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Effect of PPAR-γ Agonist on Adiponectin Levels in the Metabolic Syndrome: Lessons From the High Fructose Fed Rat Model

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Background: The health hazard of the metabolic syndrome (MS) is increasing, yet there is no effective pharmacologic treatment to this entity as a whole. Recently, hypoadiponectinemia was found to play an important role in the development of MS. We studied the effect of the PPAR-γ agonist rosiglitazone on adiponectin and the metabolic profile in the fructose-induced hypertensive, hyperinsulinemic, hypertriglyceridemic rat model.

Methods: Thirty male Sprague-Dawley rats were divided into three groups. Ten were fed standard rat chow for 5 weeks, 10, a fructose-enriched diet for 5 weeks, and 10, a fructose-enriched diet for 5 weeks, with rosiglitazone 10 mg/kg/d added during the last 2 weeks. Blood pressure (BP), oral glucose tolerance test (OGTT), plasma insulin, triglycerides, and adiponectin were recorded, as well as mRNA levels of the adiponectin gene in visceral adipose tissue.

Results: Fructose-fed rats developed MS as manifested by the increase in systolic BP (from 139 ± 3 to 158 ± 4 mm Hg, $P < .05$), insulin (from 26 ± 1.6 to 40 ± 2.5 μU/mL, $P < .05$), triglycerides (from 91 ± 9 to 304 ± 24 mg/dL, $P < .05$), and impaired OGTT (area under the curve from 13,894 ± 246 to 17,725 ± 700 mg/dL/min). Treatment with rosiglitazone reversed these effects and reduced BP to 133 ± 7 mm Hg, insulin levels to 30 ± 2.8 μU/mL, triglycerides to 116 ± 9 mg/dL, and the OGTT to 15,415 ± 372 mg/dL/min ($P < .05$ for all variables). In addition, rosiglitazone increased plasma levels of adiponectin fourfold from 4.3 ± 0.1 to 18.4 ± 0.6 μg/mL ($P < .05$). This increase was coupled with 3.8-fold increase in adiponectin mRNA in visceral adipose tissue.

Conclusions: This study shows for the first time that in an animal model of MS, the insulin sensitizer, rosiglitazone, improves the metabolic profile and increases plasma levels of adiponectin and its gene expression. It is possible therefore that rosiglitazone exerts its beneficial effects by increasing the levels of adiponectin. Am J Hypertens 2007;20:206–210 © 2007 American Journal of Hypertension, Ltd.

Key Words: Metabolic syndrome, adiponectin, PPAR gamma, rosiglitazone.

The prevalence of the metabolic syndrome (MS) and its cardiovascular consequences has increased dramatically during the past decade, yet effective pharmacologic treatment to this entity as a whole is still in search. The underlying key processes are obesity and the associated insulin resistance. Among adipose tissue hormones, adiponectin plays a central and unique role in the pathophysiology of the MS and its cardiovascular complications.[1,2] Adiponectin possesses antidiabetic, antiatherogenic, and anti-inflammatory properties that collectively establish its important vascular protective role.[3] Unfortunately, plasma levels of adiponectin are decreased in the MS in contrast to other known adipocyte-derived hormones.[4] Accordingly, therapeutic strategies aimed at increasing plasma adiponectin concentrations could potentially be beneficial in preventing the complications of the MS.[3]

Thiazolidinedions (TZDs) are specific synthetic ligand activators of peroxisome proliferators-activated receptor gamma (PPAR-γ) that improve glucose tolerance and insulin sensitivity in type 2 diabetic patients and in animal models of insulin resistance through mechanisms that are not completely understood. The PPAR-γ agonists are known to increase plasma levels of adiponectin in diabe-

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tes. However, the effect of PPAR-γ agonist on adiponectin levels in an animal model of the prediabetic MS has not been studied yet. Furthermore, its effect on the metabolic profile in such model of a prediabetic state needs further elucidation. For this purpose the fructose fed rat model was used. When given a high fructose diet for 3 weeks the genetically predisposed Sprague-Dawley rats develop the MS manifested by hypertension, hyperinsulinemia, and hypertriglyceridemia, as well as an impaired oral glucose tolerance test (OGTT). The aim of the study is to determine the effect of rosiglitazone on adiponectin levels, blood pressure (BP), and the metabolic profile in a rat model of the MS.

