal volume of 0.5% carboxymethyl cellulose. Body weight was measured before treatment (baseline) and then weekly throughout the study. Adiposity was measured at the end of the 4-week study period by weighing the retroperitoneal and epididymal fat pads and summing them.

Blood Sampling and Tissue Preparations

For blood sampling and tissue preparation, the rats were anesthetized with ketamine hydrochloride (40 mg/kg intraperitoneally) after 4 hours of fasting. Blood was obtained using cardiac puncture. Plasma was separated using centrifugation at 3000*g* at room temperature for 15 minutes and stored at –80°C until assay for ≤3 months. Livers and kidneys were removed and immediately washed in ice-cold 0.09% sodium chloride solution, blotted dry, and frozen in

liquid nitrogen with subsequent storage at –80°C. The abdominal fat pads were removed and weighed.

Blood Biochemistry

Plasma glucose, total cholesterol, and triglyceride levels were determined using Aeroset clinical chemistry kits (Abbott Laboratories, Abbott Park, Illinois). Plasma insulin was assayed using radioimmunoassay kits (LINCO Research, Inc., St. Charles, Missouri).

Measurements of Enzyme Activities in Liver and Kidney Homogenates

Livers and kidneys were homogenized with 3 volumes of 50 mM potassium phosphate buffer, pH 7.4, in an Ultra-Turrax homogenizer (GENEQ inc., Montreal, Quebec, Canada) and centrifuged at 14,000*g* at 4°C for 20 minutes, and supernatants were used for assays.

Enzyme activities and protein determinations were studied using an LKB Ultraspec Plus (4054 UV/visible) spectrophotometer (Analytical Instruments, LLC, Golden Valley, Minnesota). All assays were run in triplicate for each supernatant of the liver.

Glucose-6-Phosphate Dehydrogenase Activity

G6PD activity was determined by monitoring NADPH production at 340 nmol/L and at 37°C.[17] The assay mixture contained 10 mM magnesium chloride, 0.2 mM $NADPH^+$, and 0.6 mM G6PD in 100 mM Tris-hydrochloride buffer solution, pH 8.0. Assays were carried out in duplicate and the activities were followed up for 40 seconds. One unit of activity (U) is the amount of enzyme required to reduce 1 µmol/min of $NADPH^+$ under the assay conditions. Specific activity is defined as units per gram of protein.

6-Phosphogluconate Dehydrogenase Activity

6-PGD activity was measured by substituting 0.6 mmol/L 6-phosphogluconate as substrate in the previously mentioned assay mixture for G6PD measurement.[18] Because 6-PGD also catalyzed the production of NADPH, in the earlier steps of the purification, both G6PD and 6-PGD activities were determined as total, and the initial velocity of G6PD was calculated by subtracting the 6-PGD activities.

Glutathione-S-Transferase Activity

GST activity was determined using 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 0.1 M potassium phosphate buffer, pH 6.5, and tissue supernatants. The linear increase in absorbance at 340 nm was monitored at 37°C. One enzyme unit is defined as 1 µmol CDNB-conjugate formed per minute (extinction coefficient of 9.6 mM·cm).[19]

Glutathione Reductase Activity

GR activity was measured according to a modified Stall method.[20] The incubation mixture contained 100 mM sodium phosphate buffer, pH 7.4, 1 mM GSSG, 100 μmol/L reduced NADPH, and the tissue supernatants. Decrease in the absorbance of NADPH at 340 nm was monitored using spectrophotometry at 37°C. A unit of activity (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH in 1 minute under these conditions.

Protein Determination

Proteins were measured using the Bradford method.[21] Bovine serum albumin was used as the standard.

Statistical Analysis

Statistical analyses were performed using the paired *t* test to compare the mean baseline body weight with the mean final body weight. Analysis of variance was performed, followed by the Tukey test to estimate the significance of differences for between-group comparisons. Results were considered significantly different if $P \leq 0.05$. All analyses were performed using GraphPad Prism software version 3 (GraphPad Software, Inc., San Diego, California).

RESULTS

Liver Enzyme Activity

Significantly less G6PD, 6-PGD, GR, and GST activity was measured in groups F, R, and FR compared with group C (all $P < 0.05$; **Table I**).

