



## **Effect of Obestatin on Gastric Acid Secretion and Mucin Expression in Normal and Streptozocin Induced Diabetic Rats**

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### **Abstract**

**Background:** Obestatin is a peptide hormone derived from the same peptide precursor as ghrelin, it regulates food intake and gastric motility, however, to date, no studies conducted about the effects of obestatin on gastric acid secretion and gastric mucin expression. **Aim:** investigate effects of obestatin on gastric acid secretion and mucin expression in normal and streptozocin (STZ) induced diabetic male rats. **Material and Methods:** 32 adult male albino rats were divided into group I (normal) and group II STZ induced diabetic rats, each group further subdivided into two equal subgroups: group a (received intraperitoneal (ip) saline daily for 21 days) and group b (daily received ip obestatin "10 nmol/kg" for 21 days). In all groups, gastric secretion was collected, then total acid output, ghrelin and volume of gastric secretion were measured, real time polymerase chain reaction (PCR) for gastric mucin (MUC5AC) and gastric H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit gene expression were measured, blood samples were obtained for insulin and glucose measurement. **Results:** in both normal and diabetic rats, obestatin increased total acid output, ghrelin, volume of gastric secretion, gastric H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit and MUC5AC gene expression. There was none significant change in blood glucose and insulin in normal rats, while significant increase in insulin level and significant decrease in blood glucose in STZ induced diabetic rats. **Conclusion:** obestatin on chronic run increased gastric acidity, ghrelin and volume of gastric secretion in normal and diabetic rats, obestatin can be considered as gastro- protective agent as it increased gastric mucin in normal and diabetic rats.

**Keywords:** Obestatin, Gastric secretion, Acid, Diabetes, Ghrelin, H<sup>+</sup>-K<sup>+</sup>-ATPase

### **1. Introduction**

Obestatin is a peptide hormone formed of 23-amino-acid, it is mainly produced in the gastrointestinal tract and is derived from the

posttranslational cleavage of the same peptide precursor as ghrelin <sup>[1]</sup>. Obestatin regulate metabolic function at the central and the peripheral levels, which includes regulation of food intake, gastric emptying with controversial results <sup>[2-4]</sup>, pancreatic,

adipocyte, cardiac function as well as cell proliferation [1,5-7]

It is worth saying that both the glucagon-like peptide-1 receptor (GLP1R), as well as the orphan G protein-coupled receptor 39 (GPR39), can be activated by obestatin. In addition, obestatin has been shown to compete with ghrelin's binding at the growth hormone secretagogue receptor (GHS-R) in pancreatic  $\beta$ -cell lines [1,8,9], it can also be hypothesized that the pharmacological interaction between both peptides are mediated through the GHS-R. [5]. Obestatin is found in the gastrointestinal (GI) tract, within the A-like cells and oxyntic glands of the gastric mucosa and cholinergic neurons of the myenteric plexus, where it is co-localized with its precursor peptide, preproghrelin [10,11], this may indicate that obestatin may have local autocrine/paracrine roles in gastric mucosa, moreover, in addition, obestatin binding to GPR39 has been demonstrated in gastric mucosa [1,12], as intra-peritoneal injection of obestatin increased c-fos staining in the nuclei of gastric mucosa [1], these data suggest a possible effect of obestatin on gastric secretion.

In diabetic conditions, there is a decrease in gastric acid secretion due to autonomic neuropathy [13], in addition, there is necrosis of gastric mucosal cells and a decrease in gastric mucin (MUC) which is secreted by the epithelial cells of the gastrointestinal mucosal cells that form a dynamic and interactive mucosal defense system on the gastrointestinal mucosal surface [14-16].

It is interesting that obestatin has a promising role in the improvement of diabetes mellitus symptoms [17,18]

**Aim of the work:** From the above data, the present study aimed to investigate the role of chronic obestatin administration on gastric secretion in normal and streptozotocin (STZ) induced diabetic rat model.

