ORIGINAL ARTICLE

GLUCOSE LOWERING EFFECT OF VISFATIN IN OBESE AND INSULIN DEPENDENT DIABETES MELLITUS

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Background: Human obesity-related diabetes and the accompanying metabolic disorders have been specifically linked to increased visceral adipose tissue mass. Visfatin (pre-B-cell colony enhancing factor, PBEF) is a novel adipokine that appears to be preferentially produced by visceral adipose tissue that has insulin-mimetic actions. This study investigated if visfatin administration decreases blood glucose in both obese and insulin dependent diabetic mice. **Methods:** One hundred and twenty balb/c strain albino mice were divided into 4 groups. Animals in Group I and Group II were made obese by feeding high fat/high carbohydrate diet whereas Group III and Group IV were made insulin dependent diabetic by injecting streptozotocin. Group I and Group III served as controls whereas Group II and Group IV were administered visfatin injection intravenously as a single bolus dose. Blood samples were collected to measure the blood glucose and visfatin levels. **Results:** Visfatin levels were found increased in obese mice, while administration of recombinant-histidine soluble (mice) visfatin significantly decreased the blood glucose level in obese and insulin dependent diabetic mice ($p<0.001$). **Conclusion:** The glucose lowering effect of visfatin in insulin resistant and insulin dependent diabetic mice is observed.

Keywords: Visfatin, obesity, adipocytokines, insulin dependent diabetes mellitus

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia and dyslipidemia resulting from decreased in insulin secretion, resistance to insulin action or both.[1] The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million by 2030 in the world.[2,3] More than 10% of the adult population in Pakistan suffers from diabetes.[3,4] Obesity is the most important risk factor in the development of insulin resistance and type 2 Diabetes Mellitus. High fat diet fed mouse have been recognised as the robust model for induction of type 2 (insulin resistant) diabetes mellitus.[5] Adipose tissue produces several proteins (adipocytokines) such as leptin, adiponectin, resistin, tumour necrotic factor alpha (TNF-α), and interleukin (IL-6) that modulate insulin sensitivity and appear to play an important role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, atherosclerosis, and atherosclerosis.[6–8]

Visfatin is a recently acknowledged adipocytokine secreted by the visceral fat of both human and mice. Like insulin, it increases glucose transport and lipogenesis by adipocyte and myocyte and decreases glucose production by hepatocyte.[9,10] It binds to insulin receptor but at a different binding site than insulin itself. The affinities of visfatin and insulin for insulin receptors are similar but circulating visfatin concentration is at least 10 times lower than that of insulin in mice.[10] The molecular mechanisms revealed that visfatin activates intracellular cascade for insulin signalling, including tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1/2 (IRS1/2) as well as downstream activation of protein kinase B. Interestingly, however visfatin activates the insulin receptor in a manner distinct from that of insulin.[10]

This study was designed to evaluate the glucose lowering effect of visfatin in obese and insulin dependent diabetic mice.

MATERIAL AND METHODS

The study was conducted from March to December 2007 at Physiology Department, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad.

One hundred and twenty albino mice of balb/C strain having age between 6−12 weeks and weight 20−40 gram, were procured from the animal house of NIH, Islamabad. By convenience sampling, the mice (n=120) were divided into 4 equal groups (n=30 in each group). Weight of the animals was recorded and blood samples for baseline fasting blood glucose and serum visfatin levels were taken by tail bleed. Animals in Group I and II were given a high fat/high carbohydrate diet, for 4 months. The feed was composed of fat 58%, carbohydrates 25.6% and proteins 16.4%.[5] Group I served as obese control and was not given any treatment whereas mice in Group II were treated with 240 ρmol of recombinant-histidine soluble (mice) visfatin injection (ALEXIS Biochemical-USA) intravenously as a single bolus injection after they became obese.

