EFFECT OF VISFATIN ON TESTOSTERONE LEVELS IN INSULIN RESISTANT DIABETIC MALE SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To study the effect of visfatin on testosterone levels in insulin resistant diabetic male Sprague Dawley rats.

Methods: Eighty male Sprague Dawley rats were randomly divided into four groups with twenty rats in each group. Group I served as control. Group II served as control insulin resistant diabetic. Rats in group III were made insulin resistant diabetics and were treated with visfatin. Rats in group IV were treated with visfatin and resistin after making them insulin resistant diabetics. Blood samples were taken and analyzed for serum testosterone, LH and FSH by ELISA.

Results: There was a significant rise in testosterone, FSH and LH levels in insulin resistant diabetic rats treated with visfatin. Co-administration of resistin did not result in an elevation of testosterone levels.

Conclusion: Visfatin increased testosterone levels and stimulated LH and FSH secretion from anterior pituitary.

Keywords: Visfatin, adipocytokines, diabetes, testosterone.

INTRODUCTION

There is an inverse relationship between fasting insulin levels and serum testosterone in men[1]. Testosterone levels in men suffering from insulin resistant diabetes (type II) mellitus are lower than age-matched normal weight and non-diabetic controls[2]. The inverse relationship between testosterone and insulin has been suggested to be due to obesity[3,4].

The synthesis of testosterone by Leydig cells of the testes is controlled by hormones, growth factors, and cytokines[5]. Testosterone production in Leydig cell cultures is stimulated by insulin[6,7] and insulin receptors have been identified on Leydig cells[6]. A variety of adipokines are secreted by adipose tissue including leptin and the newly discovered hormones adiponectin, resistin and visfatin.

Resistin is an adipocytokine whose effects on glucose metabolism are antagonistic to those of insulin[8]. It suppresses insulin-stimulated glucose uptake and induces insulin resistance.

Visfatin, described in 2005 by Fukuhara et al[9] has an insulin-mimetic action i.e., it reduces the blood glucose level. Although visfatin binds to the insulin receptors yet it does not compete with insulin, suggesting that the two proteins bind to different sites on the receptor. A high-fat diet results in increased plasma visfatin levels suggesting that it may have a role in diet or obesity-induced insulin resistance[10]. The effect of visfatin on testicular steroidogenesis is yet to be elucidated. It is proposed that visfatin can increase testosterone secretion due to its insulin mimetic action. The present study was designed to study the effect of visfatin in an in vivo model using insulin resistant diabetic male sprague dawley rats.

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MATERIALS AND METHODS

Chemicals and kits

Visfatin, Soluble (rat) (recombinant) (His) from ALEXIS Biochemicals, AXXORA, USA.

Resistin, Soluble (rat) (recombinant) (ALEXIS Biochemicals, AXXORA, USA).

Rat Visfatin ELISA Kit-ALPCO DIAGNOSTICS, Cat No. 44-VISFTH-0523.

Rat Resistin ELISAKit- BIOVENDOR Research and Diagnostic Products, Cat No. RD391016200R.

Rat Testosterone EIA (In vivo) Kit - ADALTIS-EIAgen, Cat No. Testosterone- LI 4011 K.

Rat FSH EIAKit - IBL AMERICA, Cat No. = IB19103.

Rat LH EIAKit - ALPCO DIAGNOSTICS, Cat No. = 11-LUTHU-E01.

These lab based randomized controlled trials were conducted in the Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad. Eighty male, healthy Sprague Dawley rats, at least 60 days old were included in the 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^{0}C. Food and water was available ad libitum. The rooms were well-ventilated and 12-hour light-dark cycle was maintained.

