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Effect of High Fat Diet and High Protein Diet on Fasting Serum Ghrelin Level in Type-1 Diabetes

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Abstract

Ghrelin is a multifunctional orexigenic peptide that is related to the regulation of the feeding behavior, energy homeostasis, growth hormone release and alsoit has evidenced relation with insulin. In normal weight and non-diabetic conditions, the ghrelin response to food depends completely on the type of macronutrients, but this relation with the type of macroneutrient is not clear in obese or diabetic conditions. In this work we studied the effect of high fat diet (HFD) and high protein diet (HPD) on ghrelin level in type I diabetic lean and obese rats aiming to clarify the role of these diets. The work was conducted on 5 groups of rats each consisted of 10 rats. Body weight was measured at the onset of the study, after 2 weeks then at the end of the study period; 8 weeks. After 8 weeks blood samples were collected and analyzed for fasting serum glucose and ghrelin levels. Results showed a significant reduction in the serum ghrelin level in the HFD diabetic group as compared to both the control diabetic and HFD fed group while the HPD diabetic rats showed significant increase in serum ghrelin level compared to both the control and HFD groups and insignificant difference compared to the control diabetic group. We concluded that HFD significantly reduces ghrelin in diabetic rats while HPD in spite of being more satiating, its effects on ghrelin secretion is not clear in type 1 diabetes.

Keywords: Ghrelin, body weight, high fat diet, high protein diet, Type-1 diabetes mellitus.

Introduction

Ghrelin is a peptide hormone formed of 28 amino acids. It is secreted from different organs in the body, mainly the stomach. It has 2 forms the acylated and the non-acylated forms. It has strong orexigenic properties also it stimulates the release of growth hormone through its action on theGrowth Hormone secretagogue Receptor 1a (GHSR-1a). Ghrelin has important role in energy homeostasis and in regulating food intake. Its level is high in anorexic subjects and low in obese. In subjects with normal body weight, its secretion is stimulated by fasting and decreased by intake of food and by oral glucose load[1].

Most of the metabolic effects of ghrelin are due to its effects on the Agouti Gene Related Protein (AGRP). Acylation of ghrelin on the serine in position 3 is catalyzed by ghrelin O-acyltransferase (GOAT) and is essential for its activity. The ghrelin-GOAT system is involved in insulin resistance, lipid metabolism dysfunction, and inflammation[2].

Although accumulating evidence has shown crucial roles of ghrelin and insulin in food intake and energy metabolism, the exact relationship between these hormones remains unclear with some researchers suggesting that higher ghrelin levels decrease insulin secretion, while others claim an opposite relationship. So, there is still an ongoing debate about the relation between ghrelin and insulin. Earlier studies however, have generated conflicting results in that ghrelin has been shown to inhibit insulin release in some experimental situations[3] and to stimulate insulin secretion in others[4]. However, these inconsistencies may reflect species differences and / or differences in experimental design as different doses, different times of observation, *in vitro* versus *in vivo* experiments.

Type-1 diabetes is usually linked to abnormal secretion of ghrelin as its level is low before insulin injection also the response to test meals is not present. This relation shows that abnormal secretion of ghrelin plays an important role the metabolic balance in type[5].

Higher protein diets are very effective and a preferable dietary regimen in fighting obesity than traditional reduced energy diets as they have valuable effects in reducing food intake, body weight, fat mass while preserving lean body mass , and improving satiety in overweight and/or obese individuals[6].

In normal weight and non-diabetic conditions, the ghrelin response to food depends completely on the type of macronutrients. Due to their rapid rate of absorption and insulin-secreting effect, carbohydratessuppress ghrelin secretion maximally. Proteins are the most satiating macronutrient as they effectively suppress ghrelin while fats have weak ghrelin-suppression capacity. The mediators responsible for meal-induced ghrelin regulation are glucose, insulin, gastrointestinal hormones, gastric emptying rate, changes in intestinal osmolarity and vagal activity. However, this relation is not

clear in obese or diabetic conditions[7].