Kidney Enzyme Activity

G6PD, 6-PGD, and GR activity was found to be significantly less in group R compared with the other groups (all $P < 0.05$). Kidney GST activity was not statistically different between the groups (**Table II**).

Table I. Mean (SEM) enzyme activities (U/mg protein) in liver study rats (N = 24).

Enzyme	Fat (n = 6)	Rosiglitazone (n = 6)	Fat + Rosiglitazone (n = 6)	Control (n = 6)
G6PD	0.0267 (0.0036)*	0.0237 (0.0016)*	0.0216 (0.0022)*	0.0497 (0.0038)
6-PGD	0.0467 (0.0026)*	0.0434 (0.0023)*	0.0369 (0.0027)*	0.0678 (0.0045)
GST	0.8750 (0.0229)†	0.8936 (0.0566)†	0.8160 (0.1370)†	1.4297 (0.1511)
GR	0.0763 (0.0043)†	0.0767 (0.0055)†	0.0747 (0.0047)†	0.1198 (0.0160)

G6PD = glucose-6-phosphate dehydrogenase; 6-PGD = 6-phosphogluconate dehydrogenase; GST = glutathione-*S*-transferase; GR = glutathione reductase.
*$P < 0.001$ versus control group (analysis of variance [ANOVA]).
†$P < 0.01$ versus control group (ANOVA).

Table II. Mean (SEM) enzyme activities (U/mg protein) in kidney study rats (N = 24).

Enzyme	Fat (n = 6)	Rosiglitazone (n = 6)	Fat + Rosiglitazone (n = 6)	Control (n = 6)
G6PD	0.0706 (0.0021)*	0.0403 (0.0019)	0.0695 (0.0049)†	0.0715 (0.0088)*
6-PGD	0.0617 (0.0040)*	0.0407 (0.0007)	0.0636 (0.0056)*	0.0601 (0.0040)*
GST	0.4781 (0.0074)	0.3175 (0.0159)	0.4308 (0.3330)	0.4585 (0.0954)
GR	0.3039 (0.0102)‡	0.1701 (0.0073)	0.2847 (0.0205)‡	0.2635 (0.0384)‡

G6PD = glucose-6-phosphate dehydrogenase; 6-PGD = 6-phosphogluconate dehydrogenase; GST = glutathione-*S*-transferase; GR = glutathione reductase.
*$P < 0.01$ versus rosiglitazone group (analysis of variance [ANOVA]).
†$P < 0.001$ versus rosiglitazone group (ANOVA).
‡$P < 0.05$ versus rosiglitazone group (ANOVA).

Body Weights and Adiposity

Mean baseline body weights were similar between the groups (**Table III**). At the end of the 4 weeks, however, body weight was significantly increased in all groups (all $P < 0.01$) except group C. No significant differences in percentages of body weight gain were found between groups R (36.45%), FR (55.72%), and F (42.96%), but all were significantly higher compared with group C (8.08%) ($P < 0.05$). After the 4-week period, adiposity was found to be significantly increased in groups F and FR ($P < 0.05$); however, it did not significantly change in group R compared with group C.

Blood Biochemistry

Plasma glucose, total cholesterol, triglyceride, and insulin levels after 4-hour fasting, and between-group comparisons, are shown in **Table IV**. At the end of

Table III. Mean (SEM) body weights (g) and adiposity (g) of study rats (N = 24).

Parameter	Fat (n = 6)	Rosiglitazone (n = 6)	Fat + Rosiglitazone (n = 6)	Control (n = 6)
Baseline body weight*	220.0 (10.3)	216.6 (9.8)	218.3 (5.1)	218.3 (6.2)
Final body weight	313.3 (12.0)†[a]	301.6 (12.7)†[a]	340.0 (6.9)†[a]	236.0 (10.2)†[bcd]
Total adiposity	13.4 (1.7)	9.0 (0.8)‡	16.4 (1.4)	6.6 (0.4)†[bd]

*No significant between-group differences were found (analysis of variance [ANOVA]).
†$P < 0.01$.
[a]Versus baseline (paired *t* test).
[b]Versus fat group (ANOVA).
[c]Versus rosiglitazone group (ANOVA).
[d]Versus fat + rosiglitazone group (ANOVA).
‡$P < 0.05$ versus fat + rosiglitazone group (ANOVA).

