Relation between Plasma Levels of Ghrelin and Hyperphagia in Streptozotocin-Induced Diabetes in Male Albino Rats

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Abstract

Background: Ghrelin, a hormone identified in the stomach, is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) and the peripheral or central administration of ghrelin increases food intake and body weight. Ghrelin release and gene expression are regulated by nutrient flux, such that plasma ghrelin levels increase steadily before meal onset and fall rapidly after food ingestion. Some studies have suggested that tonic vagal activity may play a role in maintaining baseline plasma ghrelin level or may cause increase in the plasma ghrelin level in case of food deprivation.

Material and Methods: 72 normal adult male albino rats were divided into three groups 24 rats each. Group (I): Control group (n=24), Group (II): Induced diabetic group (n=24) and Group (III): Induced diabetic group treated with insulin (n=24). Each of these groups was divided into three subgroups 8 rats each. Group (a): Injected with saline IP once daily throughout the experimental period (n=8), Group (b): Injected with GHS-R antagonist (n=8) once daily throughout the experimental period and Group (c): Vagotomized group (n=8).

Results: Data showed a significant increase in fasting ghrelin level in diabetic group (group II). This was also accompanied by significant increase in food intake and decrease in body weight. These changes were reversed after treatment with insulin in controlled diabetic group (groupIII). Also, peripherally administered GHS-R antagonist significantly decreased food intake and body weight in all study groups. Treatment with GHS-R antagonist decreased blood glucose level in control group (group I) and controlled diabetic group (group III). Data also showed a significant decrease in basal ghrelin levels in vagotomized rats in all groups. Vagotomy also caused a significant decrease in food intake and body weight in all study groups.

Conclusion: Ghrelin plays a stimulatory role in the hyperphagia associated with STZ-DM. Also, food intake was significantly decreased by treatment with GHS-R antagonist and vagotomy. In addition, vagotomy contributed to the dissociation of the peripheral signaling of ghrelin, causing significant decrease in food intake and body weight in STZ-DM in rats.

Key Words: Hyperphagia – Ghrelin – STZ-DM – Vagotomy.

Introduction

STREPTOZOTOCIN-induced diabetes mellitus (STZ-DM) is characterized by hyperphagia. Researchers have examined the contribution of hypothalamic neuropeptides to the feeding response of diabetic animals and hypothesized that two orexigenic peptides, hypothalamic neuropeptide Y (NPY) and agouti-related protein (AGRP) are involved in diabetic hyperphagia [1].

Appetite in mammals is regulated by a well designed homeostatic network made up of central and peripheral components that maintain the balance between energy intake and energy expenditure. Leptin, insulin and ghrelin are known to be main hormones involved in the control of food intake, with opposing effects [2].

Ghrelin, a hormone identified in the stomach, is an endogenous ligand for the growth hormone secretagogue receptor and the peripheral or central administration of ghrelin increases food intake and body weight [3]. It was subsequently demonstrated that ghrelin is an orexigenic hormone that potently increases food intake, body weight, and adiposity via its activation of ARC NPY/AgRP neurons [4].

Ghrelin release and gene expression are regulated by nutrient flux, such that plasma ghrelin levels increase steadily before meal onset and fall rapidly after food ingestion. Researchers have suggested a role for ghrelin in hyperphagia in STZ-DM rats [5]. Recent evidence has accumulated suggesting that ghrelin is part of the mechanism regulating β-cell function and glucose homeostasis.

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Studies have established ghrelin as a novel islet hormone that inhibits insulin release and thereby upwardly regulates blood glucose [6].

Peripheral ghrelin does not cross the blood brain barrier. Thus peripheral ghrelin must activate the appropriate hypothalamic regions via an indirect pathway. The detection of ghrelin receptors on vagal afferent neurons in the rat nodose ganglion suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve [7].

Some studies have suggested that tonic vagal activity may play a role in maintaining baseline plasma ghrelin level or may cause increase in the plasma ghrelin level in case of food deprivation [8,9].

A key question is whether there is a correlation between ghrelin level and diabetic hyperphagia or not?

Another key question is what could be the possible role of the vagus nerve in controlling the peripheral signaling and level of ghrelin in case of diabetic hyperphagia.

Aim of the work:
In the current study we are going to investigate:
1- Relation between plasma ghrelin level, food intake, body weight and glucose levels in control, STZ-DM and insulin treated diabetic rats.
2- Effect of GHS-R antagonist on possible actions of ghrelin.
3- Role of the vagus nerve in controlling the peripheral signaling and level of ghrelin in case of diabetic hyperphagia.
4- Effect of insulin treatment on pre and postprandial plasma levels of ghrelin.