Material and Methods

After approval of the "Animal Ethical Committee of the college of Pharmacy and Health Sciences" and following the Principles of Laboratory Animal Care, a total of 50 adult male albino mice divided into 5 groups, were included in this study,rats were housed in their cages with *ad libitum* access to the regular rat diet and water. The rats were maintained under 12:12 h light – dark cycle. After one week of habituation to housing conditions, they were divided into 5 groups:

Group I: (Control group) rats were maintained on normal rat dietand water *ad libitum* for 8 weeks and received a single intraperitoneal (IP) dose of Sodium Citrate buffer (pH =4.5) 2 weeks before the end of the work period; by the end of 6 weeks[8].

Group II: (Control HFD group) rats were maintained on high fat diet for 8 weeks.

Group III: (Control diabetic group) rats maintained on normal rat diet and water *ad libitum*for 6 weeks then diabetes was induced by a single IP injection of Streptozotocin (STZ) dissolved in 0.01 mmol/L Sodium Citrate buffer at pH=4.5 in a dose of 50 mg/kg body weight[9] and maintained on the same diet for 2 more weeks after induction of diabetes.

Group IV: (Diabetic on HPD group) rats were fed HPD for 6 weeks then diabetes was induced as before and they were kept on the same diet for the next 2 weeks

Group V: (Diabetic on HFD group) rats were maintained on HFD for 6 weeks then diabetes was induced as before, then rats were fed the same HFD for the next 2 weeks.

After 8 weeks blood samples were collected and analyzed for fasting serum ghrelin, insulin and leptin. For all diabetic rats, blood glucose level was measured 2 weeks after injection and rats with blood glucose level > 200 mg/dl were accepted to be diabetic[10].

Composition of HFD: HFD consisted of butter (40%), corn oil (20%), sucrose (23%) and casein (17% Nestlé ® condensed milk) respectively[11]. Sixty percent of calories are from fat. The body weight was checked after 2 weeks then at the end of experiment period i.e. 8 weeks. Rats should attain at least 15% higher body weight within 2 weeks. Rats which gained more than 80% increase in body weight after 8 weeks were only included in the study[12].

Composition of HPD: HPD is made of 40% protein (Harlan Teklad custom research diets, product No. TD 90018). This diet is isocaloric rodent diet. It has the following composition, table-1

For all the 50 rats, blood samples were collected by a cardiac puncture after an overnight fast, centrifuged and serum was separated and analyzed for: Fasting blood glucose level. (By an Enzymatic calorimetric method (GOD-PAP), and Fasting serum total ghrelin using the ELISA kits.

Table-1
Composition of high protein diet

Formula	g/kg
Casein	460.0
Sucrose	231.82
Corn Starch	200.0
Corn Oil	50.0
Vitamin Mix, Teklad (40060)	15.0
Ethoxyguin, antioxidant	10.0
Mineral Mix,Ca-P deficient. (79055)	0.01
Calcium Phosphate, dibasic.	13.37
Calcium carbonate.	9.6

Statistical analysis: Our results were statistically analyzed using the following statistical tests and formulae: Arithmetic mean (X), Standard deviation (SD), t- test of significance, Correlation co-efficiency.

Results and Discussion

The initial body weight of all rats ranged between 100 and 130 gm and showed insignificant difference between the different studied groups table-2 and figure-1

Table-2
Initial body weight (g) in different studied groups

	Group I Control	Group II HFD Control	Group III Diabetic control	Group IV Diabetic on HPD	Group V Diabetic on HFD
Min	100	100	100	105	105
Max	135	130	130	125	130
Mean	114.5	114.5	114	116	116.5
SD ±	12.12	9.56	9.07	8.10	7.47
F	0.133				
p	0.969 N.S.				

F = ANOVA test, N.S. = Not significant

After 2 and 8 weeks of consumption of the different types of macronutrient, HFD fed rats (group II and V) showed a high significant increase in body weight also in the percentage change of body weight compared to the rats fed on control or HPD (group I, II, IV), while rats fed on the HPD showed significant increase in body weight compared to the control diet fed group (group I and II) which is most probably due to increase muscle mass. However, HPD fed group showed significant decrease to the HFD fed groups (group II,IV) in both the body weight and the percentage change of body weight ($p< 0.00001$). Table-3,4,5,6 and figure-2,3,4,5,6.

