ORIGINAL ARTICLE

EFFECT OF THIAZOLIDINEDIONES ON ADIPOCYTOKINES AND LIPID PROFILE 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 been positively correlated 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 observe the effects of thiazolidinediones on lipid profile and levels of adipocytokines (visfatin and resistin) in insulin resistant Sprague Dawley rats. **Methods:** Ninety Sprague Dawley rats were randomly divided into three groups. Group I served as control. Rats in Group II and III were made insulin resistant diabetics. Group III was treated with rosiglitazone after development of diabetes. Plasma glucose, serum triglycerides (TG), HDL, TG: HDL ratio and serum adipocytokines (visfatin, resistin) levels were analyzed. **Results:** Body weight and plasma glucose were significantly increased ($p<0.05$) along with TG: HDL ratio ($p<0.05$) in group II and group III at the end of 4th week. Serum visfatin and resistin levels also increased significantly ($p<0.05$) in group II and III at the end of 4th week. Treatment of group III with rosiglitazone led to improvement in insulin resistance with decrease in serum resistin levels ($p<0.05$) and increase in serum visfatin levels ($p<0.05$). Rosiglitazone treatment decreased serum TG level and increased serum HDL level. **Conclusions:** Deranged lipid profile and increased serum resistin levels indicate insulin resistance and impending hyperglycaemia. Thiazolidinediones augment sensitivity of insulin to restore normoglycemia by improving lipid profile, decreasing serum resistin levels and improving serum visfatin levels.

Keywords: Thiazolidinediones, adipocytokines, visfatin, resistin, insulin resistance, type 2 diabetes, HDL, TG

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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 disease.[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.[3] Insulin resistance appears to develop as an interaction of environmental factors with specific genetic predisposition.[4] A dose-dependent glucose lowering effect of visfatin has been observed when mice were injected with recombinant visfatin. Visfatin expression in different mice models significantly lowers plasma glucose concentrations.[2]

Thiazolidinediones have shown remarkable efficacy in improving insulin sensitivity in animal models of insulin resistance. These frequently restore insulin sensitivity to normal in insulin resistant humans.[5] Resistin is one of the factors which affect peripheral insulin resistance. Serum resistin levels were found to be elevated in two different genetic models of mice and in a diet-induced model of insulin resistance and obesity.[6]

Resistin *m*RNA levels were increased in the fructose-fed rat model of insulin resistance.[7] Mice exhibiting decreased adiposity and improved insulin sensitivity were found to have decreased serum resistin levels.[8] Most individuals appear to develop insulin resistance when environmental factors interact with specific genetic predispositions.[4] Obesity is one of the key environmental factors responsible for insulin resistance development as it is source of adipocytokines like visfatin and resistin. This indicates the role of resistin in regulating insulin sensitivity. If resistin is involved in the pathogenesis of insulin resistance, it can be predicted that insulin sensitizing agents like thiazolidinediones may decrease resistin expression. Resistin *m*RNA and protein are down regulated by antidiabetic thiazolidinediones (TZDs) in adipocytes.[9–11] The present study was designed to evaluate the effects of thiazolidinediones on serum TG, HDL, serum visfatin and resistin levels in insulin resistant Sprague Dawley rats.

MATERIAL AND METHODS

The project and relevant regulations governing the study were approved by the Committee for Postgraduate Studies, Army Medical College, Rawalpindi. This study was conducted at the Department of Physiology, Army Medical College, Rawalpindi in collaboration with