Animals in Group III and Group IV were made insulin dependent diabetics by giving 40 mg/Kg body weight streptozotocin injection intraperitoneally.[2] On 5th day, their fasting blood glucose levels were measured by tail bleed. Mice with blood glucose levels

of 11.1 mmol/l and above were considered as diabetic. The animals were given food and water *ad libitum* for the next 10 days. Good hygienic conditions and optimum temperature (22−24 °C) was maintained in the animal house. Group III served as diabetic control and mice in Group IV were treated with 240 ρmol of visfatin injection intravenously as a single bolus injection on the 11th day after they became diabetic.

In Group II and IV, 30 minutes after visfatin injection, blood sample was taken under ether anaesthesia by intracardiac puncture. Out of 3 ml blood drawn, 1 ml was transferred to the vacutainer containing potassium fluoride and was used for glucose determination in plasma. Two ml blood was transferred to a tube containing spray-coated silica and a polymer gel for serum separation and estimation of visfatin levels. Similarly blood samples of Group I (obese control) and Group III (insulin dependent diabetic control) mice were taken. Blood glucose was measured by glucose oxidase method using the commercially available kit. For the measurement of serum visfatin, the samples were centrifuged to separate the serum which was then stored at -80 °C till analysis. Visfatin levels were estimated by Mouse Visfatin/PBEF ELISA Kit.

Independent sample *t*-test was applied for comparison of quantitative variables between different groups followed by POSTHOC TUKEY HSD test. The *p*-value <0.05 was considered as significant.

RESULTS

The weight (Mean±SD) of the mice (n=120) at the start of the study was 24.0±1.48 grams (Table-1) and their fasting blood glucose level was 4.4±0.38 mmol/l while visfatin level was 1.03±0.03 ηg/ml. The weight of mice in Group I and II increased to 58.4±2.01 gm, fasting glucose levels increased to 14.16±1.38 mmol/l, while visfatin levels of these mice was also found increased to 2.62±0.10 ηg/ml (Table-1). There was no significant gain in weight in Streptozotocin induced insulin dependent diabetic mice (Group III and IV) and average weight of these animals was 27.38±2.28 gm. However, their fasting glucose levels increased to 13.5±1.40 mmol/l and visfatin level in groups III and IV increased significantly to 2.41±0.10 (Table-1).

Administration of visfatin in Groups II and IV resulted in significant decrease in blood glucose levels to 8.08±0.60 mmol/l and 7.22±0.50 mmol/l respectively (Table-2).

Table-1: Base line plasma glucose, serum visfatin and body weight of mice (Mean±SD)

Parameters	Baseline Value (n=120)	Obese Group (n=60)	Insulin Dependent Diabetic Group (n=60)
Fasting Glucose (mmol/l)	4.40±0.38	14.16±1.38	13.5±1.40
Visfatin (ηg/ml)	1.00±0.03	2.62±0.10	2.41±0.10
Weight (gm)	24.00±1.48	58.4±2.01	27.38±2.28

Table-2: Plasma glucose levels after administration of visfatin in obese and diabetic mice (Mean±SD)

Parameter	Group I (n=30)	Group II (n=30)	Group III (n=30)	Group IV (n=30)	*P*
Glucose (mmol/l)	14.16±1.38	8.08±0.60	13.5±1.40	7.22±0.50	<0.001*

*highly significant

DISCUSSION

The association between obesity, insulin resistance, and cardiovascular diseases has been established since long but the mechanisms for their interrelationship are vague. In study, there was statistically significant ($p<0.05$) increase in visfatin levels by 1.62±0.02 ηg/ml (Table-3) in obese mice (Group I and II) fed on a high fat-carbohydrate diet for 4 months and their body weight increased by 34.4±0.48 grams in both the groups of obese mice. It has been documented that obesity induces the release of visfatin from adipocytes[12] which is more expressed in visceral fat as compared to the subcutaneous fat[11]. It is suggested that increase in visceral fat of these obese mice have resulted in substantial increase in visfatin levels whereas hyperglycemia was due to insulin resistant diabetes mellitus.[12] However, it is yet to be proven whether visfatin production is a compensatory response to tissue-specific insulin resistance or the marker of the action of tissue-specific inflammatory cytokines. Chen *et al*[13] have demonstrated that visfatin expression is regulated by cytokines and development of insulin resistance has been associated to TNF-α, which increased during obesity, a reason that can be associated to the obese group of mice in the present study.