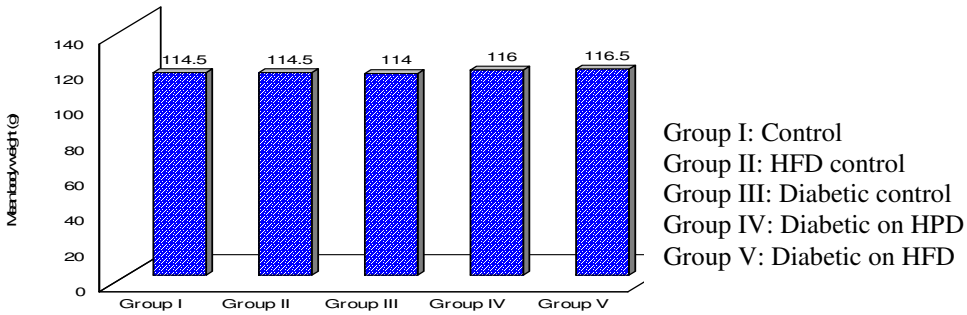

Figure- 1
Initial body weight (g) in the different studied groups

Table-3
Body Weight (g) after 2 weeks in the different studied groups

	Group I Control	Group II HFD Control	Group III Diabetic control	Group IV Diabetic on HPD	Group V Diabetic on HFD
Min	110	150	110	115	150
Max	145	190	140	150	180
Mean	122.5[a]	167.5[b]	124.58[a]	130.5[a]	165[b]
SD ±	12.30	12.75	11.17	10.92	10.00
F	37.204				
p	0.0001*				

* Significant, p < 0.05, a Significance versus group II, V, b Significance versus group I, III and IV

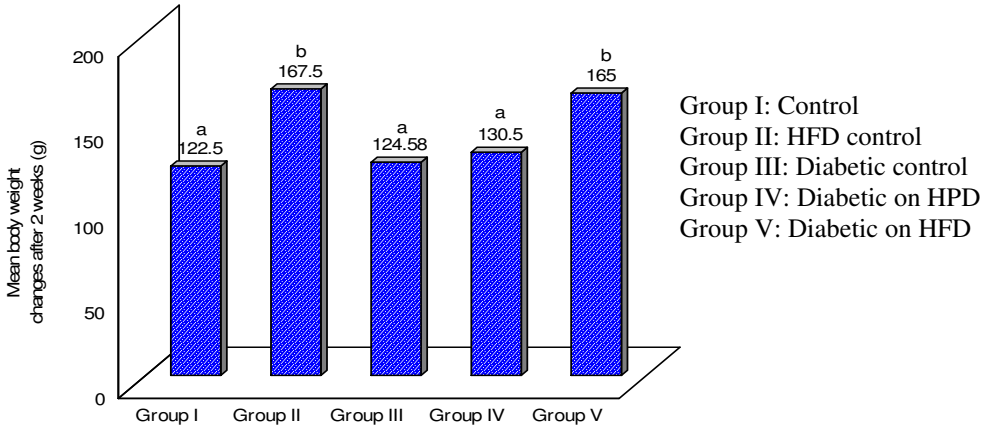

Figure-2
Body weight (g) after 2 weeks in the different studied groups.
a Significance versus group II, V, b Significance versus group I, III and IV

Table-4
Percent change in body weight after 2 weeks in the different studied groups

	Group I Control	Group II HFD control	Group III Diabetic control	Group IV Diabetic on HPD	Group V Diabetic on HFD
Min	3.85	36.00	4.55	8.00	33.33
Max	10.00	63.64	17.39	20.00	47.83
Mean	7.07 [a]	46.63 [b]	9.30 [ac]	12.47 [c]	41.71 [b]
SD ±	2.46	9.34	4.34	4.42	4.07
F	115.37				
p	0.0001*				

***** Significant, p < 0.05, a Significance versus group II, IV and V, b Significance versus group I, III and IV, c Significance versus group I, II, V, ac Significance versus group II, V

