EFFECTS OF PEROXISOMES PROLIFERATOR ACTIVATED RECEPTOR GAMMA AGONIST ON OXIDATIVE STRESS BIOMARKERS AND INSULIN RESISTANCE IN HIGH FAT DIET INDUCED OBESITY

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ABSTRACT

Obesity is considered as an epidemic pathology. The present study hypothesized that Peroxisomes proliferator activated receptor gamma agonist (Rosiglitazone) would be beneficial to ameliorate the oxidative stress and Insulin resistance associated with the dysregulated carbohydrate metabolism in High fat diet induced obesity.

Male Wistar rats (180-220 g body weight) were categorized into three groups. Group 1: Control animals remain fed on a normal rat diet, Group 2: High fat diet fed obese group, Group 3: Obese + RSG treated group, treated with Rosiglitazone (RSG) (2 mg/kg) for 7 days. The Obese model was developed using a high fat diet over a period of 8 weeks. Anthropometric parameters were determined; Tissues Malondialdehyde (MDA), Catalase (CAT) and Superoxide dismutase (SOD) assessed as markers of oxidative stress and serum Insulin level and HOMA-IR were assessed as a marker for Insulin resistance.

Obese treated group showed a significant decrease tissue Malondialdehyde formation, while the activities of the anti-oxidant enzymes such as Catalase and superoxide dismutase were enhanced significantly, FBG, serum insulin level and HOMA-IR score were markedly decreased compared with untreated counterparts. The results suggested that oxidative stress and Insulin resistance mediated by HFD is restored by using PPAR-γ agonist.

Key-words: High fat diet, Reactive Oxygen Species, Oxidative stress, PPAR-γ agonist, Insulin resistance, HOMA-IR.

INTRODUCTION

Diet is considered as the most important contributor in the etiology of obesity (Levin, 2005). Obesity is an epidemic pathology characterized by excessive fat deposition, high oxidative stress, increased cardiovascular risk factors, metabolic syndrome, Insulin resistance, diabetes, and cancer (van Baak, 2013).

Epidemiological evidence demonstrated that there is increased production of reactive oxygen species (ROS) in obesity that may be the consequences of endothelial dysfunction (Galili *et al*., 2007). It has marked influence on β-cells, skeletal muscles and hepatocytes thus exacerbate insulin resistance (Gregor *et al*., 2009; Cinti *et al*., 2005). Several studies showed that oxidative stress has a critical role in the prognosis and pathogenesis of insulin resistance (Furukawa *et al*., 2004), which has been widely estimated in research by using the homeostasis model assessment-estimated insulin resistance (HOMA-IR) (Matthews *et al*., 1985).

Peroxisomes proliferator activated receptor gamma (PPAR-γ) belongs to the nuclear receptor family that has an important role in homeostasis of lipid metabolism and regulates the adipocyte differentiation. Rosiglitazone (RSG) member of Thiazolidinediones (TZDs) class of oral hypoglycemic agents serves as insulin sensitizers. It acts by stimulating PPAR-γ. PPAR-γ is present abundantly in adipose tissues, but also found in pancreatic beta- cells, liver, macrophages, bone and other tissues (Yau *et al*., 2013). TZDs play a significant role in lipolysis of triglyceride, free fatty acid transportation and cause improvement in endothelial function in humans and animals (Naka *et al*., 2011; Tian *et al*., 2010). Rosiglitazone increases cognitive performance, functions of β-cell and revokes insulin resistance (Abbatecola *et al*., 2010; Awara *et al*., 2005; Hanley *et al*., 2010)by altering the expression of glucoregulatory genes. HFD-induced insulin resistance is provoked by production of ROS and has been reported as an important therapeutic target for impeding obesity and increasing insulin sensitivity. Data relevant to the effectiveness of Rosiglitazone on diet induced obesity and oxidative stress is limited.

The aim of the study was to evaluate the preventive effect of PPAR-γ agonist on tissue oxidative stress and insulin resistance in high fat diet induced obesity.

MATERIALS AND METHODS
Animals:

Thirty male albino wistar rats with body weight of 180- 220 g were purchased from ICCBS (International Center for chemical and biological sciences), Animal care facility, University of Karachi. The rats were maintained

at an ambient temperature (23 ± 4°C) with 12-h light-dark cycles and habituated to housing conditions for 1 week before the experiments. Rats had free access to the standard rat diet and water. Experimental work was carried out with institutional ethical guidelines.