Rats were randomly divided into four groups of 20 each using random numbers table. Group I served as control group and received standard diet. Group II served as control insulin resistant diabetic and were made insulin resistant diabetics (Type II diabetes mellitus) by being fed a diet rich in sucrose (Table 1) for 3 weeks[11]. Insulin resistance was measured by the triglycerides-high density lipoproteins (TG/HDL) ratio[12]. Initial blood sampling was done at the end of 3rd week by tail bleed to confirm the presence of insulin resistance. At the end of 4th week terminal sampling was done by intra cardiac bleed under ether anesthesia and the animals were euthanized. Rats in group III were made insulin resistant diabetic, as previously described[12], and at the end of 4th week they were given 500 pmol recombinant visfatin hormone by a single intraperitoneal injection. Rats in group IV were also made insulin resistant diabetic as previously described, and at the end of 4th week they were given 500 pmol recombinant visfatin hormone and 10 µg recombinant resistin hormone by a single intraperitoneal injection. In both groups III and IV blood was collected 30 minutes after administration of visfatin and resistin by intracardiac sampling under ether anesthesia and the animals were euthanized. After clotting, samples were first centrifuged at 4,000 rpm at 4 ^{o}C in the cold centrifuge. Then serum was pipetted out and stored in Eppendorf storage tubes at -70 ^{o}C till analysis. Serum testosterone, LH and FSH were determined by ELISA.

Data were analysed on SPSS-13. The arithmetic mean and standard deviation (SD) of quantitative variables were calculated. Difference in mean among control and treated groups was calculated by ANOVA. The difference was considered significant if p value was found to be less than 0.05.

Table-1: Composition of vitamins /minerals /amino acids (premix) mixed in the diet for rats.

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 grms/Kg
Dibasic Calcium Phosphate	9.5 grms/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

RESULTS

The animals in this study remained healthy and active throughout study period and took their feed properly.

The TG/HDL ratio of more than 3.0 in groups II, III and IV at the end of three weeks confirmed the presence of insulin resistant diabetes (Table 2). There was a decline in serum testosterone, LH and FSH level in groups II, III and IV at the end of 3 weeks (Table 3). Visfatin administration significantly (p<0.001)(Table 5) improved testosterone levels in group III (Table 4) and also improved LH and FSH levels (p<0.05)(table5). Co administration of visfatin and resistin did not improve LH, FSH or testosterone levels (p>0.05).

DISCUSSION

Visfatin was originally identified as a pre β

Table-2: Body weight, plasma glucose and TG:HDL in groups at the end of 3 weeks.

Variables	Body weight (grams)	Plasma glucose (mg/dl)	Serum TG (mg/dl)	Serum HDL (mg/dl)	TG : HDL
Group I n = 20	254.35 ± 5.20	103.2 ± 2.05	105.45 ± 9.45	67.35 ± 2.86	1.37 ± 0.45
Group II n = 20	310.65 ± 7.34	154.1 ± 3.05	184.66 ± 6.24	60.56 ± 3.86	3.01 ± 0.46
Group III n = 20	309.55 ± 6.45	155.0 ± 3.55	183.63 ± 5.31	61.53 ± 3.45	3.00 ± 0.46
Group IV n = 20	311.45 ± 7.24	153.2 ± 2.15	183.45 ± 5.86	61.24 ± 3.96	3.02 ± 0.24

Table-3: Summary of in vivo results in groups at the end of 3 weeks.

Variables	Visfatin (ng/ml)	Serum Resistin (ng/ml)	Serum LH (IU/l)	Serum FSH (mIU/l))	Testosterone (ng/ml)
Group I n = 20	1.0 ± 0.06	12.14 ± 1.28	1.42 ± 0.16	1.47 ± 0.34	2.42 ± 1.18
Group II n = 20	2.41.0 ± 0.03	21.02 ± 1.02	1.27 ± 0.11	1.40 ± 0.29	1.02 ± 0.05
Group III n = 20	2.40.0 ± 0.05	14.02 ± 1.24	1.27 ± 0.12	1.42 ± 0.29	1.03 ± 0.07
Group IV n = 20	2.43.0 ± 0.05	21.10 ± 1.16	1.28 ± 0.12	1.41 ± 0.31	1.02 ± 0.06

Table-4: Summary of in vivo results in groups at the end of 4 weeks.

Variables	Visfatin (ng/ml)	Serum Resistin (ng/ml)	Serum LH (IU/l)	Serum FSH (mIU/l))	Testosterone (ng/ml)
Group I n = 20	1.0 ± 0.06	12.14 ± 1.28	1.42 ± 0.16	1.47 ± 0.34	2.42 ± 1.18
Group II n = 20	2.41 ± 0.05	21.02 ± 1.12	1.27 ± 0.16	1.40 ± 0.30	1.02 ± 0.09
Group III n = 20	2.51 ± 0.13	21.02 ± 1.12	1.39 ± 0.12	1.46 ± 0.27	2.36 ± 1.16
Group IV n = 10	2.50 ± 0.12	21.14± 1.37	1.29 ± 0.23	1.41 ± 0.21	1.02 ± 0.12

Table-5: Statistical comparison of various groups of rats.