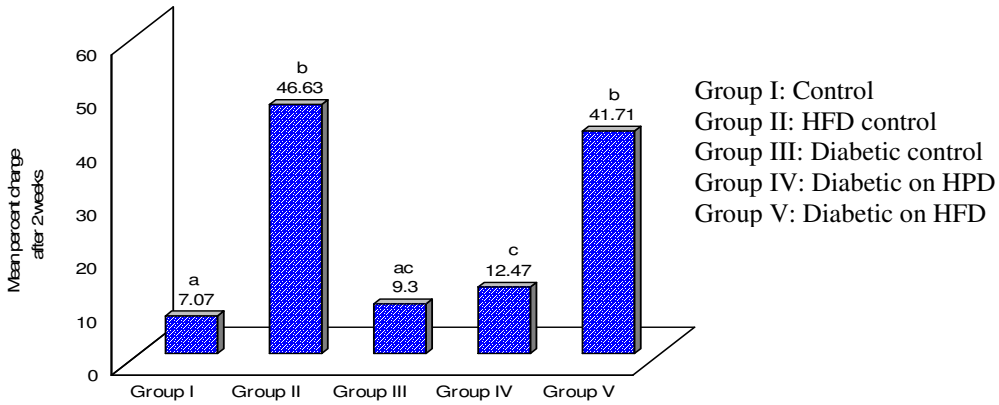

Figure- 3

The percent change in body weight after 2 weeks in the different studied groups.

A Significance versus group II, IV and V, b Significance versus group I, III and IV, c Significance versus group I, II, V, ac Significance versus group II, V

Table- 5

Body Weight (g) after 8 weeks in the different studied groups.

	Group I Control	**Group II HFD control**	**Group III Diabetic control**	**Group IV Diabetic on HPD**	**Group V Diabetic on HFD**
Min	115	190	120	125	205
Max	155	255	155	160	250
Mean	130.5[a]	222.5[b]	134.5[a]	140.5[a]	225.5[b]
SD ±	12.79	18.75	13.63	11.17	15.36
F	112.17				
p	0.00001*				

* Significant p < 0.05, a Significance versus group II andV, b Significance versus group I, III and IV

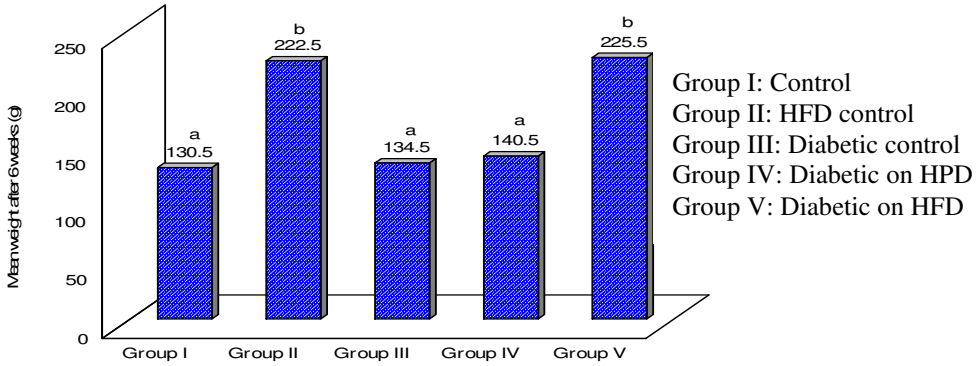

Figure- 4

Body weight (g) after 8 weeks in the different studied groups

a Significance versus group II andV, b Significance versus group I, III and IV

Table- 6

Percent change in body weight after 8 weeks in the different studied groups

	Group I Control	**Group II HFD control**	**Group III Diabetic control**	**Group IV Diabetic on HPD**	**Group V Diabetic on HFD**
Min	7.69	80.00	9.09	12.50	79.17
Max	20.00	109.09	24.00	28.00	104.17
Mean	14.12 [a]	94.43 [b]	17.88 [ac]	21.14 [c]	93.62 [b]
SD ±	3.67	7.48	5.07	4.85	6.57
F	542.17				
p	0.00001*				

* Significant , p < 0.05, a Significance versus group II, IV and V, b Significance versus group I, III and IV, c Significance, versus group I, II, V, ac Significance versus group II, V