Development of insulin resistance in obese mice has resulted in an increased blood glucose levels by 9.76±0.23 mmol/l in both Group I and II. Although visfatin levels had increased in these groups yet it was perhaps in sufficient to control blood glucose or it was due to the visfatin resistance developed similar to that of insulin resistance because visfatin uses the same receptors for its action as used by the insulin.[10] In present study exogenous administration of recombinant-histidine soluble (mice) visfatin proved effective in decreasing the blood glucose levels significantly, in the range of normoglycemia. In insulin resistant diabetes mellitus, various adipocytokines have been documented to be released such as resistin, which have been proposed to oppose the action of insulin.[7] It is suggested that increased levels of visfatin in obesity and insulin resistant diabetes mellitus might have been neutralised by other adipocytokines and have resulted in visfatin resistance and hyperglycaemia because visfatin acts on the same receptor as that for the insulin.

The increased levels of visfatin and its glucose lowering effect in obese mice of present study are comparable to the work of Fukuhara *et al* in Japan, who suggested the insulin mimetic effect of visfatin. They

further established that visfatin used the system of tyrosine phosphorylation-dependent signaling for its action comparable to that of the insulin receptor.[11]

In insulin dependent diabetic mice (Group III and IV) the visfatin levels were increased by 1.41±0.02 ηg/ml (Table-4), which was relatively close to that of obese mice and statistically significant (p<0.05). Lo´pez-Bermejo *et al*[14] have documented that visfatin secretion increases with progressive β-cell dysfunction of pancreas, while destruction of β-cells of pancreas leads to development of hyperglycaemia and insulin resistant diabetes mellitus. However, the presence of hyperglycaemia along with an increase in the level of visfatin is yet not clear. It has been suggested that possible increase in levels of visfatin observed in hyperglycaemic conditions could be due to the enhanced oxidative stress.[15,16] It has been documented that hyperglycaemia results in release of reactive oxygen species (ROS) and nitrogen species (RNS)[17], induces apoptosis and activation of cytochrome c-activated caspase-3 pathway.[18,19]

In our study, the administration of exogenous recombinant-histidine soluble (mice) visfatin in obese and diabetic groups of mice resulted in significant reduction (p<0.001) in their fasting blood glucose levels (Tables-1, 2) but it remained higher than the glucose levels of normoglycaemic mice. In obesity and insulin resistant diabetes mellitus, hyperinsulinemia is commonly seen. Since visfatin acts at the same receptor as that for insulin, exogenous visfatin administration could have increased its level as well as upregulation of insulin receptors which might have resulted in reduction in blood glucose levels in obese and insulin dependent mice.[10]

It is yet not established that how much quantity (dose) of exogenous visfatin would be required to be administered in diabetic mice to bring the blood glucose back to normal. This is a prospective avenue for further studies whereby therapeutic dose of visfatin could be determined.

Studies on adipocytes have demonstrated that visfatin release is regulated by hormones and cytokines which influence glucose homeostasis.[22,23] The insulin like effect of visfatin revealed by the present study is in accordance with the work conducted in China by Xie *et al*[24] in which visfatin was found to have insulin like effect on human osteoblasts. Apart from the hypoglycaemic action, visfatin causes proliferation and production of type I collagen similar to that mediated by the insulin receptor transduction pathway. Considering the visfatin mimetic effects of insulin through insulin receptor in cell cultures of adipocytes, myocytes and hepatocytes, it may be possible that osteoblasts be the future target for studies on visfatin action.[24] However, further studies would establish the beneficial effects of visfatin in diabetes mellitus. It could also be a useful target for the prospective development of anti-diabetic drug therapy.