National Institute of Health, Islamabad. Ninety male, healthy Sprague Dawley rats, at least 60 days old, with an average weight of 250±50 gm, were included in the study. Rats were obtained from the National Institute of Health (NIH), Islamabad. They were kept in animal house facility where the temperature was maintained at 22±3 °C. Food and water were available *ad libitum*. The rooms were well ventilated and 12-hours light-dark cycle was maintained. By convenient sampling, the rats were divided into 3 equal groups. Weight of each animal was recorded and blood samples for baseline fasting blood glucose and serum visfatin and resistin levels, triglycerides and HDL were taken by tail bleed. Group I served as control without any intervention and was fed on normal diet. Rats in group II and III were fed on a high sucrose diet for 4 weeks to induce insulin resistance. Insulin resistance was established by the triglycerides-to-high density lipoproteins (TG/HDL) ratio considered as one of the surrogate markers for insulin resistance. The cut-off value of TG:HDL ratio= 1.8 was used to mark the presence of insulin resistance (Table-1).[12] 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 centrifuged at 4,000 rpm at 4 °C in the cold centrifuge. Serum was pipetted out and stored in Eppendorf storage tubes at -80 °C till assay of plasma glucose, serum triglycerides, HDL, TG:HDL ratio, serum visfatin and resistin 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 Analysis of Variance (ANOVA). Tukey test was used for Post Hoc Comparison and $p<0.05$ was taken as statistically significant.

RESULTS

The animals in this study remained healthy and active throughout study period and took their feed properly. Body weight measured at the end of 4th week increased significantly ($p<0.05$) in the insulin resistant group II and rosiglitazone treated group III compared to control group I (Table-1). All values have been expressed as mean±SD.

TG levels were found elevated in group II and group III at the end of 4th week, compared to control group. At the end of 4th week, the HDL levels were found decreased ($p<0.05$) in group II and group III compared to control group. TG:HDL ratio in group II and III was higher than the cut-off value of 1.8 as opposed to controls.

At the end of 5th week, 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 (Table-2).

Serum visfatin levels in groups II and III were found to be significantly increased ($p<0.05$) at the end of 4 weeks (Table-1). Rosiglitazone treatment significantly increased ($p<0.05$) serum visfatin levels in group III (Table-2).

Serum resistin levels in groups II and III were found to be significantly increased ($p<0.05$) at the end of 4 weeks (Table-1). Rosiglitazone treatment significantly decreased ($p<0.05$) resistin levels in group III (Table-2).

Tables-3 and 4 represent group wise comparison of studied parameters for significant changes.

Table-1: Body weight, plasma glucose, TG:HDL ratio and serum visfatin/resistin levels in the three groups at the end of 4 weeks

Variables	Group I n=30	Group II n=30	Group III n=30	*p*
Body weight (gm)	251.1±2.48	310.8±14.62	312.23±14.42	0.001
Plasma glucose (mg/dl)	102.8±4.98	145.5±7.79	145.76±7.86	0.001
Serum TG (mg/dl)	105.9±9.95	150.0±13.99	150.42±12.63	0.001
Serum HDL (mg/dl)	77.44±4.92	71.76±3.58	72.18±3.07	0.001
TG: HDL ratio	1.37	2.09	2.08	0.001
Serum visfatin (ηg/ml)	1.05±0.32	2.41±0.40	2.51±0.33	0.001
Serum resistin (ηg/ml)	12.11±3.01	21.75±2.92	20.94±2.29	0.001

Table-2: Body weight, plasma glucose, TG:HDL ratio and serum visfatin/resistin levels in the three groups at the end of 5 weeks

Variables	Group I n=30	Group II n=30	Group III n=30	*p*
Body weight (gm)	251.1±2.48	310.4±15.39	310.8±14.62	0.001
Plasma glucose (mg/dl)	102.8±4.98	144.73±8.14	121.53±4.63	0.001
Serum TG (mg/dl)	105.9±9.95	150.04±13.99	131.69±7.77	0.001
Serum HDL (mg/dl)	77.44±4.92	71.76±3.59	73.54±3.89	0.001
TG: HDL ratio	1.37	2.09	1.79	0.001
Serum visfatin (ηg/ml)	1.05±0.32	2.42±0.40	3.55±0.44	0.001
Serum resistin (ηg/ml)	12.11±3.01	21.71±2.88	14.48±2.19	0.001

Table-3: Pairwise comparison of study variables at the end of 4th week

Variables	Pairwise comparison (*p*-values)		
	Control vs Insulin resistant	Control vs Rosiglitazone	Insulin resistant vs Rosiglitazone
Body weight (grams)	0.001	0.001	0.727
Plasma glucose (mg/dl)	0.001	0.001	0.931
Serum TG (mg/dl)	0.001	0.001	0.915
Serum HDL (mg/dl)	0.001	0.001	0.658
TG:HDL ratio	0.001	0.001	0.880
Serum visfatin (ηg/ml)	0.001	0.001	0.328
Serum resistin (ηg/ml)	0.001	0.001	0.239