Table IV. Mean (SEM) plasma lipid and insulin levels in study rats (N = 24).

Component	Fat (n = 6)	Rosiglitazone (n = 6)	Fat + Rosiglitazone (n = 6)	Control (n = 6)
Glucose, mg/dL	207.0 (17.1)	156.7 (7.8)*	162.1 (7.2)*	141.8 (6.3)*
Total cholesterol, mg/dL	50.8 (1.4)	51.6 (0.8)	47.2 (0.9)	56.3 (5.8)
Triglyceride, mg/dL	111.8 (9.4)	58.1 (6.0)†	57.8 (7.4)†	75.6 (6.1)*
Insulin, ng/mL	15.2 (2.0)	3.8 (1.0)†	3.1 (1.0)†	4.2 (0.9)†

*$P < 0.01$ versus fat group (analysis of variance [ANOVA]).
†$P < 0.001$ versus fat group (ANOVA).

the 4-week period, significantly higher plasma glucose, triglyceride, and insulin levels were detected in group F compared with the other groups (all $P < 0.05$). These parameters were not statistically different in group FR compared with group C. Plasma total cholesterol concentrations were similar between the groups.

DISCUSSION

In this study, we found that plasma glucose, triglyceride, and insulin concentrations were increased when the rats were fed a high-fat diet, as described in a previous study.[7] When given to normal (control) rats fed standard chow, rosiglitazone did not cause any changes in plasma glucose, triglyceride, or insulin concentrations. It has been shown that rosiglitazone induces body weight gain and adiposity in animals[22,23] and humans.[24] Similarly, we found that rosiglitazone treatment alone caused body weight gain and resulted in increased adiposity. Because of unchanged intra-abdominal fat pad mass (epididymal, mesenteric, and perirenal) after rosiglitazone treatment, this increased weight gain may be attributed to the increased subcutaneous adiposity in our study.

The activities of G6PD, 6-PGD, GR, and GST were found to be significantly decreased in the livers of rats fed a high-fat diet. Lipogenesis may affect the pentose phosphate pathway enzymes and leads to the reduction in G6PD and 6-PGD activity[25] that was observed in rats fed a high-fat diet in our study. Accordingly, high-fat diets have been shown to suppress the activity of lipogenic enzymes such as G6PD and malic enzymes, resulting in hepatic lipogenesis.[8] Another study[9] showed that the activity of antioxidant enzymes was decreased in liver microsomes from *ob/ob* mice characterized by obesity. In the kidney of rats, these enzymes maintain their normal activity levels despite a high-fat diet with or without rosiglitazone.

A novel finding of this study is the effect of rosiglitazone treatment alone. We observed that when the control rats were treated with only rosiglitazone, the

enzyme activity related to the pentose phosphate pathway or glutathione-dependent metabolism was decreased. Because the regeneration of GSH from GSSG depends on the presence of GR and NADPH in the environment, the low activity of both GR and the NADPH-generating enzymes, G6PD and 6-PGD, may lead to the reduction in GSH levels. The decrease in the activity of hepatic GR, G6PD, and 6-PGD in the presence of high fat, rosiglitazone, or both, may induce an increase in the tissue level of hydrogen peroxide, consequently creating oxidative stress because of decreased GSH.[14]

In support of our findings, it has been shown that the treatment of rats with peroxisome proliferators results in a decrease in the activity of GSH-related enzymes.[26] Peroxisome proliferators increase beta-oxidation in hepatic peroxisomes by means of activation of PPAR-α, leading to an increase in intracellular hydrogen peroxide concentration. Hydrogen peroxide may induce the formation of hydroxyl radicals and DNA damage by escaping from peroxisomes.[27] Although studies have emphasized the increasing effects of troglitazone on superoxide generation, membrane peroxidation, and intracellular GSH depletion in several cell types,[15,28] only limited information is available[29,30] on the propensity of rosiglitazone to act as a pro-oxidant. Moreover, rosiglitazone has been shown to neither increase membrane peroxidation nor induce loss of cell viability.[15] However, in vitro studies in cryopreserved human hepatocytes[29] and in fetal primary brown adipocytes[30] indicate the role of reactive oxygen species and depletion of intracellular GSH in rosiglitazone-induced cytotoxicity.

