ORIGINAL ARTICLE

MODULATION OF VISFATIN LEVELS BY THIAZOLIDINEDIONES IN INSULIN RESISTANT SPRAGUE DAWLEY RATS

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Background: Insulin resistance is manifested by decreased effect of fixed quantity of insulin on glucose metabolism leading to type 2 diabetes mellitus. Visceral obesity has a positive correlation with insulin resistance but its mechanism is not fully defined. Insulin resistance may be the consequence of adipocytokines including visfatin and resistin. This study was designed to see the effect of thiazolidinediones on visfatin levels in insulin resistant rats. **Methods:** Ninety Sprague Dawley rats were divided into 3 groups of 30 each. Group I served as control. Rats in Group II and III were made insulin resistant diabetics with high sucrose diet. Group III was treated with thiazolidinediones after development of diabetes. Plasma glucose, serum triglycerides, HDL, TG:HDL ratio and serum visfatin levels were analysed. **Results:** Body weight and plasma glucose, visfatin levels and TG:HDL ratio were significantly increased ($p<0.05$) in group II and group III at the end of 4th week. Treatment of group III with thiazolidinediones led to improvement in insulin resistance with increase in serum visfatin levels ($p<0.05$). **Conclusion:** Thiazolidinediones augment sensitivity of insulin to restore normoglycaemia by increasing serum visfatin level.

Keywords: visfatin, adipocytokines, insulin resistance, type 2 diabetes, HDL

Pak J Physiol 2017;13(1):33–5

INTRODUCTION

Adipose tissue secretes various adipocytokines, including adiponectin, interleukin-6, leptin, resistin, TNF-α and visfatin which play an important role in pathogenesis of insulin resistance and cardiovascular diseases.[1] These adipocytokines act at both local (autocrine/paracrine) and systemic (endocrine) levels. Visfatin is expressed by visceral adipose tissue and mimics the effects of insulin by binding to the insulin receptor at a site different from that of insulin.[2] Visfatin plasma levels are significantly increased in insulin resistance compared with controls.[3] Most individuals appear to develop insulin resistance when environmental factors interact with specific genetic predispositions that confer susceptibility.[4] A dose-dependent glucose lowering effect of visfatin has been observed when mice were injected with recombinant visfatin. The same effect was found in KKAy mice, an animal model for insulin resistance. Visfatin expression in different mice models significantly lowers plasma glucose concentrations.[2] Thiazolidinediones have remarkable efficacy in improving insulin sensitivity in animal models of insulin resistance. These frequently restore insulin sensitivity to normal. When administered in insulin resistant humans, thiazolidinediones have modest effect.[5]

The present study was designed to evaluate the effects of thiazolidinediones on visfatin levels in insulin resistant diabetes.

MATERIAL AND METHODS

This study was conducted in the Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad. The project and relevant regulations governing the study were approved by the Committee for Postgraduate Studies, Army Medical College, Rawalpindi. Ninety male, healthy Sprague Dawley rats, at least 60 days old, with an average weight of 250±50 gm were included in study. Rats were obtained from the National Institute of Health (NIH), Islamabad. They were kept in its animal house facility where the temperature was maintained at 22±3 °C. Food and water was available *ad libitum*. The rooms were well ventilated and 12-hour light-dark cycle was maintained.

By convenient sampling, the rats were divided into 3 equal groups. Weight of the animals was recorded and blood samples for baseline fasting blood glucose and serum visfatin levels, triglycerides and HDL were taken by tail bleed. Group I served as control without any intervention and fed on normal diet. Rats in group II and III were fed on a high sucrose diet (Table-1) for 4 weeks to induce insulin resistance (Type II diabetes mellitus). Insulin resistance was established by the triglycerides to high density lipoproteins (TG/HDL) ratio, considered as one of the surrogate markers for insulin resistance.

Blood sampling was done at the end of 4th week by tail bleed to confirm the presence of insulin resistance. After confirmation of development of insulin resistance, rats in group III were treated with rosiglitazone, injected intraperitoneally, 5 mg/Kg body weight for seven days. At the end of 5th week terminal sampling was done by intra cardiac bleed under ether anaesthesia and the animals were sacrificed. After clotting, samples were first centrifuged at 4,000 rpm at

4 ºC in the cold centrifuge. Then serum was pipetted out and stored in Eppendorf storage tubes at -80 ºC till assay of plasma glucose, serum triglycerides, HDL, TG:HDL ratio, and visfatin levels.

Data were analyzed on SPSS-21. The arithmetic mean and standard deviation of all samples were calculated. Difference in mean among control and treated groups was calculated by 'Independent sample *t*-test'. Groups were compared using ANOVA. Tukey's test was used for Post Hoc Comparison, and $p \leq 0.05$ was taken as statistically significant.

Table-1: Composition of high sucrose diet for rats

Ingredients	gm/Kg
Casein	200
DL-Methionine	3
Sucrose	650
Cellulose	50
Corn oil	50
Salt mix	35
Vitamin mix	10
Choline bitartrate	2

Sucrose= 68%, Protein= 20%, Fat= 12%

RESULTS

The animals in this study remained healthy and active throughout the study period and took feed properly. The triglycerides to high density lipoproteins (TG/HDL) ratio of more than 3.0 in groups II and III at the end of three weeks confirmed the presence of insulin resistant diabetes (Table-2).