## 2. Materials and Methods

**Groups of animals:** Forty five adult male Wistar albino rats weighing 180-220g supplied from the faculty of Veterinary Medicine, Zagazig University, were enrolled in the present study. All animals received care according to the guide and ethical

regulations for the care and use of laboratory animals according to Institute of Laboratory Animal Resources [19], the animals were housed in plastic cages under controlled hygienic conditions with an ambient temperature ( $22\pm 2^\circ\text{C}$ ) and a 12 hours light-cycle. All animals were subjected to a 7 day period for acclimatization. Rats were divided into two main groups: Group I (normal  $n=16$ ) which was subdivided into 2 equal subgroups: Subgroup (1a) Each rat received intra-peritoneal (ip) injection of 0.5 ml saline daily for 3 weeks. Subgroup (1b) (obestatin-treated normal group): Each rat received ip daily dose of obestatin (1nmol/ 100gm) (Sigma Aldrich Co.-USA) for 3 weeks [20]. Group II (diabetic  $n= 29$ ): to induce diabetes, for every rat ip single injection of freshly prepared solution of streptozotocin (Sigma Aldrich Co.-USA) 50 mg/kg dissolved in 0.2 mmol/L sodium citrate, at pH 4.5 [21], 0.1- 0.2 u/kg of insulin were given to rats per day to prevent ketosis and reduce mortality without making blood glucose level normal [22] (10 rats died and 16 with high blood glucose levels were selected). Rats were ready for the experiment after development of gastric neuropathy three weeks after induction of diabetes [23], It was subdivided into 2 equal subgroups (subgroup II a) each rat received ip injection of 0.5 ml of saline daily for 3 weeks. (Subgroup II b) (obestatin-treated diabetic group): Each rat received ip daily dose of obestatin (1nmol/ 100gm) for 3 weeks [20].

**Methods:** Collection of gastric secretion: At day 21 of experiment rats were fasted for 24 hours, rats were anaesthetized, using ether, a midline abdominal incision was performed, the pyloric portion of the stomach was gently mobilized and carefully ligated with a silk suture around the pyloric sphincter taking care not to interfere with gastric blood supply. Abdominal incision was sutured and the animals were allowed to recover from anesthesia, collection of gastric juice was done after 1 hour of pylorus ligation [24]. Gastric juice from each animal was centrifuged to remove any solid debris and the volume of the supernatant was measured by measuring pipettes, gastric acid output was determined by titration of samples with 0.1 N NaOH to pH 7.0 using phenolphthalein (2 %) as an indicator [25]. Acid output was calculated by multiplying the volume of gastric juice by the acid

concentration<sup>[26]</sup>. Ghrelin level was measured by the method of Kojima et al.<sup>[27]</sup>.

Gene expression of MUC5ac and H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit by real time PCR according to Wallace et al.<sup>[28]</sup>

The stomachs of animals were removed, opened along the greater curvature, rinsed with physiological saline, and pinned out in ice-cold saline, one hundred milligrams of gastric mucosal tissues were excised, snap-frozen in liquid nitrogen, and stored at -80°C until required for processing. RNA isolation was performed using TRIzol (Invitrogen) according to the manufacturer's recommendations. RNA was DNase treated prior to cDNA synthesis using the iScript™ cDNA synthesis kit (BioRad). Real-time PCR amplification and analysis were performed using SYBR Green Master Mix (Applied Biosystems) in a Primer sequences that were synthesized by (MWGBiotech, Germany) as follows:

MUC-5AC

Forward primer 5--CAACTGTCCGCAGGGCCAGG--3,

Reverse primer 5--ATGCTGT-CCCCGCAGTTGCC--3.

H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$  -subunit

Forward primer: 5' -TAT GAA TTG TAC TCA GTG GA-3'

Reverse primer: 5-TGG TCT GGT ACT TCT GCT-3'

Amplification conditions were: incubation at 95°C for 10min, 40 two-step cycles with 15s at 95°C for denaturation, and annealing-extension at 60°C for 1 min.

Relative expression of studied genes mRNA was normalized with control gene (beta actin which was used as the control housekeeping gene

Beta actin

Forward primer :5'--GGTCGGTGTGAACGGATTTGG -3

Reverse primer :5'-ATGTAGGCCATGAGGTCCACC-3.