Material and Methods

I- Experimental animals:
Seventy two normal adult male albino rats (weight range 150-200g) were included in the present study. Rats were kept in suitable cages at animal house of Faculty of Oral and Dental Medicine, Misr International University, from Sept. 2011 to Nov. 2011 at room temperature, with adjusted light-dark cycle, maintained on special formulated diet consisting of mixed commercial laboratory chow, tap water was freely allowed.

Animals were randomly divided into three groups as following:
Group (I): Control group (n=24).
Group (II): Induced diabetic group (n=24).
Group (III): Induced diabetic group treated with insulin (n=24).

Each of these groups was divided into three subgroups as following:
Group (a): Injected with saline IP once daily throughout the experimental period (n=8).
Group (b): Injected with GHS-R antagonist (n=8) once daily throughout the experimental period.
Group (c): Vagotomized group (n=8).

II- Study protocol:

- Diabetes was induced at day 0 to rats of group II and group III.
- Two weeks before STZ injection vagotomy was done for 24 rats in groups Ic, IIc, IIIc.
- Body weight, food intake and blood glucose were measured in all animals daily (from day 1 to day 14) throughout the experiment period.
- At day 14 of the experiment, after overnight fasting, blood samples were collected from each rat for chemical analysis of plasma fasting ghrelin, and postprandial ghrelin (one hour after eating).
- GHR-S antagonist was injected as a single daily IP injection of 1ml of 100nm/L for 14 days for group Ib, IIb and IIIb [10].
- 48 hours after STZ injection (day 2), group III received NPH human insulin, (1 unit) by sc injection, twice a day, between 8:00-9:00 and 16:00-17:00.
- Collection of blood samples: At day 14, blood was collected (5ml of blood) from the retro-orbital plexus, using heparinized capillary inserted in the medial canthus of the eye globe. Plasma was sucked out into eppendorf tubes and stored frozen at –70 ºC until required for determination of plasma Ghrelin [11].

III- Experimental procedures:

1- Induction of diabetes:
At day 0, Group II and Group III (total of 48 rats) were injected with single IP injection of Streptozotocin (STZ) at the dose of 50mg/kg after 3 weeks of high fat diet [8]. STZ was dissolved in ice cold citrate buffer (pH 4.5) in 0.9% saline solution. Rats were fasting 6 hours before STZ injection. Control animals received subcutaneous injections of citrate buffer only [12]. (Shah et al., 2008). STZ was supplied by Sigma Pharmaceutical Industries (Menofeya, Egypt). Animals showing
fasting blood glucose higher than 10mmol/L were considered as diabetic and used for further study.

2- Vagotomy:

Two weeks before STZ injection, 24 Rats (weighing 150-200gm) were anesthetized with a single IP injection of sodium pentobarbital (0.06g/kg body). During total subdiaphragmatic vagotomy, connective tissue between the liver, stomach and esophagus was carefully removed and a 0.5cm segment of the vagal dorsal and ventral subdiaphragmatic branches were isolated from the esophagus and excised by cautarization. Then the laparotomy had been stitched and disinfected with betadine and local antibiotic on the surface incision [13].

The flowing parameters were measured:

- Ghrelin assay:

  The Enzyme Immunoassay kit is designed to detect a specific peptide and its related peptides based on the principle of “competitive” enzyme immunoassay [14].

- Blood glucose measurement:

  Daily blood samples were measured in one drop blood from the rat tail vein and determined using a handheld glucometer (Accu-Check Go, GJ04337330). Random Samples were collected at a fixed time daily (12pm) before STZ injection and throughout 14 days after STZ injection [15].

- Food intake:

  On the basis of preliminary study of food consumption of each group during the acclimatization period, the amount of the served food was divided into two meals: In the morning and before darkness. To facilitate measures of food intake, rats were housed conventionally in individual plastic cages with stainless steel cover, with food and tap water provided ad libitum. On arrival, rats were placed immediately into their respective experimental conditions and allowed access to a pre-weighed amount of food. Food (pellets) was placed over the stainless steel cover to be freely accessible for rats, and the amount of food remaining was recorded. Intake was calculated as the weight (in grams) of food provided less that recovered [16].

- Bodyweight:

  Every 24h, rats were briefly removed from their cages and weighed in grams.