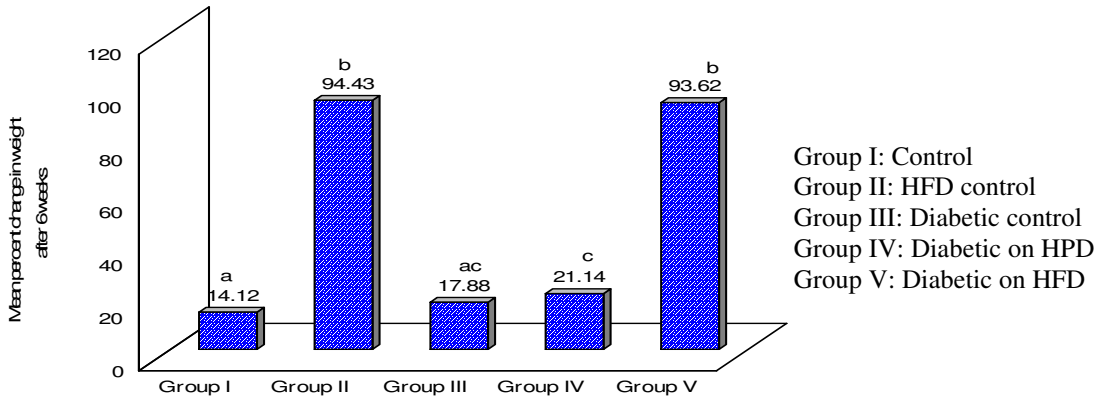

Figure- 5

The percent change in body weight after 8 weeks in the different studied groups.

A Significance versus group II, IV and V, b Significance versus group I, III and IV, c Significance versus group I, II, V, ac Significance versus group II, V

Table-7

Summary of body weight changes in the different studied groups

Group		Initial body weight g	Body weight after 2 weeks. g	% change after 2 weeks	Body weight after 6 weeks. g	% change after 6 weeks
Control I	Mean	114.5	122.5	7.707	130.5	14.12
	Sd ±	12.12	12.30	2.46	12.79	3.67
HFD control II	Mean	114.5	167.5*	46.63*	222.5*	94.43*
	Sd ±	9.56	12.75	9.34	18.75	7.48
Diabetic control III	Mean	114.0	123.5	8.29	134.5	17.88
	Sd ±	9.07	12.26	5.65	13.63	5.07
Diabetic on HPD IV	Mean	116.0	130.5	12.47*	140.5	21.14*
	Sd ±	8.10	10.92	4.42	11.17	4.85
Diabetic on HFD V	Mean	116.5	165.0*	41.71*	225.5*	93.62*
	Sd ±	7.47	10.0	4.07	15.36	6.57

* indicates significant difference to the control group, $P < 0.05$

Our results proved that HFD induced obesity and significant reduction in the ghrelin level compared to control diet fed rats and also to diabetic rats fed on control diet. These results were supported by previous studies showing that consumption of high caloric or hypercaloric diet is associated with overweight, obesity and hypertension in animals and humans. Also it was found that feeding of high-fat hypercaloric diet makes normal Wistar male adult rat obese and it is associated with hyperlipidemia, hyperinsulinemia, and glucose intolerance[13].

A previous study proved that high fat diet induces obesity by increasing fat storage without stimulation of its oxidation resulting in positive energy balance. Protein diet requires the highest metabolic cost to be converted to stored fat as it has the highest thermic effect (25- 30%) while it is only 6-8% for carbohydrates[14].

Fasting serum Glucose level (mg/dl): By the end of the eighth week, 2 weeks after STZ injection, the 3 diabetics groups; the diabetic control (group III) , the diabetic on HPD (group IV) and the diabetic on HFD (group V) showed significant increase in the fasting serum glucose level compared to the control (group I) and HFD (group II) groups. ($p< 0.00001$) table-8 and figure-6

Table-8

Fasting Serum Glucose level (mg/dl) in the different studied groups

No.	Group I Control	Group II HFD control	Group III Diabetic control	Group IV Diabetic on HPD	Group V Diabetic on HFD
Min	72	88	220	255	215
Max	102	112	400	315	333
Mean	83.8 [a]	101.0 [a]	302.6 [b]	290.3 [b]	289.7 [b]
SD±	9.08	8.77	49.75	20.68	33.19
F	147.49				
p	0.00001*				

* Significant, $p < 0.05$, a Significance versus group III, IVand V, b Significance versus group I and II