Because of the metabolism of xenobiotics by GST and the peroxidase activity of GST against organic hydroperoxides,[31] the decreased activity of hepatic GST as observed in our study may lead to insufficient detoxification. It has been shown that dietary lipids modulate toxicities and metabolism of xenobiotics,[32] and that obesity may induce some alterations in the oxidation and conjugation pathways in hepatocytes.[33] In addition, both hepatocyte GST activity and the level of GSH have been found to be substantially lower in obese mice than in nonobese ones.[34] Furthermore, more severe hepatic damage due to hepatotoxins has been observed in obese rats than in nonobese ones.[35]

On the other hand, rosiglitazone may indirectly reduce the activity of GST in rat liver, probably by causing obesity. Although we did not find a statistical difference among the groups, most likely owing to the small number of animals studied, it seems that rosiglitazone also inhibits GST activity in the kidney and in the liver. But, unlike the liver, the kidney may have a property that protects itself against degenerative factors in the presence of obesity, by maintaining normal activity of GST, G6PD, and 6-PGD.

Our study demonstrates that rosiglitazone is able to affect the cellular reducing equivalence homeostasis. This finding may have direct implications for obesity, the metabolic syndrome, and other pathologies where reductive/oxidant imbalance are considered major factors contributing to metabolic disorders, and where rosiglitazone is used therapeutically. These findings call for systematic investigations with large studies.

CONCLUSIONS

In this study, high-fat diet in rats decreased the enzyme activities responsible for pentose phosphate pathway and glutathione-dependent metabolism in liver but not in kidney. Similarly, these enzyme activities were inhibited with rosiglitazone treatment alone in both liver and kidney.

REFERENCES

1. Trevisan M, Browne R, Ram M, et al. Correlates of markers of oxidative status in the general population. *Am J Epidemiol.* 2001;154:348–356.
2. Sevanian A, Hochstein P. Mechanisms and consequences of lipid peroxidation in biological systems. *Annu Rev Nutr.* 1985;5:365–390.
3. Basaga HS. Biochemical aspects of free radicals. *Biochem Cell Biol.* 1990;68:989–998.
4. Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res.* 1999;31: 273–300.
5. Deneke SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol.* 1989;257: L163–L173.
6. Wood T. Physiological functions of the pentose phosphate pathway. *Cell Biochem Funct.* 1986;4:241–247.
7. Tallman DL, Taylor CG. Effects of dietary fat and zinc on adiposity, serum leptin and adipose fatty acid composition in C57BL/6J mice. *J Nutr Biochem.* 2003;14:17–23.
8. Chen LC, Boissonneault G, Hayek MG, Chow CK. Dietary fat effects on hepatic lipid peroxidation and enzymes of H_2O_2 metabolism and NADPH generation. *Lipids.* 1993; 28:657–662.
9. Watson AM, Poloyac SM, Howard G, Blouin RA. Effect of leptin on cytochrome P-450, conjugation, and antioxidant enzymes in the *ob/ob* mouse. *Drug Metab Dispos.* 1999; 27:695–700.
10. Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes.* 1996;45:1661–1669.
11. Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu Rev Biochem.* 2001;70:341–367.
12. Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: A critical review [published correction appears in *Clin Ther.* 2001;23:309]. *Clin Ther.* 2000;22:1151–1168.
13. Chen LC, Tatum V, Glauert HP, Chow CK. Peroxisome proliferator perfluorodecanoic acid alters glutathione and related enzymes. *J Biochem Mol Toxicol.* 2001;15:107–113.
14. O'Brien ML, Twaroski TP, Cunningham ML, et al. Effects of peroxisome proliferators on antioxidant enzymes and antioxidant vitamins in rats and hamsters. *Toxicol Sci.* 2001;60:271–278.
15. Narayanan PK, Hart T, Elcock F, et al. Troglitazone-induced intracellular oxidative stress in rat hepatoma cells: A flow cytometric assessment. *Cytometry.* 2003;52A:28–35.
16. Lennon AM, Ramauge M, Dessouroux A, Pierre M. MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-Delta(12-14)-prostaglandin J(2) and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor

gamma-independent mechanisms involving reactive oxygenated species. *J Biol Chem.* 2002;277:29681–29685.