Methods

Animals and Study Design

An interventional comparative study was designed. The experiments were conducted according to the Guidelines for Animal Care and Treatment of the hospital's Animal Ethics Committee. Thirty male Sprague-Dawley rats (Harlan, Israel), weight 200 ± 20 g, were maintained in a temperature-controlled room (22°C) and kept on a 14/10 h light/dark cycle. Food and water were available ad libitum.

Rats were divided into three groups. The first group ($n = 10$) was fed a high fructose diet for 5 weeks. The second group ($n = 10$) was fed a high fructose diet for 5 weeks and for the last 2 weeks rosiglitazone (Glaxo-Smith-Klein, Middlesex, UK) at a dose of 10 mg/kg/d. The drug was administered with the high fructose food. The third group ($n = 10$) was fed regular chow diet for 5 weeks and served as the control group. The standard chow diet (Koffolk, Israel) was composed of 50% starch, 21% protein, 4% fat, 4.5% cellulose, and standard vitamins and mineral mix. The high fructose diet (Harlan Teklad, Madison, WI) was composed of 60% fructose, 21% protein, 5% fat, 8% cellulose, and standard vitamins and mineral mix.

Biophysical and Biochemical Measurements

Blood pressure, triglycerides, OGTT, in addition to plasma insulin and adiponectin levels were measured at baseline, after 3 weeks, and at the end of the study at week 5. The BP was measured in conscious rats using the tail–cuff technique (Narco Biosystems, Houston, TX). Rats were prewarmed at 37°C for 30 min before measurements were taken. The mean of five consecutive readings was recorded as systolic BP. Blood samples were taken from the retro-orbital sinus under light anesthesia. Samples for insulin, triglycerides, and adiponectin were taken after 5 h of fasting. The OGTT was performed after the administration of 3.5 g of glucose/kg body weight using nasogastric gavages after 12 h of fasting. Blood glucose levels were measured 5, 10, 15, 30, 60, and 120 min after the glucose load. Plasma insulin levels were assayed using a radioimmunoassay (RIA) kit (DiaSorin, Saluggia, Italy). Plasma adiponectin levels were assayed using a RIA kit (Linco Research, St. Charles, MO). Triglycerides levels were assayed with an automated analyzer using an enzymatic colorimetric reaction (Olympus AU 270, Hamburg, Germany).

Quantitative Polymerase Chain Reaction Analysis of Adiponectin Gene Expression

Tissue preparation and total RNA extraction: At the end of 5 weeks of feeding, animals were euthanized with excess of ether. The abdominal visceral fat tissues were isolated, weighed, and frozen immediately in liquid nitrogen and stored at −80°C. Total RNA was extracted from adipose tissue using a commercial kit (RNeasy midi kit, Qiagen, CA) following the manufacturer's instructions. RNA was quantified spectrophotometrically. RNA (2 μg) was reversibly transcribed to complementary DNA (cDNA) using a commercial kit (Reverse iTTM, ABgene, Surrey, UK). Real Time Quantitative Reverse Transcriptase polymerase chain reaction (PCR), using ABI Prism 7900 Sequence Detection System (Applied Biosystems, CA) was performed for quantification of adiponectin mRNA. The amounts of rat adiponectin mRNA was determined by amplification the cDNA target using the primers and TaqMan probes designed from Assay-on-Demand Gene Expression product (Applied Biosystems). To normalize the quantification of adiponectin mRNA for possible differences in the amount of each cDNA template, β-actin served as a housekeeping gene, which was detected using Assay-on-Demand for rat β-actin mRNA (Applied Biosystems). The PCR amplifications of adiponectin and β-actin genes were carried out in parallel. The reaction tube contained 50 ng (in a volume of up to 9 μL) cDNA product as template, 10 μL of 1X TaqMan Universal PCR Master Mix, and 1 μL of 20 × rat adiponectin Assay-on-Demand Gene Expression Assay Mix. The final volume of each well was 20 μL. Each cDNA sample was tested in triplicates. The following temperature parameters were cycled for 50 times: 15 sec at 95°C, 1 min at 60°C. RNA amounts were calculated manually using the Comparative Ct method for the target gene and β-actin mRNA. The amount of adiponectin mRNA was normalized by division by the amount of β-actin mRNA in each sample.