Table-4: Pairwise comparison of study variables at the end of 5th week

Variables	Pairwise comparison (*p*-values)		
	Control vs Insulin resistant	Control vs Rosiglitazone	Insulin resistant vs Rosiglitazone
Body weight (grams)	0.001	0.001	0.62
Plasma glucose (mg/dl)	0.001	0.001	0.001
Serum TG (mg/dl)	0.001	0.001	0.001
Serum HDL (mg/dl)	0.001	0.001	0.43
TG: HDL ratio	0.001	0.001	0.001
Serum visfatin (ηg/ml)	0.001	0.001	0.001
Serum resistin (ηg/ml)	0.001	0.001	0.001

DISCUSSION

The use of high sucrose diet is simple and convenient method to induce insulin resistance in Sprague Dawley rats within short period of four weeks. High sucrose diet increased body weight by increasing the mass of adipose tissue. Plasma glucose and TG: HDL ratios in 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.[12]

The relationship between obesity, insulin resistance, and cardiovascular diseases has been extensively published but the mechanisms of their interrelationship are yet to be elucidated. Adipocytes are the source of adipocytokines including visfatin and resistin. Serum visfatin levels were increased in insulin resistant rats due to the increase in adipose tissue mass and body weight. It is well established that adipocytes secrete visfatin and other adipocytokines. As evidenced 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.[13–15]

Plasma visfatin levels in humans have been correlated with obesity, visceral fat mass, type 2 diabetes and presence of the metabolic syndrome.[10] 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 visfatin release leading thereby to insulin resistance alleviation. Dominik and his colleagues demonstrated that rosiglitazone treatment increased 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 anti-oxidant 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.[16] The marked effect of insulin resistance treatment on adipocyte visfatin secretion has been documented by Choi and his colleagues that are in agreement with the previous data[17] 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 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 attempted 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. Resistin has been documented to antagonize the actions of insulin. The demonstration in this study that sucrose feeding produces insulin resistance is similar 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.[12,18,19] Serum resistin 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 resistin. It is possible that resistin could lead to insulin resistance. According to Curat*et al*[19], resistin expression *in vivo* is specific to white adipose tissue and it is found in the serum of normal mice. Resistin is an adipocyte derived factor that contributes to the development of insulin resistance in vivo and has been supported by the studies on adipocytes where neutralization with resistin antiserum enhanced insulin-stimulated glucose uptake. Administration of resistin to mice impairs the glucose tolerance without reducing insulin levels and decreases sensitivity to the effects of insulin. Our study supported these findings that resistin is a hormone having effects on glucose metabolism which is antagonistic to those of insulin and thiazolidinediones improve insulin resistance by decreasing resistin levels. A significant correlation does exist between fasting glucose and serum resistin level. These findings are comparable to the previous study by Lazar *et al*[20] on the rodent model in which fasting blood glucose level was higher in resistin transgenic mice than in their non-transgenic littermates and glucose tolerance was impaired in the hyper-resistinemic mice.

Thiazolidinediones represent a crucial milestone in the therapy of insulin resistance and their ability to down regulate resistin level suggests that there exists a link among resistin, insulin resistance and the mechanism of action of thiazolidinediones. Therefore, potential complications of PPARγ activation in tissues[21] may be avoided by making resistin the target of insulin resistance therapy if regulation and properties of human resistin are similar to those of rat resistin.

CONCLUSION

Our results strongly reinforce that thiazolidinediones augment sensitivity of insulin by increasing the serum level of visfatin and decreasing the serum level of resistin. Thiazolidinediones also improve TG:HDL ratio.

RECOMMENDATIONS

Further studies may be carried out in patients to administer visfatin as an adjunct to insulin in diabetic patients and to monitor the levels of resistin to improve their glycaemic control.

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