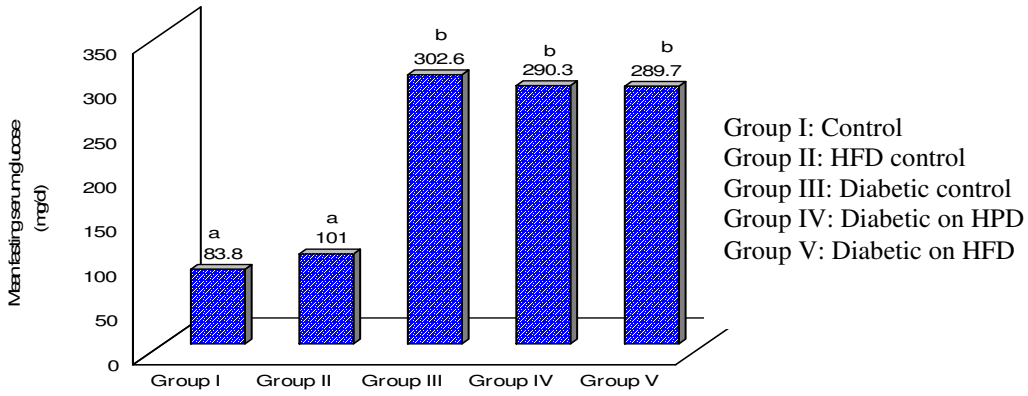

Figure-6
Fasting Serum Glucose level (mg/dl) in the different studied groups
A Significance versus group III, IVand V, b Significance versus group I and II

In the present study Fasting serum glucose concentration was found to be significantly higher in the rats of group III, IV and V (i.e. the control diabetic, the diabetic on HPD and the diabetic on HFD groups) as compared to the normal control group (group I). These findings agreed with those in previous reports[15].

However, no significant difference was detected between the three studied groups (III, IV and V) as regards the fasting serum glucose. This means that in our study the effect of the different types of food did not protect the β cells from the destructive effect of STZ and may indicate that high protein diet did not have an effect on reducing the serum glucose level.

Fasting Serum Ghrelin level (ng/ml) in the different studied groups: By the end of the study period i.e. after 8 weeks, Fasting Serum Ghrelin level showed significant decrease in the HFD control group (group II), (mean 1.32ng/ml, SD ± 0.33) compared to the control, diabetic control, diabetic on HPD and diabetic on HFD groups (group I, III, IV and V respectively) with mean values of 2.22, 4.47, 4.33 and 2.49 ng/ml and SD values of ± 0.31, ±0.77, ±0.71and ± 0.44 respectively. The diabetic on HFD (group V) showed significant increase compared to both the diabetic control (group III) and the diabetic on HPD group (group IV). $P < 0.0001$

On the other hand the diabetic rats fed on HPD (group IV) showed significant increase compared to the control (group I), the HFD control (group II) and the diabetic on HFD group (group V) $p < 0.0001$, while it showed insignificant difference to the diabetic control group (group III).

Correlation between the fasting serum ghrelin (ng /ml) and glucose (mg/dl) in the different study groups: Our results proved that fasting serum ghrelin showed a significant positive correlation with the fasting serum glucose level in the different study groups ($r = 0.739$, $p < 0.0001$). figure-8.

In the present study, it was found that fasting serum ghrelin levels were significantly higher in both the diabetic rats on regular rat diet (group III) and the diabetic rats on HPD (group IV) as compared to the control rats (group I).

Our results were confirmed by a previous study that suggested a negative association between systemic ghrelin and insulin levels. It has also suggested that ghrelin inhibits insulin secretion both in vitro and in most human or animal studies[16].

Table-9
Fasting Serum Ghrelin level (ng/ml) in the different studied groups

No.	Group I Control	Group II HFD control	Group III Diabetic control	Group IV Diabetic on HPD	Group V Diabetic on HFD
Min	1.8	0.8	3.2	3.3	1.8
Max	2.8	1.8	5.7	5.4	3.1
Mean	2.22 [a]	1.32 [b]	4.47 [c]	4.33 [c]	2.49 [a]
SD ±	0.31	0.33	0.77	0.71	0.44
F	63.25				
p	0.0001*				

*Significant, p< 0.05, a Significance versus group II, III and IV, b Significance versus group I, III, IVand V, c Significance versus group I , II and V

