ORIGINAL ARTICLE

EFFECT OF GHRELIN ANTAGONIST ON FOOD AND WATER INTAKE IN OBESE AND TYPE 2 DIABETIC MICE

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Background: Ghrelin is the most common orexigenic hormone which acts as starvation signal by acting on hypothalamic neurons. It acts as adipogenic agent. Increased food intake caused by high levels of ghrelin causes increase fat mass. We investigated the possible beneficial role of ghrelin antagonist on food and water intake in obese and type 2 diabetic mice. **Methods:** This study was carried out at Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, from Jan to Jun 2013. A total of 50 healthy male BALB/c mice were divided into 5 groups. Group I served as control, groups II and III were obese, and group IV and V were diabetic. Group II, III, IV and V were fed high-fat diet for 4 weeks. Group IV and V were given intraperitoneal (IP) injection of streptozotocin to induce type 2 diabetes mellitus. Ghrelin antagonist was injected IP to Group III and V for 6 days. Food and water intake were measured daily. Body weights were measured twice a week and at the end of experiment. Terminal intracardiac blood extraction was done and samples were analyzed for fasting plasma glucose levels. **Results:** Food intake decreased significantly in group III (obese with Ghrelin antagonist) and group V (diabetic with Ghrelin antagonist) as compared to controls after injection of ghrelin antagonist. **Conclusion:** Ghrelin antagonist decreases the food and water intake significantly in obese and type 2 diabetic mice.

Keywords: Ghrelin, Ghrelin antagonist, Obesity, Diabetes, T2DM

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INTRODUCTION

Obesity is a major threat for health.[1] It is associated with many diseases such as diabetes mellitus, hypertension and heart diseases. Sedentary lifestyle and unhealthy eating habits have increased the incidence of obesity to almost double in past two decades.[2,3]

Obesity is associated to type 2 diabetes mellitus characterized by hyperglycaemia, hyperinsulinemia and insulin resistance.[4] In obesity, glucose transporter type 4 lose their function leading to increase glucose resistance.[5] Insulin resistance can be improved by weight reduction, thus improving the glucose homeostasis in the body.[6]

Ghrelin is a 28 amino acids peptide produced from oxyntic cells in stomach. It was extracted from the stomach of the rats for the first time in 1999.[7] It is known to stimulate hunger. The ghrelin receptors, which are G protein coupled receptors, are present in many tissues including, stomach, pancreas, pituitary gland, intestines, thymus, gonads and thyroid glands.[8]

Ghrelin increases the appetite by stimulating the neurons in hypothalamus. The orexigenic effect of ghrelin is through NPY and the levels of mRNA for NPY were increased after ghrelin secretion.[7] This orexigenic effect of ghrelin was demonstrated in mice, which showed weight gain after ghrelin administration.[9]

D-Lys^3 GHRP6 (His-D-Lys-Trp-D-Phe-Lys-NH_2) is a ghrelin receptor antagonist. It antagonizes the actions of ghrelin by acting on ghrelin receptor GHS-R1a. There is some weak evidence in literature that shows ghrelin antagonist decreases appetite and causes weight reduction.[9] This study was designed to find out the probable role of ghrelin receptor antagonist (D-Lys^3) in obese and diabetic mice, and to see its effects on appetite, body weight and blood glucose levels.[9,10]

MATERIAL AND METHODS

This study was conducted at Department of Physiology, Army Medical College, Rawalpindi, National University of Science & Technology (NUST) in collaboration with National Institute of Health (NIH), Islamabad, Pakistan. A formal approval was taken from animal ethics committee of Army Medical College/NUST before starting experiment. All animals were handled with due care.

Ghrelin antagonist and Streptozotocin (STZ) were obtained from Sigma Chemicals and Bioplus Fine Research Chemicals, USA respectively. Glucometer (On Call EZ II glucometer Catalogue No. G113-152, Acon Laboratories, inc., USA) was used to measure serum glucose levels. Food and water was measured daily with the help of electrical balance.