Blood sampling: samples were obtained from all rats by scarification, serum was separated by centrifugation of blood at 3000 rpm for 15 minutes and examined for: Serum glucose level: by the method of Tietz et al.<sup>[29]</sup> using glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotechnology, Egypt). Serum insulin level: according to Temple et al.<sup>[30]</sup>, by a solid phase enzyme amplified sensitivity immunoassay.

STATISTICAL ANALYSIS: Data were expressed as mean SD for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). One way analysis of variance (ANOVA) was done followed by LSD test to compare means of the different groups. P value <0.05 was considered statistically significant.

### 3. Results

Table 1 showed the effect of obestatin administration on gastric total acid output , ghrelin level and volume of gastric secretion:

.In subgroup Ib (normal obestatin treated) there was significant increase in ghrelin level, total acid output, volume of gastric secretion in comparison with that of Subgroup Ia (normal control) (P < 0.05).

In Subgroup IIa (diabetic control) there was significant decrease in ghrelin level, total acid output (P < 0.001), in comparison with that of Subgroup Ia and Ib, significant decrease in volume of gastric secretion (P < 0.05) in comparison with that of Subgroup Ia and Ib.

In Subgroup IIb (diabetic obestatin treated) there was significant increase in ghrelin level, total acid output, volume of gastric secretion in comparison with that of subgroup IIa (P < 0.05), while there was significant decrease in ghrelin level, total acid output in comparison with that of subgroup Ia(P < 0.05) and subgroup Ib(P < 0.001), significant decrease in volume of gastric secretion (P < 0.05) in comparison with that of subgroup Ib.

In addition, In subgroup Ia, subgroup Ib and Subgroup IIb there was significant positive correlation between ghrelin and total acid output.

**Table 1: Effect of obestatin administration on ghrelin level, acidity and volume of gastric secretion in all studied groups**

	GROUP I (NORMAL)		GROUP II (DIABETIC)	
	Subgroup Ia (normal control)	Subgroup Ib (normal obestatin treated)	Subgroup IIa (Diabetic control)	Subgroup IIb (Diabetic obestatin treated)
Ghrelin(pg/ml)	59.9 ± 5.4	69.1± 3.4 a *	40.3 ± 1.2 a b ***	51.7± 3.4 a c * b ***
Total acid output (mEq / L /1 hour)	18 ± 1.9 r = 0.93**	22.3± 2.6 a * r = 0.91**	12±2.3 a b *** r = 0.56	15.9 ± 6.3 a c * b *** r = 0.8*
Volume (ml/ 100 gm/ 1 hour)	1.2 ± 0.1 r =0.61	1.9± 0.25 a * r =0.58	0.88 ±0.3 ab* r =0.51	0.99±0.24 b *c* r =0.39

(<sup>a</sup>) = significant vs. group Ia, (<sup>b</sup>) = significant vs. group Ib, (<sup>c</sup>) = significant vs. group IIa, r= correlation with ghrelin

Table 2 showed the effect of obestatin administration on gene expression of MUC5AC and H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit by real time PCR:

In subgroup Ib, there was a significant increase in MUC5AC and H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit gene expression (P<0.05) in comparison to subgroup Ia. In subgroup IIa, there was a significant decrease in

mentioned parameters (P<0.05), in comparison to both subgroup Ia and Ib. In subgroup IIb, there was a significant increase in mentioned parameters (P<0.05) in comparison to subgroup IIa, however, there was a significant decrease in mentioned parameters (P<0.05), in comparison to subgroup Ib.

**Table 2: Effect of obestatin administration on gene expression of MUC5AC and H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit by real time PCR in all groups**

	GROUP I (NORMAL)		GROUP II (DIABETIC)	
	Subgroup Ia (normal control)	Subgroup Ib (normal obestatin treated)	Subgroup IIa (Diabetic control)	Subgroup IIb (Diabetic obestatin treated)
MUC5AC	0.99 ±0.39	1.4±0.28 <sup>a</sup> *	0.65±0.13 <sup>a b</sup> *	0.95±0.17 <sup>b c</sup> *
H <sup>+</sup> -K <sup>+</sup> ATPase $\alpha$ -subunit	1 ±0.2	1.3 ±0.3 <sup>a</sup> *	0.78 ±0.4 <sup>a b</sup> *	0.93 ±0.1 <sup>b c</sup> *

(<sup>a</sup>) = significant vs. group Ia, (<sup>b</sup>) = significant vs. group Ib, (<sup>c</sup>) = significant vs. group IIa

Table 3 showed the effect of obestatin administration on serum metabolic parameters:

In subgroup Ib (normal obestatin treated), there was non significant change in serum glucose level, serum insulin level, in comparison with that of Subgroup IA (normal control) ( $P > 0.05$ ).