Statistical analysis:

Data were analyzed using the statistical package SPSS version 15. Values were expressed as mean ± standard deviation (SD). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non para metrical Mann-Whitney test was used for non normally distributed quantitative variables. p-values less than 0.05 were considered as statistically significant [17].

Results

- Fasting and postprandial Ghrelin level:

  After induction of diabetes, there was a significant increase in fasting ghrelin level in group IIa compared to control group Ia (p<0.05). Treatment with insulin significantly decreased fasting ghrelin level in group IIIa when compared to IIa but were still higher than value of control group Ia.

  Similarly in group IIb, administration of receptor blocker significantly increased fasting ghrelin level in group IIb compared to control group Ib (p<0.05). Treatment with insulin did not show a significant difference in fasting ghrelin level in group IIIb over group IIb (p>0.05).

  On the contrary, subdiaphragmatic vagotomy prevented the rise of fasting ghrelin levels induced by diabetes in group IIc and IIIc where there was no significant difference compared to group Ic (p>0.05) (Table 1, Fig. 1).

  As regarding postprandial Ghrelin Level, the mean value of PP ghrelin level showed a significant decrease compared to the fasting ghrelin level in control group Ia (p<0.05) Fig. (2). The mean value of PP ghrelin level in group Ib group Ic showed a significant decrease compared to the fasting ghrelin level. However, there was no significant changes in postprandial levels of ghrelin between the three groups (Ia, Ib, Ic) (p>0.05) and diabetic groups IIa, IIb and IIc.

  In diabetic group IIb, no significant changes in postprandial levels of ghrelin as compared to diabetic group IIa (p>0.05). However, in vagotomized diabetic group IIc the mean value pp ghrelin level was significantly lower than diabetic group IIa (p<0.05) Fig. (3).

  In group IIIb PP ghrelin level was not significantly different from control group IIIa. However, in group IIIc (vagotomized) there was a significant decrease in PP ghrelin levels when compared to group IIIa and IIIb (p<0.05) Fig. (4).
Table (1): Mean value (±SD) of Fasting Ghrelin Levels (pg/ml) in day 14 in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>Ib</th>
<th>Ic</th>
<th>IIa</th>
<th>IIb</th>
<th>IIc</th>
<th>IIIa</th>
<th>IIIb</th>
<th>IIIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.62±</td>
<td>10.12±</td>
<td>10±</td>
<td>13.5±</td>
<td>13±</td>
<td>9.25±</td>
<td>11.3±</td>
<td>12.87±</td>
<td>9.43±</td>
</tr>
<tr>
<td>Fasting Ghrelin</td>
<td>1.06</td>
<td>1.35</td>
<td>0.7</td>
<td>1.15#</td>
<td>1.6#</td>
<td>0.8%@</td>
<td>0.8%@</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

# Significant when compared to its correspondent in group I (p<0.05).
@ Significant when compared to its correspondent in group II (p<0.05).

- **Blood glucose:**

After injection of group IIa by STZ for induction of diabetes, blood glucose levels significantly increased when compared to control group Ia, with values of (+280%) in group IIa vs. (+2.3%) in group Ia (p<0.05). As expected, treatment with insulin (group IIIa) significantly decreased blood glucose level towards normal level in group Ia (Table 2, Fig. 5).

Similarly, in group IIb (receiving GHS-R antagonist) and group IIc (vagotomized), blood glucose level significantly increased after injection...
by STZ (+299% and +288% respectively) and returned to control values in insulin treated groups (−20% and −10% respectively).

There is significant positive correlation between Fasting Ghrelin at Day 14 and blood glucose at Day 14 in diabetic group Fig. (6).

Table (2): Mean value (±SD) of Blood Glucose Level (mg %) in day 1, day 14 and percentage change in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>Ib</th>
<th>Ic</th>
<th>IIa</th>
<th>IIb</th>
<th>IIc</th>
<th>IIIa</th>
<th>IIIb</th>
<th>IIIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD:</td>
<td>126.12±</td>
<td>127.12±</td>
<td>118.37±</td>
<td>126±</td>
<td>121.62±</td>
<td>124.6±</td>
<td>126.2±</td>
<td>129.6±</td>
<td>124.6±</td>
</tr>
<tr>
<td>Day 1</td>
<td>6.5</td>
<td>4.6</td>
<td>4.92</td>
<td>8.2</td>
<td>6.36</td>
<td>6.34</td>
<td>16.4</td>
<td>7.38</td>
<td>5.31</td>
</tr>
<tr>
<td>Mean ± SD:</td>
<td>129.37±</td>
<td>110±</td>
<td>111.25±</td>
<td>479.5±</td>
<td>485.5±</td>
<td>483.5±</td>
<td>113.75±</td>
<td>103.75±</td>
<td>112.73±</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.3</td>
<td>5.26</td>
<td>3.45</td>
<td>3.16</td>
<td>5.2</td>
<td>6.11</td>
<td>11.38</td>
<td>1.39</td>
<td>5.9</td>
</tr>
<tr>
<td>% Change</td>
<td>+2.3%</td>
<td>−13.47%</td>
<td>−6%</td>
<td>+280%@</td>
<td>+299%@</td>
<td>+288%@</td>
<td>−9.8%^</td>
<td>−20%@</td>
<td>−10%^</td>
</tr>
</tbody>
</table>