High Fat Diet preparation:

High fat diet was prepared following the guidelines provided by Khalifa *et al*. (2009). Briefly, Casein 30g, wheat flour 7g, glucose 10g, raw beef fat (suet) 40g, salt mixture 6g, vitamin mixture 3%, methionine 200 mg/g, bran 4gwere mixed with enough water and make small balls of weight 12g. Almost 54 % of daily ingested calories through this diet were from fat content (Khalifa *et al*., 2009).

Experimental Design:

Thirty male Albino wistar rats were allocated into following three groups.
Group 1(n=10): Control group, animals remain fed on a normal rat diet.
Group 2(n=10): High fat diet fed obese group, animals fed on high fat diet daily for eight weeks.
Group 3(n=10): High Fat Diet Fed obese treated with PPAR-γ agonist (RSG), animals fed with high fat diet daily for eight weeks and treated with PPAR-γ agonist (Rosiglitazone) (2mg/mL//kg body weight for last 7 days).

Animals from all experimental groups were weighed and their food intake with normal activity was regularly monitored per week.

Sample collection

Animals were decapitated by cervical dislocation after 24 hours of last dose. Blood was sampled in the heparinized tubes. The blood sample was centrifuged at 3000 rpm for 15 min to collect plasma. For serum, a portion of blood was collected in a clean glass tube without anticoagulant, blood cells were allowed to clot, and samples were centrifuged at 3000 rpm for 5 min. The clear supernatant was separated as serum. Both serum and plasma samples were stored at -80 °C for biochemical analysis. Liver and Kidneys were excised, removed from adhering fat and tissues, rinsed with ice chilled saline, dried and weighed. The tissues were then stored at -20 °C until the examination.

Assessment of Anthropometrical parameters

The body length (nose-to-anus) was determined in all rats at the start and end of the experiment. Anthropometrical parameters were estimated by using body weight and body length.
Body mass index (BMI) was computed as;
$(BMI) = $ body weight $(g)/$length^2 (cm^2)
Lee index was estimated as;
Lee index = cube root of body weight (g) / nose-to-anus length (cm) (Bernardis, 1970).
The difference in body weight of each rat was computed as a percentage change according to the following:
% change in body weight = final body weight- initial body weigh / initial body weight × 100

Biochemical Assay:
Estimation of blood glucose level
Fasting and fully fed blood glucose levels were measured using the Glucometer (On Call EZII) from the tail vein.

Preparation of kidney homogenate

Kidney tissue was minced and by using tissue Homogenizer, ultra taurax T-25 polytron homogenate was prepared (1:10 w/v) with 100mmol ice chilled KCl buffer (pH: 7), and centrifuged at 10,000 rpm for 20 minutes at 4 °C. 10μl BHT was added to the part of homogenate for the assessment Malondialdehyde (MDA). The supernatant used for biochemical examinations.

Preparation of post mitochondrial supernatant

Liver tissue was minced, and the homogenate (10% w/v) was prepared with ice cold sodium phosphate buffer (0.1 M, pH 7.4), containing 1.17% KCl, centrifuged the homogenate at 1,000 rpm for 10 min at 4°C. 10μl BHT was added to the part of homogenate for the assessment Malondialdehyde (MDA). For the estimation of CAT and SOD the homogenate was again centrifuged at 4°C for 20 min at 12,000 rpm to get post-mitochondrial supernatant (PMS), (Khan *et al*., 2011).

Assessment of oxidative stress:

Estimation of Malondialdehyde (MDA)
The MDA level in tissue homogenate is a measure of lipid peroxidation. Supernatant were determined by the thiobarbituric acid (TBA) reacting substance according to Okhawa *et al*. (1979).

Assessment of antioxidant status:
Estimation of Superoxide Dismutase (SOD)
The activity of Superoxide dismutase in the liver and kidney homogenate was evaluated according to the method of Kono (1978).

Estimation of Catalase (CAT)
Catalase activity in the liver and kidney supernatant was evaluated by the method of Sinha *et al*. (1972).

Estimation of Insulin
Serum insulin was estimated by using an ELISA kit (Reaves, 1983).

Assessment of HOMA-IR
HOMA-IR was used to evaluate insulin resistance (serum insulin (μU/mL)$\times$plasma glucose (mmol/ L)/22.5) (Matthews *et al*., 1985).

Statistical analysis
Results were presented as mean $\pm$ SD. The data were analyzed by student's t-test using SPSS version 20.0and a value of $P<0.05$ was chosen as the criteria of statistical significance.

