Original Article

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EFFECT OF VISFATIN ON LIPID PROFILE OF OBESE AND DIABETIC MICE

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ABSTRACT

Objective: To determine the effect of visfatin on blood lipid levels in balb/c strain of albino mice.

Design: Quasi experimental study.

Place and duration of study: The study was carried out at the department of Physiology, Army Medical College, Rawalpindi and National Institute of Health Sciences, Islamabad from April to December 2007.

Material and Methods: One hundred and twenty balb/c strain albino mice were procured from NIH, Islamabad. After taking base line blood samples, mice were divided randomly into four groups. Animals in groups I and II were made obese by feeding high fat / high carbohydrate diet whereas mice in Groups III and IV were induced diabetes mellitus by injecting streptozotocin. Groups I (obese) and III (diabetic) served as controls whereas groups II (obese treated) and IV (diabetic treated) were administered visfatin injection. Terminal intracardiac blood sample was used to measure the serum lipid and visfatin levels.

Results: Serum lipid levels were found increased in obese and diabetic groups as compared to healthy mice. The administration of recombinant-histidine soluble (mice) visfatin significantly ($p< 0.01$) decreased the serum lipid levels with concomitant increase in HDL levels ($p< 0.01$) in obese and diabetic groups of mice and were comparable with baseline normal values of healthy controls.

Conclusion: Visfatin is a potential antilipidemic adipocytokine that probably modulates insulin sensitivity and decreases atherogenic lipids (triglycerides, cholesterol, LDL and VLDL) with concomitant increase in HDL in obesity and diabetes mellitus.

Keywords: Adipocytokines, Diabetes mellitus, Obesity, Visfatin.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, defined by hyperglycemia, dyslipidemia, hypertension, abdominal obesity, and insulin resistance[1]. Diabetes affects virtually all lipids and lipoproteins, and dyslipidemia is a consistent finding in these patients manifested by the increased plasma concentrations of triglycerides, low plasma concentrations of high density lipoprotein (HDL) cholesterol, and preponderance of atherogenic small dense LDL[2]. In Pakistan, more than 10% of adult population is believed to be suffering from diabetes mellitus[3].

Obesity has been associated with an increased risk of developing insulin resistance and type 2 diabetes mellitus. Several proteins (adipocytokines) are secreted by the adipose tissue that modulate insulin sensitivity and appear to play a significant role in the pathogenesis of insulin resistance, diabetes mellitus, lipid disorders, inflammation and atherosclerosis. These include leptin, adiponectin, resistin, tumor necrotic factor alpha (TNF-alpha), Visfatin and interleukin (IL-6)[4-6]. Visfatin is a recently acknowledged adipocytokine secreted by the visceral fat of both human and mice. The studies have documented that visfatin has insulin mimetic properties whereby increases glucose transport and lipogenesis by adipocytes and myocytes and decreases glucose production by hepatocytes[7,8].

The visfatin activates intracellular signalling cascade for insulin, including tyrosine phosphorylation of insulin receptor and insulin receptor substrate-1/2 (IRS-1/2) as well as downstream activation of protein kinase B. However, activation of insulin receptor by visfatin takes place by a manner distinct from that of insulin[7,8]. The recombinant- histidine soluble visfatin administration has been documented to decrease plasma glucose[9] by

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increasing transport into the cells and lipogenesis. Hitherto, it is hypothesized that visfatin would decrease plasma lipid levels in obese and diabetic subjects. In view of the same, present study was designed to evaluate the effect of visfatin on lipid levels of obese and diabetic mice.

