EFFECT OF OBESTATIN ON GLUCOSE HOMEOSTASIS, INSULIN RESISTANCE AND SERUM INSULIN LEVELS IN TYPE 2 DIABETIC RATS

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ABSTRACT

Objective: To determine the effect of obestain administration on plasma glucose levels, serum insulin levels and insulin resistance in type 2 diabetic Sprague Dawely rats.

Study Design: Randomized controlled trail.

Place and Duration of Study: This study was carried out at Army Medical College Rawalpindi, from April 2013 to July 2013.

Material and Methods: This study was a randomized controlled trial conducted at Physiology department, Army Medical College. Forty-five healthy Sprague Dawley rats were randomly divided in to 3 groups i.e. control group (group-I) fed with normal pellet diet (NPD), diabetic group (group-II) and obestatin treated diabetic group (group-III) fed with high fat diet (HFD). Diabetic was induced by single intraperitoneal injection of streptozotocin (35mg/kg). Insulin resistance was determining by HOMA-IR. After Eight weeks, group-II was treated with obestatin (1nm01/100ml intraperitoneally) Blood samples were obtained by terminal intracardic sampling for bioassays of plasma glucose by glucose oxidase method, serum insulin level by ELISA and measurement of HOMA-IR. Mean ± SD was calculated. Statistical significance of differences across the groups was determined by one-way ANOVA by followed by post Hoc Turkey's test. A p-value <0.05 was considered significant.

Results: Obestatin supplementation in diabetic rats showed significant decrease in plasma glucose levels and insulin resistance on comparison with the non-treated control groups. Serum insulin levels were significantly increased in obestatin group when compared to non-treated diabetic group.

Conclusion: Obestatin improves the glycemic status in diabetic rats and can be used as an adjunct therapeutic tool in treatment of obesity induced type-II diabetic mellitus.

Keywords: Glucose, Homeostasis glucose level, Insulin resistance, Type-II diabetic.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by the state of chronic hyperglycemia due to the varying degrees of insulin resistance and insulin hypo secretion. It is a multi-factorial disorder with etiological basis of multiple genetic as well as environmental factors. Major fraction of diabetic population is suffering from type 2 DM. Sedentary life style and dietary factors play a key role in drastic rise in prevalence of T2DM[1]. The development of T2DM is closely linked to the concomitant obesity. Due to the greater mass of adipocytes, there occurs increased secretion of pro-inflammatory cytokines. This causes perturbed insulin signaling in the cells resulting in development of insulin resistance and development of T2DM[2]. Obestatin is a peptide hormone which is released by the parietal cells in gastrointestinal tract. This 23 amino acid polypeptide is encoded by ghrelin gene and originates from post translational modification of preproghrelin which is a precursor of ghrelin[3]. Obestatin is documented to manifest immuno-reactivity with pancreatic E cells, present in peripheral pancreas that are distinct from alpha and beta cells. It is co-localized with ghrelin which projects its role in normal functioning of pancreatic islets. It has been documented that obestatin level is decreased in obese individuals and tend to rise in patients with anorexia nervosa[4]. The proposed receptor GPR39 of obestatin had been documented to be down regulated in T2DM associated with obesity[5]. The present study has been

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conducted to outline the effect of obestatin on the glucose homeostasis, insulin resistance and serum insulin levels in diabetic Sprague Dawley Rats.

MATERIAL AND METHODS

This study was a randomized controlled trial conducted at Physiology department, Army Medical College, Rawalpindi in collaboration with National Institute of health Sciences (NIH), Islamabad from April 2013 to June 2013. Five Male Sprague Dawley rats weighing 250 to 300 grams were taken from the animal house of NIH, Islamabad. The rats were kept at standard room temperature on 12 hour light/dark cycle. The Rats were randomly divided in 3 groups I (control healthy), II (diabetic group) and III (obestatin treated diabetic group). Group I was fed on standard palleted diet for 13 weeks. Group II and III was fed with high fat diet for 2 weeks followed by a single intraperitoneal injection of streptozotocin (35mg/kg). Rats were continued on HFD and diabetes was confirmed after 2 weeks by measurement of plasma glucose and HOMA-IR. Group III was given intraperitoneal injection of obestatin (1nmol/100ml) daily for 10 days. Euthenization of rats was done by overdose of ether anesthesia at the end of 13 weeks. Terminal blood sample of rats was obtained in serum gel separator tubes through intracardiac sampling. The sample was centrifuged and serum as stored at -80C for blood assays of glucose by glucose oxidase method, Serum insulin by ELISA and calculation of HOMA-IR. Data was analyzed on SPSS version 21. Mean ± SD was calculated. ANOVA was applied to determine significance among the groups. Post-hoc Tukey test for inter-group comparison was applied.