Data Analysis

Results are presented as mean ± SE. Statistical differences between series of data were assessed by two-way paired and unpaired Student *t* tests for group comparison. $P < .05$ was considered significant.

Results

Effects of High Fructose Diet on Hemodynamic and Metabolic Parameters

The high fructose diet has induced the MS after 3 weeks (Fig. 1). Five weeks of high fructose feeding resulted in a

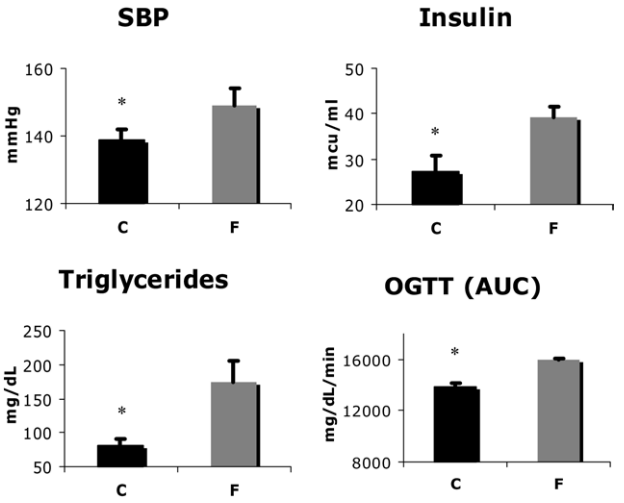

FIG. 1. Effect of high fructose (F) diet compared to control (C) diet on systolic blood pressure (SBP), insulin levels, triglycerides, and oral glucose tolerance test (OGTT) as expressed by the area under curve (AUC) measured after 3 weeks. Fructose diet was comprised of 60% fructose and the control was fed the standard chow diet with 50% starch. * denotes statistically significant difference ($P < .05$) between the fructose and the control groups.

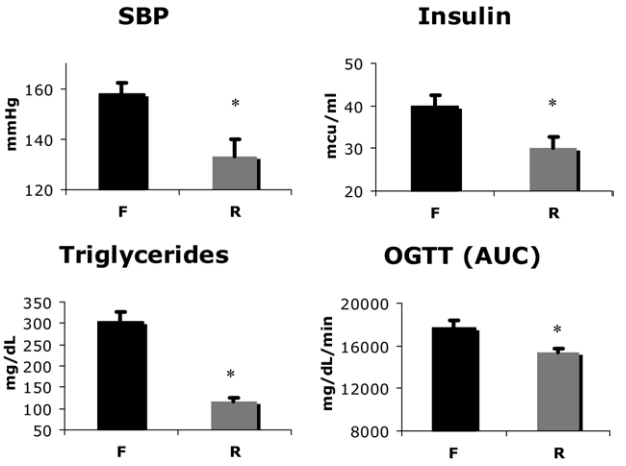

FIG. 2. Effect of rosiglitazone (R), given for 2 weeks, compared to high fructose diet (F) on systolic blood pressure (SBP), insulin levels, triglycerides, and oral glucose tolerance test (OGTT) as expressed by the area under curve (AUC). * denotes statistically significant difference ($P < 05$) between the fructose + rosiglitazone and the fructose groups.

significant increase in BP from 136 ± 3 at baseline to 158 ± 4 mm Hg ($P < .05$). In addition, the high fructose feeding progressively induced insulin resistance as expressed by the increase in the area under curve of the OGTT from 13,894 ± 246 mg/dL/min at baseline, to 17,725 ± 700 mg/dL/min at week 5 ($P < .01$) that was accompanied by an increase in the plasma insulin levels from 26 ± 5 to 40 ± 8 μU/mL ($P < .05$). The plasma triglycerides levels increased as well and only in the fructose fed rats from 91 ± 28 to 304 ± 76 mg/dL ($P < .05$). Fructose feeding did not significantly affect body weight gain and plasma adiponectin levels.