MATERIAL AND METHODS

The present study was conducted at Department of Physiology, Army Medical College, Rawalpindi with the collaboration of National Institute of Health (NIH), Islamabad. One hundred and twenty albino mice of balb/C strain (age 6-12 weeks and weight 20-40 gram), were procured from NIH. The measurement of baseline levels of plasma glucose, lipids and visfatin was carried out in blood samples taken by tail bleed. These animals were randomly divided into four groups with 30 mice in each group. Animals (n=60) in groups I and II were fed high fat / high carbohydrate diet, prepared at NIH Islamabad, for 4 months in order to make these mice obese. The feed was composed of fat 58%, carbohydrates 25.6% and proteins 16.4%. Group I served as obese control and did not receive any further treatment whereas obese mice of group II were administered 240 pico mol of recombinant-histidine soluble (mice) visfatin injection (ALEXIS Biochemical-USA) intravenously as a single bolus.

Diabetes mellitus was induced in (n=60) groups III and IV by injecting streptozotocin intraperitoneally in the dose of 40mg/Kg body weight. On fifth day, their fasting blood glucose levels were measured by tail bleed and mice with blood glucose levels of ≥ 11.1 mmol/l were considered diabetic. The animals were given food and water ad libitum for the 10 days. Optimum room temperature (22-24^{0}C) was maintained in the animal house.

Group III served as the diabetic control whereas mice in group IV were injected 240 pico mol of visfatin intravenously in a single bolus on 11th day. After 30 minutes of visfatin injection, terminal blood samples of groups II and IV were taken by intracardiac puncture after ether anesthesia. The blood was transferred into the spray-coated silica and a polymer gel vaccutainer for the separation of serum and estimation of lipid levels. Similarly blood samples from group I (obese control) and group III (diabetic control) mice were taken. Plasma glucose was measured by glucose oxidase method using the commercially available kit (Linear Chemicals S.L). Blood triglyceride levels were assayed by enzymatic colorimetric method by using the commercially available ready to use kit (Linear Chemicals S.L). HDL Cholesterol was measured by direct enzymatic colorimetric fixed time method using the ready to use kit (Linear Chemicals S.L).

For the measurement of serum visfatin, the clotted blood samples were centrifuged at 4000 rpm at 4^{0}C for 15 minutes to separate the serum which was then stored at -80^{0}C till analysis. Visfatin levels were estimated by mouse visfatin / PBEF ELISA kit, Circulex (MBL International, USA) by quantitative sandwich enzyme immunoassay technique.

Data were entered into SPSS version 13. Mean and standard deviation of all the variables were calculated. Mean of all variables among control and treated groups was compared by 'Independent sample t test. The difference was considered significant if *p*-value was found less than 0.05.

RESULTS

The body weight, plasma glucose, lipid profile and visfatin levels in mice after induction of high fat fed obesity (n=60), streptozocin induced diabetes mellitus (n=60) have been compared with base line parameters of healthy mice in table– 1. The data revealed insignificant change in body weight of diabetic group as compared to the healthy mice, whereas obese as well as diabetic groups manifested significant (p< 0.001) increase in plasma glucose, serum triglycerides, cholesterol, LDL, VLDL and visfatin levels except serum HDL levels.

The comparison of different parameters of lipid profile between group II (obese treated with visfatin) and group I (obese control) have been presented in table-2 that revealed the significant (p<0.001) reduction in serum triglycerides, cholesterol, LDL and VLDL levels with concomitant significant (p<0.001) increase

in serum HDL levels after the administration of visfatin.

The comparison of different parameters of lipid profile between diabetic mice treated with visfatin (group–IV) and diabetic control (group–III) revealed significant (p<0.001) reduction in serum triglycerides, cholesterol, LDL and VLDL levels along with concomitant significant (p<0.001) rise in serum HDL levels in diabetic mice after the administration of visfatin (Table-3).

The comparison of lipid profile between visfatin treated obese (group–II) and diabetic groups (group-IV) with healthy mice (Table-4) revealed insignificant difference between parameters of lipid profile except HDL levels which significantly increased (p<0.01) after visfatin treatment.

DISCUSSION

It is well established that obesity, particularly visceral obesity, is a major contributor of type 2 diabetes mellitus, dyslipidemia and hypertension. It has been documented that obesity induces the release of

Table-1: Comparison of body weight, plasma glucose, lipid profile and serum visfatin levels between healthy, obese and diabetic mice.