RESULTS

The study included forty-five animals divided into 3 groups with 15 in each group. At the end of study, plasma glucose, HOMA-IR and serum insulin in groups I, II and III were compared by applying ANOVA which revealed difference in their levels across all groups. On applying Post hoc Tukey's HSD, the group II manifested profound hyperglycemia as compared to group I. there was a significant decrease in plasma glucose, HOMA-IR in group III when compared to group II. A significant increase in serum insulin levels was observed in obestatin treated diabetic rats III when compared to diabetic control group (table).

Table: Comparison of Plasma glucose, Serum insulin and HOMA-IR among healthy control, diabetic group and obestatin treated group by Post Hoc Tukeys test at end of the study.

Parameters	Group-I	Group-II	Group-III
Plasma Glucose			
Mean ± SD	94.27 ± 3.61	494 ± 4.21	221.07 ± 6.19
p-value	I & II 0.015	II & III 0.045	I & III 0.033
Serum Insulin			
Mean ± SD	6.02 ± 0.85	4.08 ± 0.04	5.35 ± 0.19
p-value	I & II 0.022	II & III 0.033	I & III 0.016
HOMA-IR			
Mean ± SD	1.41 ± 0.20	4.98 ± 0.42	2.92 ± 0.11
p-value	I & II 0.015	II & III 0.01	I & III 0.025

DISCUSSION

The maintenance of glucose levels during basal or post absorptive state (10-12 hours fast) and after ingestion of a meal is essential for normal metabolic functions of the cells. Insulin promotes the glucose entry into the insulin dependent tissues including skeletal muscle (approximately 80%-85%) and adipose tissues (4-5%) while at the same time suppresses the endogenous glucose production by the liver. Although adipose tissue does not contribute much in glucose disposal, the production of free fatty acids and adipocytokines modulate insulin sensitivity in the peripheral tissues especially

liver and skeletal muscles[6]. In the present study serum insulin levels did not change by obestatin administration in healthy rats although Mony *et al* observed the direct inhibitory effect of exogenous obestatin on insulin secretion in male albino rats[7]. This could be due to the difference in dose of obestatin administered. In our study obestatin was administered IP in the dose of 1nmol/100ml for the period of 10 days whereas in the aforementioned study obestatin was administered in the dose of 64µg/kg IP for a period of 7 and 14 days. Moreover, their rats were kept fasted 16 hours before the drug administration. Fasting has been documented to cause a decline in serum insulin levels[8,9]. Obestatin has been documented to cause dual effect on insulin secretion from perfused rat pancreas when it was exposed to 1 nmol obestatin; insulin secretion was increased while 10 nmol obestatin exposure resulted in inhibition of insulin secretion in vivo. The potentiated effect of obestatin was believed to be lost in presence of diazoxide which activated the ATP sensitive K+ channels reflecting their sensitivity of these channels to obestatin[10]. It has been documented that over the course of development of T2DM, initially there is rise in insulin levels with simultaneous development of impaired glucose tolerance and insulin resistance, however overt development of T2DM is manifested later by the decreased serum insulin levels with concomitant hyperglycemia which is attributed to beta cell exhaustion[11]. In our study, the diabetic rats demonstrated the decrease in insulin levels when compared to healthy controls. Obestatin administration resulted in increased insulin levels in diabetic rats when compared to their respective non treated controls. A study conducted on neonatal Sprague Dawley rat pretreated with STZ revealed that obestatin prevented the diabetes to develop in these rats by blocking the effect of STZ on pancreas with simultaneous increase in beta cell mass with concomitant correction of plasma glucose and insulin levels. This effect was accompanied by the up regulation of insulin gene expression in pancreatic/ jejunal home box. Obestatin has been documented to act as an anti apoptotic agent by increasing the expression of anti apoptotic protein BCL 2 in STZ treated rat pancreas[12]. The obestatin administration counteracts cytokine induced apoptosis by acting through GLP 1 and promotes proliferation and survival of beta cells, hence resulting in increased insulin output from human pancreas. This effect is accompanied with simultaneous upregulation of IRS mRNA levels and GLP IR in pancreatic islets[13]. In our study, the stimulatory effect of obestatin administration on insulin levels in diabetic rats can be explained by the observation that pancreatic islets get destroyed by STZ injection, and antiapoptotic effect of obestatin might result in increased beta cells mass, hence ameliorating the destructive effect of STZ on pancreatic islets which enhance the insulin output from beta cells.