@ Significant when compared to its correspondent in group I (p<0.05).
^ Significant when compared to its correspondent in group II (p<0.05).

- Food intake:

After induction of diabetes in group II, there was a significant increase in food intake in group IIa (+36.55%) when compared to group Ia (−3.33%) (p<0.05) as shown in Table (8). Treatment with insulin in group III decreased the percentage change in food intake in group IIIa (+22.6%) compared to group IIa (+36.55%) but it was still significantly higher than group Ia (−3.33%) (p<0.05) as shown in Table (3) and Fig. (7).

In rats receiving GHS-R antagonists, the percentage reduction in food intake in untreated group IIb (−21.8%) and treated group IIIb (−25.3%) was significantly less than group Ib (−35.5%) (p<0.05).

In vagotomized rats, the percentage reduction in food intake showed no significant change between group Ic (−10%), IIc (−6.36%) & IIIc (−13.55%).

There is significant positive correlation between Fasting Ghrelin at Day 14 and food intake at Day 14 in diabetic group Fig. (8).

Table (3): Mean Value (±SD) of Food Intake (gm) in day 1, day 14 and percentage change in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>Ib</th>
<th>Ic</th>
<th>IIa</th>
<th>IIb</th>
<th>IIc</th>
<th>IIIa</th>
<th>IIIb</th>
<th>IIIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD:</td>
<td>15±</td>
<td>15.1±</td>
<td>15.12±</td>
<td>18±</td>
<td>17.75±</td>
<td>13.75±</td>
<td>17.12±</td>
<td>17.25±</td>
<td>14.75±</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.3</td>
<td>1.8</td>
<td>1.8</td>
<td>1.6</td>
<td>1.67</td>
<td>1.28</td>
<td>1.8</td>
<td>1.6</td>
<td>1.28</td>
</tr>
<tr>
<td>Mean ± SD:</td>
<td>14.5±</td>
<td>9.7±</td>
<td>13.62±</td>
<td>24.7±</td>
<td>13.8±</td>
<td>12.87±</td>
<td>21±</td>
<td>12.87±</td>
<td>12.75±</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.6</td>
<td>1.2</td>
<td>1</td>
<td>1.67</td>
<td>1.14</td>
<td>1.24</td>
<td>1.6</td>
<td>1.12</td>
<td>1</td>
</tr>
<tr>
<td>% Change</td>
<td>−3.33%</td>
<td>−35.5%</td>
<td>−10%</td>
<td>+36.55%</td>
<td>−21.8</td>
<td>−6.36%</td>
<td>+22.6%</td>
<td>−25.3%</td>
<td>−13.55%</td>
</tr>
</tbody>
</table>

@ Significant when compared to its correspondent in group I (p<0.05).
- **Body weight:**

After induction of diabetes, group IIa showed a significant decrease in body weight compared to the weight gain shown in group Ia with values of \(-32.33\%\) vs. \(+42.75\%\) \((p<0.05)\). In insulin treated group IIIa the effect was reversed significantly \(+8.30\%\) but still it was significantly lower than group Ia \(+42.75\%) \((p<0.05)\).

In group IIb (treated with GHS-R antagonist), the percentage reduction in body weight in untreated group IIb \(-50.4\%) and treated group IIIb \(-41.2\%) was significantly more than group Ib \(-30.55\%) \((p<0.05)\).

In vagotomized rats, the percentage reduction in body weight in group IIc was significantly higher \(-38.22\%) compared to group Ic \(-32.33\%) \((p<0.05)\). In insulin treated rats (group IIIc) the percentage reduction was significantly less \(-30\%) compared to group IIc \(-38.22\%) \((p<0.05)\) Table (4), Fig. (9).