In Subgroup IIa (diabetic control) there was significant decrease in serum insulin level and significant increase in serum glucose level when

compared with that of normal rats (subgroup Ia and Ib) ( $p < 0.001$ ).

In subgroup IIb (diabetic obestatin treated), there were significant increase in serum insulin and significant decrease in serum glucose levels when compared with that of subgroup IIa ( $p < 0.001$ ), while there was significant decrease in serum insulin and significant increase in serum glucose level when compared with that subgroup Ia and subgroup Ib ( $p < 0.001$ ).

**Table 3: Effect of obestatin administration on serum metabolic parameters in all studied groups**

	GROUP I (NORMAL)		GROUP II (DIABETIC)	
	Subgroup Ia (normal control)	Subgroup Ib (normal obestatin treated)	Subgroup IIa (Diabetic control)	Subgroup IIb (Diabetic obestatin treated)
Glucose (mg/ dl)	75.5±2.53	73.87±2.90	453.7±4.27 <sup>a b ***</sup>	199.37± 6.97 <sup>a bc ***</sup>
Insulin (µIU/ml)	8.85±0.716	9.04±0.50	2.17±0.18 <sup>a b ***</sup>	6.24±2.54 <sup>a bc ***</sup>

(<sup>a</sup>)= significant vs. group Ia, (<sup>b</sup>) = significant vs. group Ib, (<sup>c</sup>) = significant vs. group IIa

#### 4. Discussion

In the current study, chronic administration of obestatin increase gastric acid secretion, as it increase total acid output and increased mRNA expression of H<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit, which is an enzyme located in the apical membrane of the parietal cell and is the most critical component of the ion transport system mediating acid secretion in the stomach<sup>[31]</sup>. Many suggestions could explain this finding include, increased local ghrelin secretion as proved in the current work by a significant increase in ghrelin level in gastric secretion in obestatin treated groups. This finding can be supported by Duygu et al. <sup>[32]</sup> who stated that the number of ghrelin immunopositive cells in fundus tissue of stomach are considerably high in obestatin treated animals for 14 days. The increased ghrelin may be the mediator of obestatin to enhance gastric acid secretion, as ghrelin has a stimulatory role in regulating gastric acid secretion <sup>[33]</sup>. Furthermore the increased ghrelin gastic level was significantly positive correlated with the decreased

pH in the present results. Another suggestion is the vagal stimulation, as obestatin was co-localized with acetylcholine in the myenteric plexus of the gastrointestinal (GI) tract <sup>[34]</sup>, suggesting a role of obestatin in regulating GI tract via the cholinergic system <sup>[34]</sup>. Importantly, Kapica et al. <sup>[35]</sup> proved the obestatin capability of increasing the pancreatic protein output in rats via vagal activation.

It is interesting that ATP-dependent K<sup>+</sup>(KATP) channels are sensitive to obestatin <sup>[36]</sup>, and its stimulatory action on insulin secretion was prevented by k channel openers drugs <sup>[37]</sup>. KATP channels openers were known to interfere with Ca<sup>++</sup> ion influx and to antagonize mobilization of Ca<sup>++</sup> bound to intracellular stores leading to reduction in free cytosolic Ca<sup>++</sup> ion which is important for gastric acid and pepsin secretion <sup>[38]</sup>. So it can be hypothesized that closure of KATP channels is one of obestatin tools to stimulate gastric secretion.

Adrenal cortex may have an indirect role in the obestatin effect on gastric secretion, as obestatin acts through corticotrophin receptors (type I and II) and activated corticotrophin releasing hormone (CRH)-neurons in the hypothalamus, <sup>[39]</sup> elevating



plasma corticosterone levels [39,40]. Elevated corticosteroid level stimulate gastric glands to secrete acid and pepsin [41,42].