There were a significant decrease in glucose level and insulin resistance in our diabetic rats after obestatin treatment when compared to the diabetic control group, which could be associated with obestatin induced reduction in appetite resulting thereby in reduction of body weight in treated rats when compared to non-treated rats. However, obestatin did not to affect the serum glucose levels of healthy rats in our study. Mony *et al* documented the decline in glucose levels in obestatin treated healthy albino rats and attributed to the anorexic property of the peptide[7]. These rats were fasted for 16 hours prior to obestatin administration which would have increased the insulin sensitivity as documented by Abdelali *et al* in their study that fasting mice for the period of 16 hours increased the sensitivity of tissues to insulin manifested by vigorous phosphorylation of IRS14 which might be responsible for the fall in serum glucose levels. In our study, non-significant fall in serum glucose levels of obestatin treated rats was observed. However, there was significant decrease in serum glucose levels in diabetic rats when compared with the diabetic control group although it did not reach the normal basal levels. The reduction in plasma glucose levels could be due to the

stimulation of glucose uptake induced by obestatin either in presence or absence of insulin and increased GLUT 4 translocation resulting in rapid uptake of glucose by the skeletal muscles as reported by Granata *et al*[15]. Another mechanism of obestatin induced decline in plasma glucose level has been documented through the induction of adiponectin level[16]. Adiponectin is an adipocy-tokine which serves as a positive regulator in glucose homeostasis by decreasing gluconeo-genesis and increasing glucose uptake by the cells and enhancing insulin sensitivity[17]. Obestatin treatment resulted in significant decline in plasma glucose levels in diabetic rats when compared to their non treated controls. This manifests that the glucose lowering effect of obestatin in diabetes can be attributed to the modulation of adiponectin released by adipocytes.

The obestatin treated diabetic group also manifested decline in HOMA-IR levels when compared to their respective diabetic control group. This effect of obestatin on insulin resistance has also been documented by Granata *et al* in which obestatin treatment of HFD fed mice resulted in a decrease in insulin resistance and inflammation induced by high fat feeding. It was revealed that obestatin manifested insulin sensitizing effect by increasing Akt and AMPK phosphorylation in white adipose tissue (WAT). Infact, Akt and AMPK play a key role in insulin sensitivity and their levels have been found decreased in human and animal models of insulin resistance[16,18]. Furthermore, it was observed that obestatin blocked the accumulation of HFD induced TNF α and IL β in WAT, liver and muscles hence counteracting inflammation and reducing insulin resistance. Of 151 Ibrahim *et al* documented a significant decrease in insulin resistance on IP administration of obestatin for a period of 10 days in HFD obese male albino rats[19]. In these studies, obestatin treatment resulted in significant decrease in plasma glucose levels and insulin resistance which highlighted it as a novel peptide in glucose homeostasis in T2DM. In our study, the decline in plasma glucose levels in these groups can be attributed to the decreased insulin resistance in the diabetic rats along with increase in insulin levels in diabetic rats on treatment with obestatin.