### Table (4): Mean value (±SD) of Body Weight (gm) in day 1, day 14 and percentage change in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>Ib</th>
<th>Ic</th>
<th>IIa</th>
<th>IIb</th>
<th>IIc</th>
<th>IIIa</th>
<th>IIIb</th>
<th>IIIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>171.37±</td>
<td>173.87±</td>
<td>171.62±</td>
<td>164.62±</td>
<td>174.12±</td>
<td>173.62±</td>
<td>171.75±</td>
<td>176.25±</td>
<td>179±</td>
</tr>
<tr>
<td>Day 1</td>
<td>17.27</td>
<td>17.15</td>
<td>17.1</td>
<td>9.35</td>
<td>8.47</td>
<td>11.1</td>
<td>18.6</td>
<td>4.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>244.62±</td>
<td>120.75±</td>
<td>116.12±</td>
<td>107.25±</td>
<td>104.87±</td>
<td>107.25±</td>
<td>186±</td>
<td>103.62±</td>
<td>125.6±</td>
</tr>
<tr>
<td>Day 14</td>
<td>26.6</td>
<td>12</td>
<td>11.7</td>
<td>6.38</td>
<td>9.7</td>
<td>10.15</td>
<td>17.54</td>
<td>4.1</td>
<td>7.9</td>
</tr>
<tr>
<td>% Change</td>
<td>+42.75%</td>
<td>-30.55%</td>
<td>-32.33%</td>
<td>-32.33%</td>
<td>-50.4%</td>
<td>-38.22%</td>
<td>-8.30%</td>
<td>-41.2%</td>
<td>-30%</td>
</tr>
</tbody>
</table>

@ Significant when compared to its correspondent in group I \((p<0.05)\).

$ Significant when compared to its correspondent in group II \((p<0.05)\).
There is significant negative correlation between Fasting Ghrelin at Day 14 and body weight at Day 14 in diabetic group Fig. (10).

![Fig. (10): Regression analysis showing negative correlation between Fasting Ghrelin at Day 14 and body weight at Day 14 in diabetic group.](image)

**Discussion**

Food intake is a complex function of the brain that has been analyzed from a number of scientific perspectives, including psychological, nutritional, neuroanatomical, neurochemical, electrophysiological, endocrinological, and most recently, molecular genetics perspectives. Endocrine signals have emerged as an especially important aspect of the physiology of eating [18].

In an attempt to elucidate the possible role of ghrelin in the pathogenesis of diabetic hyperphagia, the present study investigated the relation between plasma ghrelin level, food intake, body weight and blood glucose levels in control rats, STZ-DM rats and insulin treated diabetic rats. This study also investigated the role of the vagus in controlling the peripheral signaling and level of ghrelin in the same groups of rats.

As regards the significant weight loss observed in this study despite the marked hyperphagia in the diabetic group of rats, these results are consistent with other reports that state that uncontrolled diabetes is characterized by marked behavioral and metabolic perturbations that arise as a consequence of profound insulin deficiency, including severe hyperglycemia, depletion of body fat mass, loss of weight inspite of hyperphagia [19].

Consistent with other reports, [18,20] the present study also showed significant high levels of fasting and postprandial plasma ghrelin in STZ-DM rats compared to controls. Moreover, plasma ghrelin levels were positively correlated to the food intake, blood glucose and negatively correlated to the body weight in diabetic group.

The insulin deficiency and/or other consequences of uncontrolled diabetes stimulate ghrelin secretion and raise plasma concentrations but as food intake increases, nutrient induced inhibition of ghrelin secretion partially offsets the stimulatory effect of uncontrolled diabetes and lowers plasma levels of this hormone [18].

Ghrelin is a potent growth hormone secretagogue (GHS) that binds and activates its receptor, the GHS-R [21]. Synthetic GHS-R agonists have been shown to increase food intake, fat mass and lean mass in rats even after long-term use.

In the current study, peripherally administered GHS-R antagonist abolished the stimulatory effects on feeding induced by increased ghrelin level in diabetic rats without affecting fasting plasma ghrelin levels in group Ib, IIb and IIIb. As regards food intake, GHS-R antagonist totally abolished the orexigenic actions of ghrelin and caused a significant decrease in food intake in all subgroups (Ib, IIb, IIIb) compared to their relative control subgroups (Ia, IIa, IIIa).

GHS-R antagonist significantly decreased the body weight in all subgroups (Ib, IIb, IIIb) compared to their relative control subgroups (Ia, IIa, IIIa). This effect is probably due to the anorexigenic effect of treatment with the antagonist.