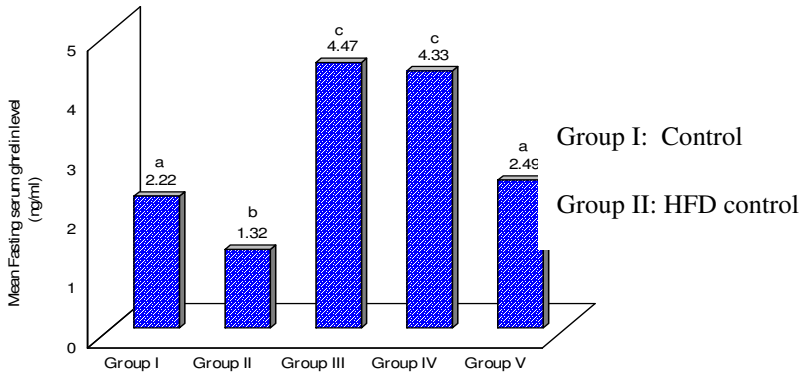

Figure-7

Fasting Serum Ghrelin level (ng/ml) in the different studied groups

A Significance versus group II, III and IV, b Significance versus group I, III, IV and V, c Significance versus group I, II and V

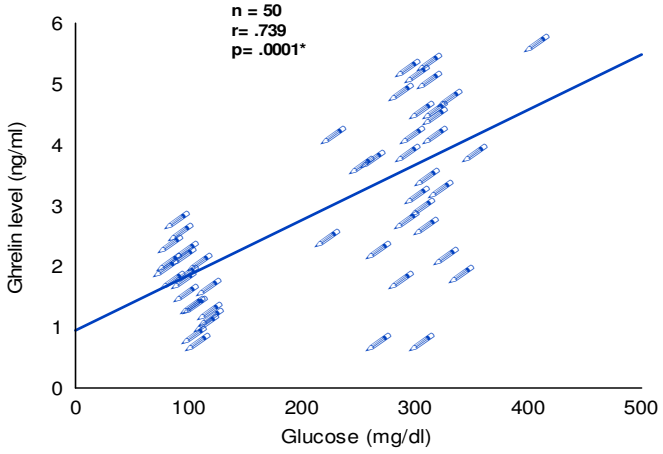

Figure- 8

Correlation between the Fasting Serum Ghrelin (ng/ ml) and Glucose (mg/dl) levels

Furthermore, our results were also verified by a previous study that proved ghrelin to be increased in STZ-induced diabetic (DM) mice, were STZ-DM mice had higher plasma ghrelin concentrations and greater ghrelin mRNA expression than control mice[17].

Many clinical studies have used variable test meals consisting of protein, fat and carbohydrate rich diet in order to examine the relative efficacy of each macronutrient to suppress postprandial ghrelin with varying results. This heterogeneity in findings may be attributed to the discrepancy in experimental design as the composition of the meal, the measured parameters, blood sampling intervals, duration of post-ingestive period[8].

Our results suggested that HFDobese rats showed a significant lower level of fasting serum ghrelin as compared to the other studied groups.

This present result was supported by a study that proved that circulating ghrelin levels are decreased in human obesity[18]. Also another study showed that circulating levels of ghrelin decrease with weight gain which may be caused by high fat diet[19]. Moreover, weight gain induced by overfeeding, was demonstrated to suppress plasma ghrelin levels in rats[20].

Ghrelin has a strong negative association with central adiposity, which is known to correlate with insulin resistance. Taken together, these findings suggest that the relationship between ghrelin and insulin may be explained by the link between ghrelin and central adiposity. It was found that chronic intravenous infusion of acylated ghrelin increased retroperitoneal and inguinal white adipose tissue (WAT) volume in rats without elevating the superficial subcutaneous fat, food intake, or circulating lipids and glucose. This increase in WAT increases hepatic steatosis, also increases the number of lipid droplets, triglyceride contents and central adiposity through stimulation of the GHS-R (1a), while this effect is not clear after infusion of non-acylated ghrelin[21].

Our results were also supported by a study that suggested that insulin and ghrelin expression in the plasma and pancreas was adversely affected by long-term high-fat/high-energy and high-protein diets[22].

Also on consumption of high fat diet, the brain GLP-1 signaling induces hyperinsulinemia and insulin resistance and decreases energy expenditure by reducing metabolic thermogenesis[23].

However, another work showed that short-term consumption of a high fat diet leads to the increase in the level of fasting plasmaCCK concentrations but does not affect ghrelin, upper gut motility and PYY[24].