CONCLUSION

Our study concludes that obestatin treatment is more beneficial in T2DM. However, failure of obestatin to return the serum glucose to basal level shows that obestatin cannot be used alone as an anti diabetic agent, however it can work as an adjunct therapeutic agent in treatment of type 2 diabetes.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(Suppl-1): S81-S90.
2. Zhang M, Li F, Wang J. Correlation analysis of serum obestatin expression with insulin resistance in childhood obesity. Genet Mol Res 2017; 16(2): 1-7.
3. Lacquaniti A, Donato V, Chirico V, Buemi A, Buemi M. Obestatin: An interesting but controversial gut hormone. Ann Nutr Metab 2011; 59(2-4): 193-9.
4. Razzaghy-Azar M, Nourbakhsh M, Pourmoteabed A, Nourbakhsh M, Ilbeigi D, Khosravi M. An evaluation of acylated ghrelin and obestatin levels in childhood obesity and their association with insulin resistance, metabolic syndrome, and oxidative stress. J Clin Med 2016; 5(7): 61.
5. Kolodziejski PA, Pruszynska Oszmalek E, Sassek M, Kaczmarek P, Szczepankiewicz D, Billert M, et al. Changes in obestatin gene and gpr39 receptor expression in peripheral tissues of rat models of obesity, type 1 and type 2 diabetes. J Diabetes 2017; 9(4): 353-61.
6. Pedersen BK. Anti inflammatory effects of exercise: Role in diabetes and cardiovascular disease. Eur J Clin Invest 2017; 47(8): 600-11.
7. Mony A, Batmanabane M, Hussein HH, Mahmoud OM, Ukwenya V, Ashaolu O, et al. Effect of obestatin on body weight, serum glucose and insulin levels in albino rats. Eur J Anat 2013; 17(2): 59-62.
8. Trepanowski JF, Bloomer RJ. The impact of religious fasting on human health. J Nutr 2010; 9(1): 57.
9. Trenkle A. Effects of short-chain fatty acids, feeding, fasting and type of diet on plasma insulin levels in sheep. J Nutr 1970; 100: 1323-30.
10. Egido EM, Hernández R, Marco J, Silvestre RA. Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. Regul Pept 2009; 152(1): 61-6.
11. Saad M, Pettitt D, Mott D, Knowler W, Nelson R, Bennett P. Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. The Lancet 1989; 333(8651): 1356-9.
12. Granata R, Volante M, Settanni F, Gauna C, Ghé C, Annunziata M, et al. Unacylated ghrelin and obestatin increase islet cell

mass and prevent diabetes in streptozotocin-treated newborn rats. J Mol Endocrinol 2010; 45(1): 9-17.
13. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, et al. Obestatin promotes survival of pancreatic β-cells and human islets and induces expression of genes involved in the regulation of β-cell mass and function. Diabetes 2008; 57(4): 967-79.
14. Agouni A, Owen C, Czopek A, Mody N, Delibegovic M. In vivo differential effects of fasting, re-feeding, insulin and insulin stimulation time course on insulin signaling pathway components in peripheral tissues. Biochem Biophys Res Commun 2010; 401(1): 104-11.
15. Granata R, Gallo D, Luque RM, Baragli A, Scarlatti F, Grande C, et al. Obestatin regulates adipocyte function and protects against diet-induced insulin resistance and inflammation. FASEB J 2012; 26(8): 3393-411.
16. Gu P, Kang D, Wang W, Chen Y, Zhao Z, Zheng H, et al. Relevance of plasma obestatin and early arteriosclerosis in patients with type 2 diabetes mellitus. J Diabetes Res 2013; 2013.
17. Dostal AM, Samavat H, Espejo L, Arikawa AY, Stendell-Hollis NR, Kurzer MS. Green tea extract and catechol-o-methyl-transferase genotype modify fasting serum insulin and plasma adiponectin concentrations in a randomized controlled trial of overweight and obese postmenopausal women. J Nutr 2016; 146(1): 38-45.
18. Hu Y, Hou Z, Yi R, Wang Z, Sun P, Li G, et al. Tartary buck-wheat flavonoids ameliorate high fructose-induced insulin resistance and oxidative stress associated with the insulin signaling and nrf2/ho-1 pathways in mice. Food & Function 2017; 8: 2803-16.
19. Ibrahim MH, Selim SA, Abd-Alaleem DI, Al-sayed RM. Effect of obestatin on gonadal functions in high fat-fed albino rats. BSM 2014; 3(3): 43-59.