17. Ulusu NN, Kus MS, Acan NL, Tezcan EF. A rapid method for the purification of glucose-6-phosphate dehydrogenase from bovine lens. *Int J Biochem Cell Biol.* 1999; 31:787–796.
18. Pearse BM, Rosemeyer MA. 6-Phosphogluconate dehydrogenase from human erythrocytes. *Methods Enzymol.* 1975;41:220–226.
19. Habig WH, Pabst MJ, Jakoby WB. Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974;249:7130–7139.
20. Acan NL, Tezcan EF. Sheep brain glutathione reductase: Purification and general properties. *FEBS Lett.* 1989;250:72–74.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72:248–254.
22. Shapiro R, Nuss ME, Kaszubska W, et al. Effects of treatment of C57BL/6J mice fed high fat vs. low fat diets with metformin or rosiglitazone on adiposity, food intake, hyperglycemia and insulin resistance. Poster presented at: 83rd Annual Meeting of the Endocrine Society; 2001; Denver, Colo.
23. Pickavance LC, Tadayyon M, Widdowson PS, et al. Therapeutic index for rosiglitazone in dietary obese rats: Separation of efficacy and haemodilution. *Br J Pharmacol.* 1999;128:1570–1576.
24. Carey DG, Galloway G, Dodrell D, et al. Rosiglitazone reduces hepatic fat and increases subcutaneous but not intra-abdominal fat depots. *Diabetologia.* 2000; 43(Suppl 1):A181. Abstract.
25. Ayala A, Fabregat I, Machado A. The role of NADPH in the regulation of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in rat adipose tissue. *Mol Cell Biochem.* 1991;105:1–5.
26. Ashby J, Brady A, Elcombe CR, et al. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum Exp Toxicol.* 1994;13(Suppl 2):S1–S117.
27. Reddy JK, Warren JR, Reddy MK, Lalwani ND. Hepatic and renal effects of peroxisome proliferators: Biological implications. *Ann N Y Acad Sci.* 1982;386:81–110.
28. Tettey JN, Maggs JL, Rapeport WG, et al. Enzyme-induction dependent bioactivation of troglitazone and troglitazone quinone in vivo. *Chem Res Toxicol.* 2001;14:965–974.
29. Lloyd S, Hayden MJ, Sakai Y, et al. Differential in vitro hepatotoxicity of troglitazone and rosiglitazone among cryopreserved human hepatocytes from 37 donors. *Chem Biol Interact.* 2002;142:57–71.
30. Teruel T, Hernandez R, Benito M, Lorenzo M. Rosiglitazone and retinoic acid induce uncoupling protein-1 (UCP-1) in a p38 mitogen-activated protein kinase-dependent manner in fetal primary brown adipocytes. *J Biol Chem.* 2003;278:263–269.
31. Lawrence RA, Parkhill LK, Burk RF. Hepatic cytosolic non selenium-dependent glutathione peroxidase activity: Its nature and the effect of selenium deficiency. *J Nutr.* 1978;108:981–987.
32. Ashida H, Nakai R, Kanazawa K, Danno G. Xenobiotic tolerance of primary cultured hepatocytes in rats fed a high-fat or high-protein diet. *J Nutr Sci Vitaminol (Tokyo).* 1998;44:89–102.

33. York JL, Wolff GL. Glutathione *S*-transferase activity and isoenzyme concentrations in obese *AVY/a* and lean *a/a* mice. *Proc Soc Exp Biol Med.* 1994;205:186–189.
34. Barnett CR, Abbott RA, Bailey CJ, et al. Cytochrome P-450-dependent mixed-function oxidase and glutathione *S*-transferase activities in spontaneous obesity-diabetes. *Biochem Pharmacol.* 1992;43:1868–1871.
35. Salazar DE, Sorge CL, Jordan SW, Corcoran GB. Obesity decreases hepatic glutathione concentrations and markedly potentiates allyl alcohol-induced periportal necrosis in the overfed rat. *Int J Obes Relat Metab Disord.* 1994;18:25–33.

Address correspondence to: Dr. Çimen Karasu, PhD, Department of Medical Pharmacology, Faculty of Medicine, Gazi University, 06510, Besevler, Ankara, Turkey. E-mail: karasu@gazi.edu.tr