High protein diet: In the present work, our results showed a significant increase in fasting serum ghrelin levels in HPD fed diabetic rats (group IV) as compared to the control group (group I), after 8 weeks. The present study revealed that HPD fed diabetic rats did not show a significant change in fasting serum ghrelin level as compared to control diabetic rats. This may be explained by the fact that diabetes was also associated with high ghrelin level. On the other hand HPD fed diabetic rats showed significant higher fasting serum ghrelin levels as compared to obese HFD fed rats (group II) also to diabetic rats on HFD (group V). This may be explained by the fact that obesity is associated with low ghrelin levels while protein diet increases the ghrelin level.

Our results were supported by a recent study which suggested that isocaloric HPD can ameliorate body composition and increase total ghrelin level[25].

They were also supported by the findings of Cremades et al who evaluated the macronutrient effect on ghrelin expression and secretion. They reported that the fasting plasma ghrelin level was especially sensitive to high protein diet. Rats receiving high protein diet had a higher ghrelin levels than the control rats. These HPD fed rats had also the highest ghrelin level among rats consuming other food types namely regular diet, high carbohydrate diet and HFD[26].

The present results were also verified by a study which found that high protein diet was associated with increased ghrelin serum peptide level and also ghrelin mRNA expression on the duodenum and the fundus of the stomach[27].

It has been reported that high protein food are more satiating and have a higher thermogenic effect than normal protein diet over the short term as well as the long term. The satiating effect of a single high protein lunch was found to be partly due to changes in plasma ghrelin levels, glucagon- like peptide 1 (GLP-1) , peptide tyrosine- tyrosine concentration and also may be due to changes in other metabolites or amino acids[28].

Acute protein intake affects satiety and ghrelin secretion, while chronic high protein intake stimulates thermogenesis, decreases the respiratory exchange rate and reduces carbohydrate oxidation[29]. A recent study suggested that protein is the most satiating macronutrient followed by carbohydrates then fat, which is least satiating. This satiating effect is most significant after HPD[30].

The mechanisms that contribute to the satiating effect of protein include increased concentration of satiety hormones, increased energy expenditure, increased concentrations of metabolites and also stimulation of gluconeogenesis. It has been found that this satiety is related to marked increase in the concentrations of the anorexigenic hormones namely Glucagon-like peptide-1 (GLP-1), Cholecystokinine (CCK) and PYY. Also it is related to the decrease in the concentration of the orexigenic ghrelin hormone. Pancreatic ghrelin acts as physiological regulator of the glucose-induced insulin secretion. Ghrelin antagonism stimulates insulin secretion and controls hyperglycemia in conditions of obesity due to HFD. This may have a potential therapeutic value in type 2 diabetes[31].

On the other hand, Leidy et al showed no effect of chronic protein intake on plasma insulin or ghrelin levels where postprandial plasma ghrelin was suppressed after a single high protein meal compared with the recommended protein meal. They concluded that the responses to acute and chronic consumption of high protein were found to be different[29].

Our results were also opposite to the study that proved that high protein breakfast decreased postprandial ghrelin concentrations more strongly over time than did the high carbohydrate breakfast. High associations between ghrelin and glucose-dependent insulinotropic polypeptide and glucagon suggest that stimulation of these peptides may mediate the postprandial ghrelin response[32]. On the other hand, a previous study proved that ghrelin and insulin were not different between high protein (30%) diet and a control diet containing of 15% protein[33].

However, this discrepancy between in the results may be attributed to different experimental periods and designs, the percentage of protein in the high protein diet, fasting or postprandial experiments and also whether the high protein diet was consumed once (as a single breakfast for example) or it was consumed over a long period of time.

Conclusion

The relation between ghrelin, appetite and body weight is crucial; ghrelin has a strong negative association with body weight. So, ghrelin or its antagonists could be a target for managing the body weight problem in conditions of obesity or cachexia. This relation is versatile yet integrated with many factors affecting it. Among these factors are the glycemic condition, the body weight and the type of macronutrient consumed whether high protein, high fat or regular diet.

Due to their effect on ghrelin level, high protein diets affect satiety and could be considered as a preferable dietary strategy in combating obesity.

Abbreviations: GHS-R (1a): Growth Hormone secretagogue Receptor 1a, GLP-1: Glucagon like peptide- 1, PYY: Peptide YY, CCK: Cholecystokinin, NPY: Neuropeptide Y, AgRP: Agouti gene Related Peptide

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