

EFFECT OF OBESTATIN SUPPLEMENTATION ON PLASMA GLUCOSE, SERUM INSULIN AND INSULIN RESISTANCE IN HIGH FAT DIET INDUCED OBESE SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To determine the effect of obestatin administration on plasma glucose levels, serum insulin levels and insulin resistance in obese Sprague Dawley Rats.

Study Design: Randomized controlled trial.

Place and Duration of Study: Army Medical College, Rawalpindi in collaboration with National Institute of Health Sciences (NIH), Islamabad from Apr 2013 to Jun 2013.

Material and Methods: This study is a randomized controlled trial conducted at Physiology Department Army Medical College. Forty five male healthy Sprague Dawley rats were randomly divided into 3 groups i.e. control group (group I) fed with normal pellet diet (NPD), obese group (group II) and obestatin treated obese group (group III) fed with high fat diet (HFD). After 10 weeks, group III was treated with obestatin (1 nmol/100ml intraperitoneally). Blood samples were obtained by terminal intracardiac sampling for bioassays of plasma glucose by glucose oxidase method, serum insulin levels by ELISA and measurement of HOMA IR. Mean + SD was calculated. Statistical significance of differences across the groups was determined by one way ANOVA followed by Post Hoc Tukey's test. A p -value < 0.05 was considered significant.

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

Conclusion: Obestatin improves the glycemic status in obese rats and can be used as an adjunct therapeutic tool in preventing the comorbid sequelae of obesity.

Keywords: Insulin resistance, Obesity, Obestatin.

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INTRODUCTION

Obesity is one of the key causes in development of multiple pathological conditions including the glucose intolerance culminating in development of diabetes mellitus¹. The term metabolic syndrome is now used in literature which includes the central obesity, dyslipidemia and glucose intolerance². Documented as a major public health challenge due to its association with alarmingly high rate of morbidity and mortality, obesity is defined in terms of a body mass index of more than 30 kg/m²³. As per the meta analysis conducted in 2016, worldwide prevalence of adults

is 13% while that of children is 17% which is around 650 million and 124 million respectively⁴.

Obestatin is a peptide hormone, which is released by the parietal cells in gastrointestinal tract. It mediates its action by acting on a G-protein-related orphan receptor 39 (GPR39) which belongs to ghrelin receptor subfamily. Obestatin is also documented to act on target tissues through its glucagon like peptide-1 (GLP-1) receptor, however the receptor is still controversial and alterations in receptor expression have been reported in diabetes mellitus (type 1 and type 2) and obesity⁵. Obestatin receptors are found in cholinergic neurons of myenteric plexus, Leydig cells of testes, stomach and small intestine of rats. Obestatin is anorexigenic in its effect and inhibitory to the gastrointestinal motility. Obesta-

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tin has been documented to decrease the obesity which is attributed to its effect on decreasing the food intake. Intensive research is being conducted in this context to use this peptide as a therapeutic agent in obese individuals⁶. Obestatin has been documented to play an essential role in energy homeostasis in conjunction with ghrelin. Increased levels of obestatin have been found in obese individuals as an adaptive process in over all energy homeostasis⁷. Insulin resistance is one of the early manifestations in obesity, studies conducted have shown that the obestatin levels are positively correlated with insulin resistance while negatively correlated with insulin levels in obese children⁸. Our study was aimed at evaluating the effects of obestatin supplementation on insulin levels, insulin resistance and plasma glucose levels in obese Sprague Dawley rats.

MATERIAL AND METHODS

These randomized controlled trials were conducted at Physiology department, Army Medical College, Rawalpindi in collaboration with National Institute of Health Sciences (NIH), Islamabad from April 2013 to June 2013. Forty five Male Sprague Dawley rats weighing 250 +50 grams were taken from the animal house of NIH, Islamabad and kept under standard conditions with daily photo period of 12 hour light and 12 hour dark cycle at ambient temperature of 24 + 2°C. The rats were randomly divided by lottery method in 3 groups each having 15 rats.

The rats in group I (control group n=15) were fed on normal pellet diet (NPD) ad libitum for 2 weeks. This NPD was prepared at NIH according to the guidelines of Universities Federation for Animal Welfare. Single intra-peritoneal (IP) injection of normal saline was given after 2 weeks. Rats were fed on the same diet for next one week followed by overnight fast for 12 hours. Tail vein sampling for blood glucose and serum insulin was taken to determine insulin resistance by HOMA-IR. The rats were continued on NPD for 8 more weeks followed by daily IP injections (100uL) of normal saline for 10 days.

The rats in group II: (Obese group n=15) were fed on high fat diet (HFD) for 2 weeks followed by single IP injection of normal saline on 15th day. Rats were continued to be fed on high fat diet for 1 week and they were subjected to overnight fast. Tail vein sampling for blood glucose and serum insulin was done to determine insulin resistance by HOMA-IR. Rats continued to take high fat diet for 8 weeks followed by the measurement of blood glucose for exclusion of diabetic rats (blood glucose >200mg/dl)⁹. All the rats included in obese group were non diabetic i.e. blood glucose <200mg/dl. The rats were continued on HFD for 8 more weeks followed by daily IP injections (100uL) of normal saline for 10 days. The rats in group III (obestatin group n=15) underwent the same treatment as group II. However, intra peritoneal injections of obestatin in the dose of 1ml/100uL were given for 10 days at the end of study.