RESULTS

Effects on Anthropometrical parameters
Table 1 compared the effects of PPAR-γ agonist (RSG) on anthropometrical parameters among control, HFD fed obese rats and obese rats treated with RSG. HFD fed group showed marked increased ($P<0.05$) body weight. There is significant increased ($P < 0.001$) BMI of HFD in comparison with control group while HFD fed obese rats treated with RSG showed significant decreased ($P < 0.05$) BMI as compared with HFD. HFD showed significant ($P< 0.01$) change in Lee index, however, HFD treated with RSG showed significant ($P< 0.01$) decreased Lee index as compared with HFD. Organ weight and relative organ weight were markedly increased in HFD rats as compared with control. However, there is no significant change in organ weight and relative organ weight in RSG treated rats.

Effects on Malondialdehyde
Table 2 and Table 3 showed that the level of renal and hepatic MDA is significantly raised in HFD fed obese rats as compared with control ($P< 0.001$). Comparison with HFD fed group treatment of PPAR-γ agonist (RSG) has been showing marked decreased MDA level in liver ($P< 0.001$) and kidney ($P<0.05$).

Effects on Superoxide Dismutase
There is marked decrease renal ($P<0.001$) and hepatic ($P<0.01$) SOD activity in HFD obese rats as compared with Control group, however HFD fed obese rats treated with RSG has been showing increased SOD activity in liver and kidney ($P < 0.001$).

Effects on Catalase
Table 2 and Table 3 showed significant lower activities of renal ($P< 0.001$) and hepatic ($P<0.01$) Catalase in HFD group as compared with Control. Treatment of HFD with RSG showed significantly enhanced the activity of renal ($P< 0.05$) and hepatic ($P < 0.01$) Catalase.

Effects on Blood Glucose
Fig. **1** showed that there is marked increased blood glucose level in HFD fed rats ($P<0.001$). However, treatment of Rosiglitazone markedly ($P<0.001$) decreased blood glucose level.

Effects on Serum Insulin
Fig. **2** showed that treatment of Rosiglitazone significantly ($P<0.05$) decreased serum insulin level, which was increased ($P<0.05$) by HFD.

Assessment of HOMA-IR

HOMA-IR was increased significantly (P<0.001) in HFD fed groups which markedly (P<0.001) decreased after RSG treatment (Fig. 3).

Table 1. Comparison of Anthropometric determination and organ weight among Control, HFD and HFD treated with Rosiglitazone.

PARAMETERS	CONTROL	HFD [a]	HFD+RSG [a, b]
Body Weight gain (g)	16.88 ± 11.46	38.17 ± 11.89	29.18 ± 6.31
%Body Weight Change	9.85 ± 7.12	19.74 ±8.02 *	14.16 ± 3.22 ***
BMI (g/cm^2)	0.471 ± 0.06	0.604 ± 0.06 ***	0.512 ± 0.08 #
Lee Index (g/cm)	0.351 ± 0.02	0.394 ± 0.03 **	0.352 ± 0.02 ##
Food Intake (g)	68.04 ± 6.84	76.07 ± 6.89	75.42 ± 7.24
Liver weight (g)	5.28 ± 0.46	7.08 ± 0.51 ***	6.82 ± 0.26 ***
Liver Weight Index (g/kg of body weight)	26.238	29.798	28.676
Kidney weight (g)	0.632 ± 0.07	0.782 ± 0.03 ***	0.761 ± 0.03 ***
Kidney Weight Index (g/kg of body weight)	3.20	3.379	3.273

Data are presented as mean ±SD; n=10; a= as compared with control; b= as compared with HFD (High fat diet)
***P< 0.001, **P< 0.01, *P< 0.05 with Control, ##P<0.01, #P<0.05 with (HFD) High fat diet, P>0.05(Non- Significant).

Table 2. Effects of Rosiglitazone treatment on renal oxidative stress markers in HFD fed rats.

Parameter	Control	HFD[a]	HFD+RSG[a,b]
KIDNEY			
MDA (µmol/g of Tissue)	7.438 ±0. 42	8.439 ±0.41***	7.338 ±0.52 #
CATALASE (mmol/mg of Tissue)	4.658±0.54	3.252±0.37***	3.799±0.17 ***, #
SOD (U/g of Tissue)	5.810±0.52	3.161±0.48***	4.465±0.23 ***, ###

Data are presented as mean ±SD; n=10; a= as compared with control; b= as compared with HFD (High fat diet)
***P< 0.001with Control, ###P<0.001; #P<0.05 with (HFD) High fat diet; P>0.05(Non- Significant)

Table 3. Effects of Rosiglitazone treatment on hepatic oxidative stress markers in HFD fed rats.