Parameters	Healthy mice (n=120)	Obese Groups (I&II) (n=60)	Diabetic Groups (III&IV) (n=60)
Weight (gm)	24.0±1.48	58.4±2.01	27.38±2.24
Glucose (mmol/l)	4.4± 0.38	14.50±1.83	13.96±1.80
Cholesterol (mmol/l)	3.0 ± 0.50	5.96±0.46	4.88±0.42
Triglycerides (mmol/l)	1.57± 0.21	2.76±0.18	2.88±0.16
HDL (mmol/l)	0.84±0.09	0.89±0.09	0.87±0.06
LDL (mmol/l)	1.51±0.51	3.78±0.86	2.96±0.18
VLDL (mmol/l)	0.51±0.11	1.30±0.18	1.08±0.06
Visfatin (ng/ml)	1.03±0.03	2.64±0.20	2.38±0.18
TG:HDL ratio	1.78±0.2	3.26±0.1	2.86±0.13

All values are expressed as Mean ± SD
p< 0.05 as compared to control
p< 0.001 as compared to control

Table-2: Comparison of lipid profile between group I (obese control) and group II (obese treated) mice.

Parameters	Group I (obese control) (n=30)	Group II (obese treated) (n=30)	p value
Triglycerides (mmol/l)	2.84 ± 0.13	1.38 ± 0.26	< 0.001
Cholesterol (mmol/l)	5.84 ± 0.18	3.54 ± 0.39	< 0.001
LDL (mmol/l)	3.72 ± 0.36	1.88 ± 0.27	< 0.001
VLDL (mmol/l)	1.29 ± 0.14	0.62 ± 0.12	< 0.001
HDL (mmol/l)	0.87 ± 0.07	1.01 ± 0.12	< 0.001
TG:HDL ratio	3.27± 0.10	1.35± 0.19	< 0.001

All values have been expressed as mean ± SD

Table-3: Comparison of lipid profile between group III (diabetic control) and group IV (visfatin treated) mice.

Parameters	Group III (Diabetic control) (n=30)	Group IV (Diabetic treated) (n=30)	p value
Triglycerides (mmol/l)	2.40 ± 0.11	1.20 ± 0.25	< 0.001
Cholesterol (mmol/l)	4.84 ± 0.107	3.36 ± 0.50	< 0.001
LDL (mmol/l)	2.91 ± 0.14	1.90 ± 0.42	< 0.001
VLDL (mmol/l)	1.07 ± 0.04	0.51 ± 0.10	< 0.001
HDL (mmol/l)	0.84 ± 0.09	0.94 ± 0.15	0.004
TG:HDL ratio	2.87± 0.11	1.27± 0.20	< 0.001

All values have been expressed as mean ± SD

Table 4 Comparison of lipid profile between healthy and visfatin treated obese and diabetic groups of mice.

Parameters	Healthy mice (n = 120)	Obese treated (n = 30)	Diabetic treated (n = 30)
Triglycerides (mmol/l)	1.57±0.21	1.38±0.26	1.20±0.25
Cholesterol (mmol/l)	3.0±0.50	3.54±0.39	3.36±0.50
LDL(mmol/l)	1.51±0.51	1.88±0.27	1.90±0.42
VLDL(mmol/l)	0.51±0.11	0.62±0.12	0.51±0.10
HDL(mmol/l)	0.84±0.09	1.01±0.12**	0.94±0.15*
TG:HDL ratio	1.78±0.2	1.35± 0.19	1.27± 0.20

All values have been expressed as mean ± SD
* $p < 0.05$ diabetic treated compared with healthy mice
** $p < 0.01$ obese treated compared with healthy mice.

visfatin from adipocytes[10] expressed in visceral fat as compared to the subcutaneous fat[8]. The increase in visfatin levels is obese (group I) and diabetic (group III) mice could be due to its release from the visceral fat of mice in present study[10]. However, it is yet to be proven whether visfatin production was a compensatory response to tissue-specific insulin resistance or the marker of the action of tissue-specific inflammatory cytokines. Chen *et al* have demonstrated that visfatin expression is regulated by cytokines and development of insulin resistance could be associated to TNF-α, which increased during obesity[11]. The same can be associated to the obese group of mice in the present study.