Table-2: Body weight, plasma glucose and TG:HDL ratio in groups at the end of 4 weeks

Group	Body weight (gm)	Plasma glucose (mg/dl)	Serum TG (mg/dl)	Serum HDL (mg/dl)	TG:HDL
I	254.35±5.20	103.2±2.05	105.45±9.45	67.35±2.86	1.37±0.45
II	296.65±7.34	154.1±3.05	184.66±6.24	60.56±3.86	3.01±0.46
III	295.55±6.45	155.0±3.55	183.63±5.31	61.53±3.45	3.00±0.46

Serum visfatin levels in groups II and III were found to be significantly increased ($p<0.05$) at the end of 4 weeks, after development of insulin resistance by high sucrose diet. Serum visfatin levels increased to 2.41±0.40 ηg/ml in group II. In group III the serum visfatin level increased to 2.51±0.33 ηg/ml ($p<0.05$) after the intake of high sucrose diet for four weeks as compared to the control (1.05±0.32 ηg/ml). Rosiglitazone treatment significantly increased ($p<0.05$) serum visfatin levels in group III from 2.51±0.33 ηg/ml to 3.55±0.44 ηg/ml (Table-3).

Table-3: Serum visfatin levels at the end of 4th and 5th week (ηg/ml)

Serum visfatin	Group I	Group II	Group III
At end of 4th week	1.05±0.32	2.41±0.40	2.51±0.33
At end of 5th week	1.05±0.32	2.42±0.40	3.55±0.44

DISCUSSION

Use of high sucrose diet is simple and less expensive procedure that requires short period of four weeks to induce insulin resistance in Sprague Dawley rats. High sucrose diet increases body weight by increasing the mass of adipose tissue. Plasma glucose and TG:HDL ratio in the sucrose fed Sprague Dawley rats were increased. The combination of fasting hyperglycaemia and increased TG:HDL ratio in sucrose fed rats was indicative of glucose intolerance and insulin resistance.[6]

The relationship between obesity, insulin resistance, and cardiovascular diseases has been recognized since long but the mechanisms for their interrelationship are indistinct. Adipocytes are the source of adipocytokines including visfatin. Serum visfatin levels were increased in insulin resistant rats due to the increase in adipose tissue mass and body weight. It is well known that adipocytes secrete visfatin and other adipocytokines. The demonstration in this study that sucrose feeding produces insulin resistance, is comparable to the findings of other studies carried out in high fructose and high sucrose fed rats, in which different techniques were used to assess insulin resistance.[7–9]

The plasma visfatin levels in humans have been correlated with obesity, visceral fat mass, type 2 diabetes, and presence of the metabolic syndrome.[3] In our study, the reduction in TG to HDL ratio is believed to be an important predictor of improvement in insulin resistance after the treatment of insulin resistant rats with rosiglitazone. A possible explanation is that insulin-sensitizer rosiglitazone triggered the visfatin release, leading thereby to insulin resistance alleviation. Dominik *et al* demonstrated that rosiglitazone treatment increased the circulating visfatin concentrations in healthy humans and induced the release of visfatin from isolated adipocytes into the supernatant medium. This effect was counteracted by FFA and could be influenced *in vitro* by antioxidant strategies. Furthermore, visfatin secretion from adipocytes by rosiglitazone involved the activation of PI 3-kinase and a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation and apoptosis.[10]

The marked effect of insulin resistance treatment on adipocyte visfatin secretion has been documented by Choi *et al*[11] that is in agreement with the previous data demonstrating that rosiglitazone increases visfatin *m*RNA expression in animal adipocyte deposits. Consequently, the action of rosiglitazone on release of the insulin-mimetic adipocytokine visfatin might contribute to the insulin sensitizing effect. This may be further influenced by the simultaneous changes in other adipocytokines. A variety of adipocytokines and peptides secreted from adipocytes have been considered

to play a crucial role in insulin resistance and present study was an effort to find the role of visfatin in glucose homeostasis. Visfatin activates insulin receptors in a manner different from insulin. To understand the role of rosiglitazone in improving insulin sensitivity via activation of PPARγ, we examined the expression of visfatin levels, in insulin resistant Sprague Dawley rats. Insulin resistance was improved significantly ($p<0.05$) while serum glucose concentration was significantly decreased ($p<0.05$) in rosiglitazone treated Sprague Dawley rats compared to the insulin resistant hyperglycaemic controls. Rosiglitazone significantly ($p<0.05$) increased the serum visfatin concentration.

These findings suggest that thiazolidinediones may improve insulin resistance by regulating visfatin secretion. Thiazolidinediones represent an important breakthrough in the therapy of insulin resistance, and their ability to manipulate visfatin level suggests that there exists a link among visfatin, insulin resistance, and the mechanism of action of thiazolidinediones.

CONCLUSION

Thiazolidinediones augment sensitivity of insulin by increasing the serum level of visfatin. Further studies may also be carried out in human subjects to give visfatin as an adjunct to insulin in diabetic patients to improve their glycaemic control.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of Higher Education Commission, Pakistan, for conducting this research project. We would also like to recognize the support provided by NIH, Islamabad for carrying out the study in their animal house.

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Received: 28 Feb 2017 Reviewed: 11 Mar 2017 Accepted: 15 Mar 2017