Parameter	Control	HFD[a]	HFD + RSG[a,b]
LIVER			
MDA (µmol/g of Tissue)	35.918± 0.90	63.925 ± 2.43 ***	54.692± 1.76 ***, ###
CATALASE (mmol/mg of Tissue)	3.912± 0.43	3.199± 0.49 **	3.873 ± 0.17 ##
SOD (U/g of Tissue)	11.202± 1.38	9.136± 0.718 **	11.589± 0.95 ###

Data are presented as mean ±SD; n=10; a= as compared with control; b= as compared with HFD (High fat diet)
***P< 0.001, **P< 0.01 with Control; ###P<0.001, ##P<0.01 with (HFD) High fat diet; P>0.05(Non- Significant)
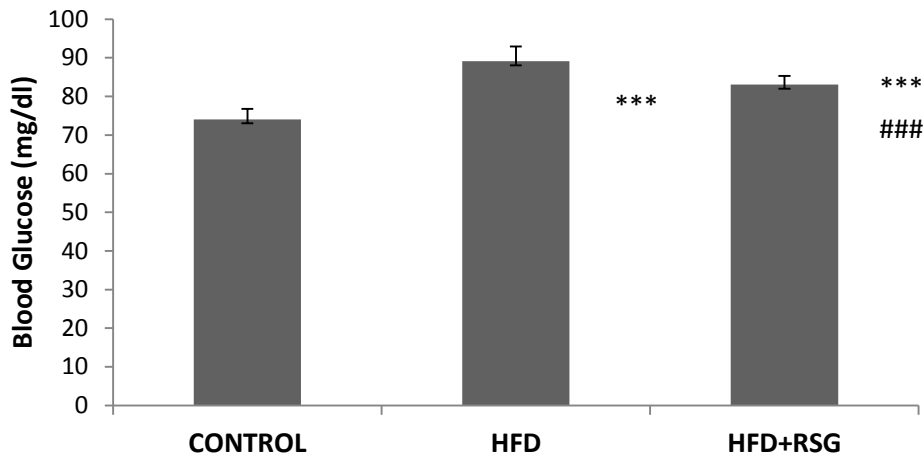
Fig. 1. Effect of PPAR-γ agonist (RSG) treatment on high fat diet fed obese rats on Blood glucose level.
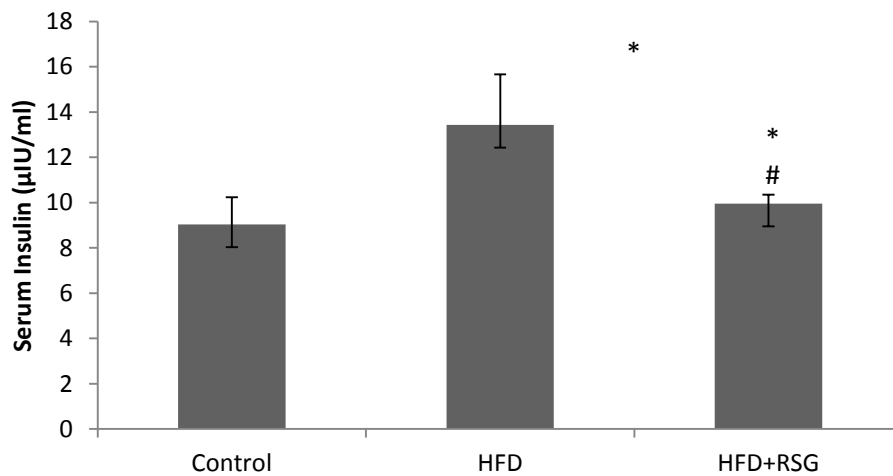n=8, values represents the mean ±SD; ***P< 0.001 with Control; ###P<0.001 with High Fat Diet