Another mechanism of the stimulatory role of obestatin could be related to the inhibition of the dopamine release from rat hypothalamic [43], as dopamine decrease the activity gastric H<sup>+</sup>K<sup>+</sup>-ATPase in acute and chronic unpredictable stress models [44].

Somatostatin which is released from gastric mucosa D cells, decreases acid secretion by direct inhibition of parietal cells or by decreasing histamine secretion from enterochromaffin cells (ECLs) and decreasing gastrin secretion from G cells [45,46]. Another explanation of obestatin action may be through affecting gastric somatostatin receptors, as obestatin reduced the expression of pituitary somatostatin receptor subtypes (sst1 and sst2) [40], it may have the same suppressing effect on gastric sst2 receptor.

It is worth saying that obestatin increase expression of nitric oxide synthase (NOS) and endothelial nitric oxide (NO) generation [47,48]. Tsuchiya et al. [49] have shown that centrally injected NO donor stimulated gastric acid secretion in both conscious and anesthetized rats. Furthermore, Kitay et al. [50] reported that NO can induce acid secretion via the H<sup>+</sup>, K<sup>+</sup> ATPase activation in resting glands, they suggest that postprandial increased levels of L-arginine in the blood can cause a continued secretion of acid from the parietal cell even in the absence of classical secretagogues.

Moreover, chronic administration of obestatin caused a significant increase in the volume of gastric secretion, this effect may be through NO production, as NO receptor antagonist markedly decreased the volume of gastric secretion [33].

In addition, real time PCR showed increased gene expression of mucin (MUC5AC) in obestatin treated groups, MUC5AC is the major mucin in the normal stomach, secreted by the surface mucus cells, it is a main component of gastric mucus protective layer [51], this effect may be due to the vasodilator effect of obestatin [52], as efficient blood supply helps improvement of mucosal defense in gastric mucosa [53]. Claudia et al. [52] stated that endothelial dependent NO/cGMP signaling. was involved in the vasodilator effect of obestatin by endothelial NO

generation, it was reported that NO has a protective effect on the surface mucus bicarbonate barrier [54]. NO increase mucosal prostaglandin (PG) [55], which play a major role in preservation of gastric mucosal integrity, and ulcer healing by induction of cytoprotective bicarbonates and mucous glycoproteins [56].

In diabetes, there is low level of NO due to oxidative stress [57] this caused diabetic vascular problems which lead to variety of neuropathy [58]. Claudia et al. [52] stated that obestatin increase endothelial NO generation, which is one of the most potent endogenous vasodilators, and widely recognized as an endogenous vasoprotective in diabetes [58], by improvement of diabetic gastropathy, this may caused increase in gastric secretion in streptozotocin induced diabetic rats when compared with control diabetic rats.

In the present study, significant reduction in serum glucose level and increase in insulin level was observed in the obestatin treated diabetic rats, these findings were in agreement with that given by Granata et al [17], who reported that obestatin counteracted all STZ-diabetic effects by reducing glucose and increasing insulin levels, this could be attributed to its role in prevention of pancreatic  $\beta$ -cell death and stimulation of its regeneration [8,59], in addition, it was observed that beta cells were more responsive to obestatin when glucose levels were high which enhance insulin release [35, 60].

The significant increase in insulin levels in this study may act as indirect preservation of gastric functions, as it was reported that insulin has the ability to reverse established diabetic neuropathy [61].

## 5. Conclusion

The present study demonstrated stimulatory effect of obestatin on gastric acid accompanied by increased mucin in both normal and STZ induced diabetic rats. This beneficial action mediated partially by increased gastric ghrelin secretion.

## Limitations

In this study, we did not studied the effect of chronic obestatin treatment on gastric acid in rats with different regimes of food restriction, future studied are required.

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## Compliance with Ethical Standards

Declaration of interest statement The authors declare that there is no conflict of interests regarding the publication of this paper

## Ethical approval

All animals received care according to the guide and ethical regulations for the care and use of laboratory animals according to Institute of Laboratory Animal Resources, all experimental procedures were approved and in accordance with the ethical standards of the Institutional Research Board of Zagazig University.

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