Group comparison	Visfatin	Resistin	LH	FSH	Testosterone
II vs I	< 0.001	< 0.001	< 0.01	< 0.05	< 0.001
III vs II	< 0.05	> 0.05	< 0.05	< 0.05	< 0.001
IV vs III	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
II vs IV	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

colony enhancing factor (PBEF), and was found to have a role in the maturation of B cell precursors[13]. Later on visfatin was characterized as an adipokine with a high expression in

visceral fat exhibiting insulin–like functions[9]. These insulin mimetic actions of visfatin are mediated through binding to the insulin receptor at a site separate from that of insulin[14].

The rats in groups II, III and IV showed a significant weight gain. The blood glucose levels along with serum TG and HDL levels were also significantly raised in these groups. The TG:HDL ratio in these groups increased above 3.0. This increased ratio along with elevated blood glucose levels confirmed the presence of insulin resistant diabetes in these groups. This finding is similar to the findings of Soria et al[15] and McLaughin et al [12] who have reported the development of insulin resitant diabetes mellitus with a TG:HDL ratio of more than 3.0 in rats fed a sucrose rich diet.

The increased levels of resistin and visfatin in group II, III and IV at the end of 3 weeks corresponded to the studies by Fukuhara et al[9] and Nogueiras et al[16] who have also shown that adipocytokine secretion increased in diabetes mellitus. The onset of diabetes increases the amount of adipose tissue and the adipocytes start production of adipocytokines. Although visfatin is an insulin mimetic hormone and decreases the blood glucose levels in isolated settings the high levels of resistin (an insulin antagonist) oppose its function. The serum LH and FSH levels in these groups were also reduced at the end of 3 weeks. The testosterone levels were significantly reduced in these groups and this corresponded to previous studies[17,18] which have shown that in insulin resistant diabetics, testosterone levels decrease.

One of the possibilities of decreased serum testosterone levels in these groups after the onset of insulin resistant diabetes is raised resistin levels. Since resistin opposes the action of insulin and previous studies[19] have shown that decreased insulin levels also lead to a decrease in testosterone levels. This action could have been either directly by its action on Leydig cells of the testis or indirectly through the pituitary gland.

Testosterone biosynthesis is regulated primarily by pulsatile secretion of LH, and compelling evidence exists that Leydig cell steroidogenesis is further modulated locally by circulating hormones, growth factors, and cytokines[20]. Serum testosterone levels reflect the integrity of the hypothalamic-pituitary gonadal (HPG) axis, and low testosterone levels noted in cases of insulin resistance may indicate a defect at one or more functional levels of the HPG axis. In the insulin-resistant state, Leydig cell function, particularly steroidogenesis, may be impaired by changes in the production of hormones and cytokines locally in the target tissue and in adipose tissue.

In insulin resistant rats treated with visfatin (group III) a significant increase in serum LH and FSH levels were observed. Similarly an increase in serum testosterone levels was also observed in this group. This indicated that visfatin administration resulted in an increased synthesis and release of pituitary FSH and LH followed by an increase in testosterone production from Leydig cells of the testis. It appears that visfatin plays a role similar to insulin signaling in the brain[21]. The effect of visfatin in increasing LH and FSH levels correlated with the study of Burcelin et al[22] who have previously demonstrated that insulin increases LH and FSH levels thereby increasing testosterone production. It is noteworthy that visfatin, being insulin mimetic, showed similar results. Visfatin administration had no effect on serum resistin levels.

The opposing action of visfatin and resistin was demonstrated in group IV where no change in serum LH, FSH or testosterone levels was observed. Thus it appears that adipocytokines may play a major role in decreased testosterone levels in diabetic patients.

CONCLUSION

Collectively, our results strongly reinforce that visfatin increases serum testosterone levels and FSH and LH levels in insulin resistant diabetic rats. Further studies need to be carried out on cultured Leydig cells to further elucidate the mechanism of action of visfatin in in vitro models. Further studies may also be carried out in human subjects to give visfatin as an adjunct to insulin in diabetic patients to improve their testosterone secretion.

CONFLICT OF INTEREST

We would like to disclose that there is no conflict of interest.

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