Fig. 2. Effect of PPAR-γ agonist (RSG) treatment on high fat diet fed obese rats on Insulin level.
n=8, values represents the mean ±SD; ***P< 0.001, **P <0.01, *P <0.05 with Control; ###P<0.001, ##P<0.01, #P<0.05 with High Fat Diet
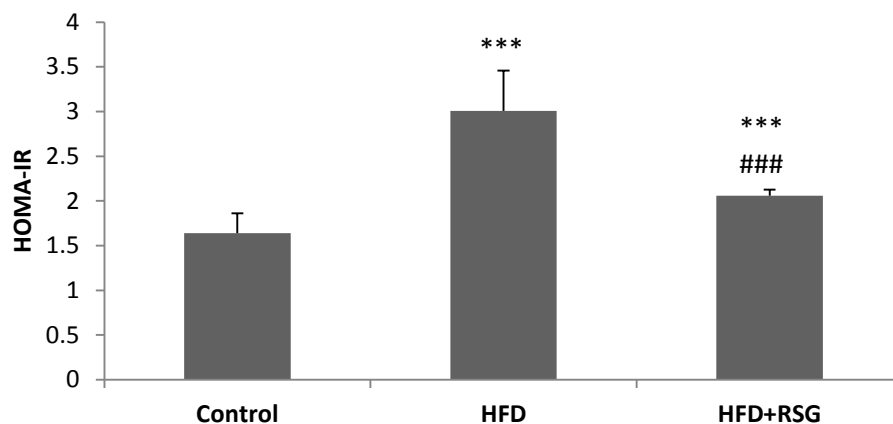

Fig. 3. Effect of PPAR-γ agonist (RSG) treatment on high fat diet fed obese rats on HOMA- IR.
n=8, values represents the mean ±SD; ***P< 0.001with Control; ###P<0.001 with High Fat Diet

DISCUSSION

High fat diet induced obesity is characterized by excessive abdominal fat deposition and weight gain. It may cause obesity, hyperglycemia, Insulin resistance, dyslipidemia that may lead to diabetes (Wouters *et al*., 2008; Stewart *et al*., 2008; Shih *et al*., 2008). HFD also provoked oxidative stress and elevate the levels of reactive oxygen species (ROS)that cause cellular damage. Highly reactive free radicals arbitrate lipid peroxidation, which may result in deposition of lipid peroxidation products in the cells thereby causing cellular injuries. It has been proved form various studies that there is increased oxidative stress, reduced Catalase (CAT), Superoxide dismutase (SOD) and glutathione Reductase in HFD fed obese mice (Shertzer *et al*., 2006) as well as obese human (Furukawa *et al*., 2004).

Presently, the effects of PPAR-γ agonist (RSG) in high fat diet induced obese rats were observed. HFD caused significant increase in body weight, BMI and Lee index. Treatment with PPAR-γ agonist (RSG) markedly decreased BMI and Lee index (Table 1).

Consequently, the results further showed that PPAR-γ agonist (RSG) significantly decreased tissue MDA (Table 2 and Table 3) and Insulin resistance (Fig. 2) in obese treated group, showing suppression of lipid peroxidation and insulin resistance in diet induced obesity. It is confirmed by several studies that oxidative stress contributes the progression of structural and functional cellular damage. Formation of ROS can cause endothelial dysfunction (Bonetti *et al*., 2003)

HFD consumption results in markedly decreased tissue SOD and Catalase which increased significantly after RSG treatment (Table 2 and Table 3). This effect of Rosiglitazone may be attributed to the inhibition of ROS generation due to the activation of AMPK. Increased NADPH oxidase activity is induced by hyperglycemia through PKC inhibition. However, AMPK activation prevents hyperactivity of NADPH oxidase (Ceolotto *et al*., 2007)

It has been proved that increased production of ROS results increased Free Fatty Acid in the plasma and liver which may lead to insulin resistance. Up-regulated gene expression for ROS production caused HFD induced oxidative damage in the adipose and hepatic tissue. These findings suggest that generation of ROS lead to HFD-induced insulin resistance (Naoto *et al*., 2008). Results showed that there are marked decreased glucose (Fig. 1) and insulin level (Fig.2) in RSG treated group as compared with HFD fed obese rats.

In brief, our study corroborates the hypothesis that RSG protects from tissue damage from high fat diet induced obesity through PPARγ- activation. Further studies of genetic mechanism will open the new therapeutic way for the treatment and prevention of Diabetes and its complications.

CONCLUSION

The findings of the present research are of merit in revealing that HFD induced obesity triggers the oxidative stress related tissue damages which is characterized by unbalanced reactive oxygen species as well as Insulin resistance. PPAR-γ agonist possesses the potential to reverse the oxidative stress and increase the insulin sensitivity thus preventing diabetes.

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