Effects of Rosiglitazone on Hemodynamic and Metabolic Parameters

The administration of rosiglitazone resulted in a significant decrease (Fig. 2) in systolic BP to 132 ± 2.2 mm Hg, triglycerides to 116 ± 8.5 mg/dL, and insulin levels to 30 ± 2.8 μU/mL ($P < .05$ for all variables) and reduction of the area under curve of the OGTT to 15,415 ± 372 mg/dL/min ($P < .001$) (Fig. 2). The plasma adiponectin levels increased significantly after 2 weeks of treatment with rosiglitazone from 4.3 ± 0.3 to 18.4 ± 1.6 μU/mL ($P < .05$). There was a significant inverse correlation between the increased plasma levels of adiponectin and the MS components ($r = -0.78$ for systolic BP, $r = -0.34$ for insulin and $r = -0.18$ for triglycerides, $P < .05$ for trend). The adipose tissue mRNA levels of adiponectin were 3.8-fold higher in the rosiglitazone group compared to the fructose and regular chow group ($P < .05$) (Fig. 3).

Discussion

The increasing prevalence of the MS calls for therapeutic strategies directed specifically to the set of metabolic errors that constitute the syndrome. Currently, the recommendations are to treat the associated hypertension, dyslipidemia, and overweight with the conventional methods, and as for the impaired glucose metabolism, no specific measures are recommended until overt diabetes is present. The pivotal place of insulin resistance and the possible role of adiponectin suggest that treatment with PPAR-γ agonist might increase adiponectin levels and thus offers a treatment to the MS as a whole and not just its components.

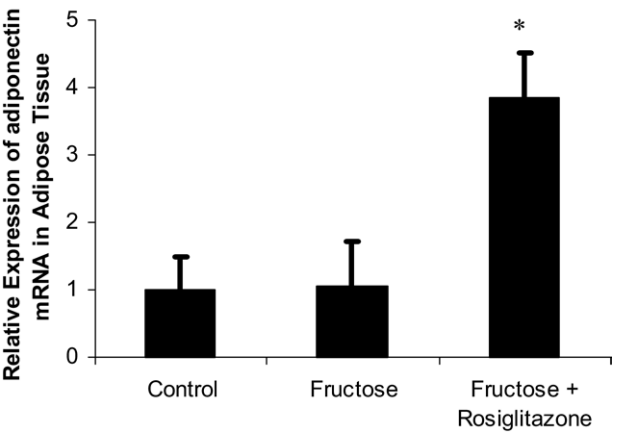

FIG. 3. The relative expression of adipose tissue mRNA of adiponectin (± SE), compared to control (β-actin) in the control, fructose, and fructose with rosiglitazone fed Sprague-Dawley rats (see text for details). * denotes statistically significant difference ($P < .05$) between the fructose + rosiglitazone and the fructose groups.

Insulin resistance is thought to be the core of the MS that connects all its components.[5–7] Hyperinsulinemia and the associated disrupted hemostatic balance[8] increased inflammation markers[9] and impaired endothelial function[10] establish the MS as a major cardiovascular risk factor.[11–14] Abdominal obesity is a hallmark of the MS. The role of adipose tissue as an active endocrine organ, secreting many bioactive substances is now well established. These adipocytokines mediate important metabolic and inflammatory processes that link obesity and the MS with its cardiovascular complications. Among these adipocytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and resistin, are upregulated in the MS and contribute to insulin resistance.[15] In contrast, adiponectin is an adipocytokine that is downregulated in correlation with visceral obesity.[16]

Adiponectin appears to be an antiatherogenic substance. It accumulates within injured vascular walls and suppresses adherence of monocytes to endothelial cells, reduces uptake of oxidized LDL by macrophages thereby inhibiting foam cell formation, and inhibits proliferation and migration of smooth muscle cells, properties that establish its vascular protective role[17] and point to promising therapeutic capabilities in the prevention and regression of atherosclerosis.