Rats were euthanized by overdose of ether anesthesia at the end of 13 weeks. Terminal blood sample of rats (4-5 mL) was obtained in serum gel separator tubes through intra cardiac puncture. The sample was centrifuged and serum as stored at -80°C 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 \pm SD was calculated. ANOVA was applied to determine significance among the groups. Post-hoc Tukey test for inter-group comparison was applied. A *p*-value of ≤ 0.05 was considered as significant value.

RESULTS

The study included forty five animals divided into 3 groups with 15 rats in each group. Group I was fed with NPD (table-I) while group II and III were fed with HFD (table-II) 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 (table-III). On applying post hoc Tukey's HSD, the group II manifested significant increase in body weight,

plasma glucose, serum insulin and HOMA-IR levels as compared to group I. However, there

Table-I: Composition of Normal Pelleted Diet (NPD).

S. No	Ingredients	Diet (g/kg)
1.	Wheat flour	285
2.	Wheat brawn	285
3.	Dried skimmed milk powder	200
4.	Soyeban oil	0.050
5.	Mollasen	15
6.	Fish meat	150
7.	Salt	5
8.	Vitamin and mineral mix	10

Table-II: Composition of High Fat Diet (HFD).

S. No	Ingredients	Diet (g/kg)
1.	Powdered NPD	365
2.	Anhydrous butter fat	310
3.	Casein	250
4.	Cholesterol	10
5.	Vitamin and mineral mix	60
6.	DL-Methionine	03
7.	Yeast powder	01
8.	Sodium chloride	01

was a significant decrease in plasma glucose, serum insulin and HOMA-IR in group III when

promotes 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 muscles¹⁰.

In our study, obese rats manifested hyperinsulinemia when compared to the healthy controls. Obestatin treated obese rats manifested the significant reduction in serum insulin levels as compared to the non-treated obese rats. This decline in serum insulin levels in obese rats can be due to the anorexigenic effect of obestatin which would have decreased the food intake by rats causing there by the decrease in plasma glucose and serum insulin levels. obestatin has also been documented to reduce insulin secretion under glucose stimulated conditions from pancreatic islets in vivo¹¹. Obestatin has been documented to cause dual effect on insulin secretion

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

Parameters	Group I (Healthy non treated) (n=15)	Group II (Obese non treated) (n=15)	Group III (Obese treated) (n=15)	p-value
Plasma glucose (mg/dl)	94.27 ± 3.61	188.03 ± 5.35	166.4 ± 6.29	<0.001
HOMA-IR	1.41 ± 0.20	3.67 ± 0.09	2.66 ± 0.07	<0.001
Serum Insulin (ng/ml)	6.02 ± 0.85	7.90 ± 0.05	6.48 ± 0.15	<0.001

Data is expressed as mean ± SD, *p<0.05

Table-IV: Comparison of Plasma glucose, Serum insulin and HOMA-IR among healthy control, obese group and obestatin treated group by ANOVA at the end of study.

Parameters	Group I & II	Group II & III	Group I & III
Plasma Glucose	0.016	0.043	0.031
Serum Insulin	0.02	0.034	0.018
HOMA-IR	0.015	0.01	0.023

*p<0.05

compared to group II (table-IV).

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

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 obestatin¹². 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 exhaustion¹³.

There was a significant decrease in glucose level and insulin resistance in obese rats of our study after obestatin treatment when compared to their healthy control group. This 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. Mony *et al* documented the decline in glucose levels in obestatin treated healthy albino rats and attributed this to the anorexic property of the peptide¹⁴. The rats in the aforementioned study were fasted for 16 hours prior to obestatin administration which would have increased the insulin sensitivity, as also reported by Agouni *et al*¹⁵. In our study, there was significant decrease in serum glucose levels in obese rats when compared with the non-treated obese groups although it did not reach the normal basal levels. Ibrahim *et al* reported significant decrease in serum glucose levels in obestatin treated HFD induced obese male albino rats when compared with the controls¹⁶. 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*¹⁷. Another mechanism of obestatin induced decline in plasma glucose level has been documented through the increase in adiponectin level by obestatin¹⁸. Adiponectin is an adipocytokine which serves as a positive regulator in glucose homeostasis by decreasing gluconeogenesis and increasing glucose uptake by the cells and enhancing insulin sensitivity as documented by Heurta *et al*¹⁹.

The obestatin treated obese group also manifested decline in HOMA-IR levels when compared to the non treated obese 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 decline in release of inflammatory cytokines induced by high fat feeding¹⁷. It was revealed that obestatin manifested insulin sensitizing effect by increasing protein kinase B (Akt) and adenosine monophosphate activated protein kinase (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 resistance²⁰. 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 resistance²¹. 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 rats¹⁶. 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 obesity. In our study, the decline in plasma glucose levels in obese group can be attributed to the decreased insulin resistance in the obese rats along with correction of hyperinsulinemia in obese rats on treatment with obestatin.

CONCLUSION

Obestatin decreases the insulin resistance, plasma glucose level and serum insulin levels in obese Sprague Dawley rats and can be used as an adjunct therapeutic tool in preventing the comorbid sequel of obesity.

CONFLICT OF INTEREST

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

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