As regards blood glucose levels, there was a significant decrease in GHS-R antagonist treated subgroups (Ib-IIIb) compared to their relative control subgroups (Ia-IIIa). As for diabetic group, blood glucose level didn't show a significant change in GHS-R antagonist treated subgroup (IIb) when compared to its correspondent control group (IIa).

Hassouna and his colleagues [22] also explained that this pharmacological dissociation is surprising considering that both GH-releasing and orexigenic actions of ghrelin are absent in GHS-R knockout mice, precluding a GHS-R dependent pathway for both actions. The GHS-R1a is indeed coupled to multiple G-protein effectors and different signaling pathways to trigger intracellular calcium mobilization in a tissue-specific manner. In somatotroph cells, ghrelin agonists are coupled to Gq proteins and activate the phospholipase C-inositol triphosphate pathway. In neuropeptide Y (NPY) cells, the increase in intracellular calcium is triggered by the cAMP/protein kinase A pathway and N-type calcium channel.
In previous studies, centrally administered GHS-R antagonist abolished the stimulatory effects on feeding induced by peripherally administered ghrelin. Previous studies also demonstrated that peripherally administered GHS-R antagonist decreased gastric emptying rate [23].

Results of the present study showed a beneficial effect of GHS-R antagonist when combined with insulin in treated diabetic groups, in accord with previous studies suggesting that the GHS-R-1a antagonists might be beneficial for the treatment of type II diabetes and obesity. In type II diabetes, remarkable reduction in glucose levels, accompanied by a moderate decrease in serum insulin levels, implicates that GHS-R antagonists play a role in the amelioration of insulin resistance [24].

In lean mice as in obese mice, GHS-R antagonist decreases food intake and reduces weight gain. Recently, orally bioavailable antagonists have been developed and lead to suppression of food intake and body weight reduction through selective loss of fat mass and glucose-lowering effects by enhancing insulin secretion [21].

Moreover, the present study showed a significant decrease in basal fasting ghrelin levels in all vagotomized groups (control, diabetic and controlled diabetic). It also shows a significant decrease in body weight and food intake in all vagotomized groups.

These present results are in accord with previous reports that indicate that functions of the vagus, although important for the short-term control of food intake, apparently are not necessary for the ghrelin response to nutrients in the gut. Vagal mediation is indicated, however, for the food deprivation-induced elevation of plasma ghrelin, and the activity of the vagus as an efferent pathway for the ghrelin release has been implicated in the starvation-induced elevation of plasma ghrelin [25].

However, our results are contradictory to those obtained by Erlanson-Albertsson and Lindqvist, [26] showing that mice subjected to vagotomy had unaffected ghrelin secretion (both total and active ghrelin) compared to sham-operated mice. In that study, they measured preprandial ghrelin levels after a two weeks period and could not explain how postprandial ghrelin is affected in the vagotomized mice. They also explained that in the long-term perspective, the loss of satiety mediated through vagotomy is compensated for by other signaling systems; blood-borne systems such as maintained peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and leptin secretion from the lower and upper intestine and white adipose tissue, respectively.

In the present study, vagotomized rats also showed a significant decrease in blood glucose level. This may be explained by the fact that vagotomized rats has slower gastric emptying rate causing gastric distension. Decrease in fasting ghrelin level and slow gastric emptying rate may both act as satiety signals causing decrease in blood glucose. Considerable evidence has accumulated to indicate that gastric distention acts as a satiety signal to inhibit food intake, and rapid gastric emptying is closely related to overeating and obesity, as is delayed gastric emptying to anorexia and cachexia [27].

Interestingly, on comparing the effects of GHS-R antagonist to the effects of vagotomy in all studied subgroups in the present study, GHS-R antagonist treated rats showed a significantly higher decrease in food intake and body weight, than vagotomized rats. This, in addition to the previously discussed changes, highlights the strong relation that ghrelin possesses as an orexigenic hormone. It also highlights the presence of some compensatory mechanisms for food intake after vagotomy, yet to be studied.

**Recommendations:**

- Further studies on the effect of ghrelin antagonism on food intake and insulin release.
- Possible uses of GHR-S antagonists as anti-obesity treatment and treatment of diabetes type 2.
- Further studies on the effect of gastric bypass surgery (removal of ghrelin producing cells from the stomach) as a method of losing weight.
- Further studies on the effect of ghrelin hormone on gastric emptying and gastric acid secretion.

**References**