CONCLUSION

We conclude that the present study has reconfirmed the glucose lowering effect of recombinant-histidine soluble (mice) visfatin in obese and insulin dependent diabetic mice to the level within normal range. Further studies are recommended to ascertain the role of visfatin as a potential antidiabetic drug/hormone that could perhaps act synergistically with insulin to control blood sugar in diabetes mellitus.

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REFERENCES

1. Coskun O, Ocakci A, Bayraktaroglu T, Kanter M. Exercise training prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Tohoku J Exp Med 2004;203:145−54.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047−53.
3. Nishtar S, Shera S, Raffique G, Mohamud KB, Ahmed A. Diabetes prevention and control: National action plan for NCD prevention, control and health promotion in Pakistan. J Pak Med Assoc 2004;54:S26−30.
4. Badruddin N, Basit A, Hydrie MZI, Hakeem R. Knowledge, attitude and practices of patients visiting diabetes care unit. Pakistan Journal of Nutrition 2002;1(2):99−102.
5. Winzell MS, Ahrén B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 2004;53 Suppl 3:S215−9.
6. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinal Metab 2004;89(6):2548−56.
7. Arner P. Insulin resistance in type 2 diabetes-role of adipokines. Curr Mol Med 2005;5:333−9.
8. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911−9.
9. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, R. Schon Michael, *et al*. Plasma visfatin concentrations and fat depot–specific mRNA expression in humans. Diabetes 2005;54:2011−6.
10. Hammarstedt A , Pihlajamaki J, Sopasakis V R, Gogg S, Jansson PA, Laakso M, *et al*. Visfatin is an aipokine, but it is not regulated by thiazolidinediones. J Clin Endocrinal Metab 2006;91(3):1181−4.
11. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, *et al*. Visfatin a protein secreted by visceral fat that mimics the effect of insulin. Science 2005;307:426−30.
12. Brendt J, Klöting N, Kralisch S, Kovacs P, Fasshauer M, Schön M, *et al*. Plasma visfatin concentrations and fat depot specific RNA expression in humans. Diabetes 2005;54:2911– 6.
13. Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, Bryant-Greenwood GD. Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. J Mol Endocrinol 2001;26(2):107−17.

14. Lopez-Bermejo A, Chico-Julia B, Fernandez-Balsells M, Recasens M, Esteve E, Casamitjana R, *et al*. Serum visfatin increases with progressive β-cell deterioration. Diabetes 2006;55:2871−5.
15. de Luis DA, Sagrado MG, Aller R, Conde R, Izaola O. Circulating visfatin in obese non-diabetic patients in relation to cardiovascular risk factors, insulin resistance, and adipocytokines: A contradictory piece of the puzzle. Nutrition 2010;26(11−12):1130−3.
16. Adeghate E. Visfatin: Structure, Function and Relation to Diabetes Mellitus and Other Dysfunctions. Curr Med Chem 2008;15(18):1851−62.
17. Cai L, Kang YJ. Oxidative stress and diabetic cardiomyopathy: a brief review. Cardiovasc Toxicol 2001;1(3):181−93.
18. Cai L, Li W, Wang G, Guo L, Jiang Y, Kang YJ. Hyperglycemia-induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway. Diabetes 2002;51(6):1938−48.
19. Adeghate E. Molecular and cellular basis of the aetiology and management of diabetic cardiomyopathy: a short review. Mol Cell Biochem 2004;261(1−2):187−91.
20. Senthi JK, Vidal Puig A. Visfatin: the missing link between intraabdominal obesity and diabetes? Trends Mol Med 2005;11:344−50.
21. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, *et al*. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2006;91(1):295−9.
22. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia 2006;49:1909−14.
23. Arner P. Visfatin –a true or false trail to type 2 diabetes mellitus. J Clin Endocrinol Metab 2006;91(1):28−30.
24. H Xie, Tang SY, Luo XH, Huang J, Cui RR, Yuan LQ, *et al*. Insulin-like effects of visfatin on human osteoblasts. Calcif Tissue Int 2007;80:201−10.

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