ORIGINAL ARTICLE

EFFECT OF THIAZOLIDINEDIONE TREATMENT ON RESISTIN LEVELS IN INSULIN RESISTANT SPRAGUE DAWLEY RATS

Iftekhar Yousaf, Waqas Hameed*, Tauseef Ahmed Rajput**

Army Medical College, Rawalpindi, *Pak International Medical College, Peshawar, **Margalla Institute of Health Sciences, Rawalpindi

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 see the effect of thiazolidinediones on levels of resistin in insulin resistant 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, HDL, TG:HDL ratio and serum resistin levels were analysed. **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 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 ($p<0.05$). **Conclusion:** Increased serum resistin level indicates insulin resistance and impending hyperglycaemia. Thiazolidinediones augment sensitivity of insulin to restore normoglycaemia by decreasing serum resistin level.

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

Pak J Physiol 2015;11(2):8–10

INTRODUCTION

Adipose tissue secretes various adipocytokines, including adiponectin, interleukin-6, leptin, resistin, TNF-α, visfatin and resistin which play an important role in pathogenesis of insulin resistance and cardiovascular disease.[1] Resistin is one of the factors which affects 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.[2] In the fructose-fed rat model of insulin resistance, resistin mRNA levels were increased.[3] Mice exhibiting decreased adiposity and improved insulin sensitivity, were found to have decreased serum resistin levels.[4]

Most individuals appear to develop insulin resistance when environmental factors interact with specific genetic predispositions that confer susceptibility.[5] These include abnormalities of nutritional intake, obesity and decreased physical activity. Obesity is one of the key environmental factors responsible for the development of insulin resistance as it leads to increased production of adipocytokines such as 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 mRNA and protein are down regulated by antidiabetic thiazolidinediones (TZDs) in adipocytes.[6–8] The present study was designed to evaluate resistin levels after treatment with thiazolidinediones and effect on insulin resistant diabetes.

SUBJECTS AND METHODS

The project and relevant regulations governing the study were approved by the committee for postgraduate studies, Army Medical College, Rawalpindi.

The study was conducted from January to July 2010 at Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Ninety healthy Sprague Dawley rats with an average weight of 250±50 gm were procured from animal house of 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 (n=90) were divided into 3 equal groups (n=30 in each group). Weight of the animals was recorded and blood samples for baseline fasting blood glucose and serum resistin 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. Insulin resistance was measured by the triglycerides-to-high density lipoproteins (TG/HDL) ratio; being 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 anesthesia and the animals sacrificed. The plasma and serum samples were stored at –80 °C till assay of plasma glucose, serum triglycerides, HDL, TG:HDL ratio, and resistin levels.

Table-1: Composition of rat diet

Ingredients	Weight*
Vitamin A	10,000 IU/Kg
Vitamin D	5,000 IU/Kg
Vitamin E	50 mg/Kg
Choline	800 mg/Kg
Methionine	500 mg/Kg
Sodium chloride	5 gm/Kg
Dibasic Calcium Phosphate	9.5 gm/Kg
Zinc Sulphate	24 mg/Kg
Potassium Iodide	3 mg/Kg

Total weight=amount of the premix added in 10 Kg of the pelleted diet prepared. *amount added/Kg of the diet prepared

Data were analysed on SPSS-13. 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 chosen as the limit for statistical significance.

RESULTS

Serum resistin levels in groups II and III were found to be significantly increased ($p<0.05$) at the end of 4 weeks (Table-2), after the development of insulin resistance by high sucrose diet. Serum resistin levels increased to 21.75±2.92 ηg/ml in group II. In group III the serum resistin level increased to 20.94±2.29 ηg/ml ($p<0.05$) after the intake of high sucrose diet for four weeks as compared to the control (12.43±3.01 ηg/ml). Rosiglitazone treatment significantly decreased ($p<0.05$) resistin levels in group III from 20.94±2.29 ηg/ml to 14.48±2.19 ηg/ml.

Table-2: Serum resistin at end of 4th and 5th week (ηg/ml)

Variables	Group I n=30	Group II n=30	Group III n=30
Serum resistin 4th week	12.11±3.01	21.75±2.92	20.94±2.29
Serum resistin 5th week	12.11±3.01	21.71±2.88	14.48±2.19

DISCUSSION

The relationship between obesity, insulin resistance, and cardiovascular diseases has been recognized since long but the mechanisms for their interrelationship are indistinct. Feeding of high sucrose diet for a period of 4 weeks led to the increased body weight, hyperglycemia and increased Tg:HDL ratio in insulin resistant model as compared to the healthy control. A decrease in insulin sensitivity accompanied the hyper triglyceridemia in Sprague Dawley rats after 4 weeks of high sucrose diet. Adipose tissue mass increased due to high sucrose diet. Adipocytes are the source of adipocytokines including resistin. Resistin has been documented to antagonize the actions of insulin. 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.[9–11] 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*[11], 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*[12] 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 represents an important breakthrough 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[13] 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

Increased serum resistin levels indicate insulin resistance and impending hyperglycemia. Thiazolidinediones augment sensitivity of insulin to restore normoglycemia by decreasing serum resistin level.

ACKNOWLEDGEMENTS

The financial support of Higher Education Commission of Pakistan (HEC) for conducting this research, and NIH, Islamabad to provide experimental animals is gratefully acknowledged.

REFERENCES

1. Samaras K, Kelly PJ, Chiano MN, Arden N, Spector TD, Campbell LV. Genes versus environment. The relationship between dietary fat and total and central abdominal fat. Diabetes Care 1998;21:2069–76.
2. Steppan CM, Bailey ST, Bhat S. The hormone resistin links obesity to diabetes. Nature 2001;409:307–12.
3. Juan CC, Au LC, Fang VS. Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. Biochem Biophys Res Commun 2001;289:1328–33.
4. Hirosumi J, Tuncman G, Chang L. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333–6.
5. Samaras K, Campbell LV. Increasing incidence of type 2 diabetes in the third millennium: is abdominal fat the central issue? Diabetes Care 2000;23:441–2.
6. Shojima N, Sakoda H, Ogihara T. Humoral regulation of resistin expression in 3T3-L1 and mouse adipose cells. Diabetes 2002;51:1737–44.
7. Haugen F, Jorgensen A, Drevon CA, Trayhurn P. Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. FEBS Lett 2001;507:105–8.
8. Hartman HB, Hu X, Tyler KX, Dalal CK, Lazar MA. Mechanisms regulating adipocyte expression of resistin. J Biol Chem 2002;277:19754–61.
9. Santure M, Pitre M, Marette A, Deshaies Y, Lemieux C, Lariviere R. Induction of insulin resistance by high sucrose feeding does not raise mean arterial blood pressure but impairs haemodynamic responses to insulin in rats. Br J Pharmacol 2002;137:185–96.
10. McLaugin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann Intern Med 2003;139:802–9.
11. Curat CA, Wegner V, Sengenes C, Miranville A, Tonus C, Busse R. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia 2006;49:744–7.
12. Rangwala SM, Rich AS, Rhoades B, Shapiro JS, Obici S, Rossetti L, *et al*. Abnormal Glucose Homeostasis due to Chronic Hyperresistinemia. Diabetes 2004;53(8):1934–41.
13. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998;93:241–52.

Address for Correspondence:

Dr Iftekhar Yousaf, Assistant Professor Physiology, Army Medical College, Rawalpindi. **Cell:** +92-334-4462920
Email: iftiyusuf41@gmail.com