Several models looked at the relationship between adiponectin and the various components of the MS in isolation and found it to be negatively associated with obesity, hypertension, hyperlipidemia, and type 2 diabetes. Accordingly, because insulin resistance is the basis of the MS, using PPAR-γ agonist may increase the plasma levels of adiponectin and improve the MS. If proven to be so, it may rationalize the use of insulin sensitizer to a prediabetic condition. Hypoadiponectinemia, insulin resistance, and hypertension are closely inter-related in the MS. Yamauchi et al[18] found that adiponectin may reverse insulin resistance by decreasing triglyceride content in muscle and liver in obese mice. Iwashima et al[19] found a direct effect of adiponectin on BP. Moreover, recently Ohashi et al[20] showed a direct vasodilatation effect of adiponectin, through activating endothelial Nitric Oxide Synthase (eNOS), in addition to its effect through improving insulin resistance, and that adiponectin reduced BP. Nevertheless, the causal relations between adiponectin and the MS components are not fully understood and await further investigation, addressing this particular issue.

The fructose fed rats model is a model that mimics the human MS in many aspects, including hypertension, hypertriglyceridemia, insulin resistance, and compensatory hyperinsulinemia. Our study is the first to look at the effect of PPAR-γ on adiponectin, using an environmental and physiologic induction of the MS and not a transgenic one. In our study, fructose-induced MS was effectively treated as a whole with PPAR-γ. Administration of rosiglitazone resulted in reversal of all of these parameters accompanied with a large increase in plasma adiponectin levels. Moreover, the increase in adiponectin levels was associated with an increase in adipose tissue gene expression of adiponectin. It is possible that the increase in adiponectin contributed to the overall beneficial effect of rosiglitazone in the MS.

Our results are in accordance with other reports that have demonstrated an increase in plasma adiponectin levels and its gene expression in response to treatment with PPAR-γ agonists, in both cell culture studies and animal models, as well as clinical observation.[21–24] However, these reports used obese, diabetic animal models and did not look at the MS as a whole. Iwaki et al[22] has proposed that the mechanism through which TZDs affect adiponectin is an activation of the peroxisome proliferators response element of the adiponectin gene and thus induce an increase in its expression. Another possible mechanism suggests an attachment of the Liver Receptor Homolog-1 factor to a different response element on the adiponectin gene and activation of its expression.[22] Our study has a limitation. Although fructose fed rat is widely used and considered a standard model for the MS, the short-term dietary intervention did not decrease adiponectin levels. The inter-relation between high fructose diet, MS, and adiponectin needs further elucidation as data concerning the effect of specifically high carbohydrate diet on adiponectin levels are scarce and inconsistent.[25,26] In our study, plasma level of adiponectin was not changed due to fructose feeding and was associated with a nonsignificant change in its mRNA level. The relation between high carbohydrate diet and adiponectin levels as well as gene expression is controversial and contradicting reports had been published.[27,28] Therefore, we do not have a clear explanation to this finding. It is possible, however, that it is related to the fact that obesity, a major determinant of adiponectin level and gene expression,[29,30] was not developed.

This is the first study that looked at the relation between a drug that increases adiponectin levels and the overall effect on the MS in its entirety. The increase in adipose tissue gene expression of adiponectin reflects an increased production of adiponectin in the adipose tissue. The exact mechanism through which rosiglitazone stimulates adipose tissue to produce adiponectin is beyond the scope of this report and needs further elucidation of the molecular interaction between PPAR-γ activation and the adiponectin gene or its promoter.

From a clinical perspective, our study may point to a way to treat the MS as a whole and not only its components separately. Human studies did not examine the effect of early administration of PPAR-γ agonists. In addition, the animal model of an environmentally induced MS does not necessarily translate to the human physiology, as the time frame as well as the type and severity of diabetes are important and may modulate the metabolic effects of PPAR-γ agonists. Yet, the main outcome of an implementation of such an approach would be the delaying of the development of type 2 diabetes or its effect on the occurrence of rigid cardiovascular end point in patients with the

MS. If proven to be beneficial in the MS, it may rationalize a potential use of insulin sensitizer in a prediabetic condition.

In summary, in the present study we have induced the MS in Spauge-Dawley rats with high fructose diet. Treatment with rosiglitazone resulted in a significant increase in adiponectin levels and its gene expression and, perhaps mediated by this increase, and the reversal of all of the deleterious metabolic indices. Further studies are needed to shed light on the molecular interaction between PPAR-γ and adiponectin. Moreover, it may rationalize a preliminary clinical trial to determine whether TZDs can be used to treat the MS and prevent its consequences.

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