Development of insulin resistance (>1.8 TG: HDL ratio) and increased level of blood lipids (triglycerides, cholesterol, LDL and VLDL) have been observed in both obese and diabetic groups (groups I & III). Despite increase in visfatin levels above normal in these groups yet it was perhaps not sufficient to bring the blood lipid levels to normal due to the insulin resistance because visfatin use the same receptor for its action as that by the insulin[7]. In present study, exogenous administration of recombinant-histidine soluble (mice) visfatin effectively decreased the blood lipid levels with concomitant increase in HDL levels comparable to the basal levels of healthy mice. It could be due to the action of visfatin through insulin receptors causing lipogenesis along with reduction in blood glucose levels in obese and diabetic mice[9]. In insulin resistant diabetes mellitus, various adipocytokines including resistin are released which oppose the action of insulin[5]. It is proposed that increased levels of visfatin in obesity might be counterproductive due to the insulin resistance induced by adipocytokines (like resistin) which would have resulted in hyperlipidemia in obese group of mice.

The lipid lowering effect of exogenous visfatin on triglycerides, cholesterol, LDL and VLDL in obese mice of present study is comparable to the work done by Fukuhara and his colleagues[10], and supported the concept of insulin mimetic effect of visfatin. They further established that visfatin treatment markedly induced the expression of genes encoding adipose markers such as peroxisome proliferator-activated receptor–gamma (PPAR-gamma), fatty acid synthase (FAS), diacylglycerol O-acyltransferase–1 (DGAT-1), adipose P2 (aP2), and adiponectin. In addition, visfatin used the system of tyrosine phosphorylation-dependent signaling for its action comparable to that of the insulin receptor[10].

In present study, the induction of diabetes mellitus in mice resulted in increased levels of visfatin (2.41 ± 0.02 ng/ml) from its basal levels in healthy mice (1.3± 0.03 ng/ml). Lo´pez-Bermejo *et al*. have documented that visfatin secretion increases with progressive β-cell dysfunction of pancreas[12] as that in streptozotocin induced diabetic mice. While increased levels of visfatin observed in hyperglycemic conditions could be associated to the enhanced oxidative stress following the release of reactive oxygen species (ROS) and nitrogen species (RNS)[13] which induce

apoptosis and activation of cytochrome c-activated caspase-3 pathway[14,15].

In a recent in vivo study the effect of visfatin/PBEF/Nampt on insulin sensitivity, glucose and lipid metabolism before and after visfatin administration has been documented[16]. On 4th day after visfatin injection, it resulted in hypercholesterolemia in both normal-chow and high-fat fed animals[17]. They also observed the up regulation of insulin receptors substance (IRS-I) tyrosine phosphorylation, the mRNA expression of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and sterol regulatory element-binding proteins 2 (SREBP-2) in the liver and adipose tissues of these animals[17].

A study on Asian-Indian immigrants revealed the positive correlation of visfatin with HDL cholesterol[18], as found in present study. It suggests that reduction in atherogenic (LDL and VLDL) lipid levels with concomitant increase in cardio protective (HDL) lipid would markedly reduce cardiovascular risk in diabetic and non-diabetic patients. Future research would define the threshold for treatment of these patients and the desired target lipid concentrations to achieve primary prevention. Interestingly, the observed actions of visfatin indicate that this adipocytokine could be an interesting therapeutic target.

CONCLUSIONS

We conclude that visfatin is a potential antilipidemic adipocytokine that probably modulates the sensitivity of insulin and decreases atherogenic lipids (triglycerides, cholesterol, LDL and VLDL) with concomitant increase in HDL in obesity and diabetes mellitus.

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