The study was conducted on 5 weeks old, 50 healthy BALB/c male mice obtained from animal house of NIH, Islamabad. Average weight of mouse was 28.07±0.1 g. Mice were divided into 5 equal groups. They were given free access to food and water. The room was well ventilated with controlled

temperature range of 20–22 °C and 12 hours light and dark cycles were maintained.

After acclimatization the animals were randomly divided into 5 equal groups. Group 1 (control) was fed normal pellet diet, while group II–V were given high fat diet *ad libitum* (to induce obesity and insulin resistance) for 4 weeks. After 4 weeks, group IV and V were given intraperitoneal injections of streptozotocin 50 mg/Kg body weight for 5 days, to induce type 2 diabetes. Streptozotocin induces rapid and extensive degradation of cellular deoxyribonucleic acid (DNA) of beta cells of pancreas.[11,12] All other groups were injected normal saline intraperitoneally (IP).

Tail vein blood samples were used to measure fasting blood glucose levels and to confirm diabetes. The blood glucose level >252 mg/dl was taken as the cut-off value for confirming diabetes mellitus.[12] This was followed by intraperitoneal injection of ghrelin antagonist 10 μmol/Kg for 6 days[13] to group III and V. At the end of 8th week, terminal blood samples were collected by intracardiac puncture. Blood was collected and serum was separated by centrifugation at 3,000 rpm.[14] The samples were stored at -20 ºC for analysis.

Data was analysed using SPSS-20. Results were expressed as Mean±SD. The statistical significance of differences across the groups was determined by applying Post-hoc ANOVA and Tukey's HSD to find the difference in various pairs of groups and $p<0.05$ was considered statistically significant.

RESULTS

The average weight of the mice was 28.07±0.10 gm. At the start of experiment the body weight of mice in all groups were nearly same without any significant difference. The mice continue to gain weight throughout the study period.

Significant weight reduction was observed in group III (obese mice treated with ghrelin antagonist) as compared with group II (obese mice) (p=0.00). Similarly, significant weight reduction was also observed in group V (diabetic mice treated with ghrelin antagonist) as compared to group IV group (diabetic) (p=0.00).

There was no significant difference in the blood glucose level at the start of the experiment. After 8 weeks of study fasting blood glucose levels were significantly high in group IV and group V compared with the group I (control) (p=0.00). There was no significant difference between other groups.

Blood glucose level in terminal blood samples were raised in group II (obese mice) as compared to the Group I and group III (p=0.00) Significant difference in blood glucose was also seen in group IV in comparison to Group I and V (p=0.00) Ghrelin antagonist significantly reduced the blood glucose levels in group III and Group V compared with group II and group IV respectively showing beneficial role of ghrelin antagonist in reducing blood glucose levels in obese and diabetics.

After 8 weeks, and at the end of the experiment, significant difference in food intake was observed between control group (group I) and study groups (II, III, IV and V). Difference in food intake among group II and III was significant (p=0.00) while insignificant among group IV and V, thus indicating Ghrelin antagonist significantly reduced the food intake in obese, but in diabetic groups the use of ghrelin antagonist did not cause any decrease in food intake.

After 8 weeks there was no significant difference in water intake between group I and group II, III, IV and V, while there was a significant difference between group III and IV. However At the end of the experiment there was significant difference between water intake in group II as compared to group III and IV (p=0.033 and p=0.045 respectively) and between group IV and Group V (p=0.011) thus indicating Ghrelin antagonist had significant effect on water intake in diabetic mice (Table-1 & 2).

Table-1: Comparison of body weight, blood glucose levels, food and water intake in Group I, II and III

Parameter	Time	Control Group I	Obese Group II	*p*	Obese with ghrelin antagonist Group III	*p*
Weight of mice (Grams)	Start of experiment	28.1±0.27	28.09±0.45	0.997	28.18±0.21	0.982
	At 8th week	32.57±1.80	37.78±0.14	0.00	38.35±0.53	0.00
	At end	32.81±1.56	39±0.68	0.00	36.7±0.62	0.00
Blood glucose (mg/dl)	Start of experiment	97.2±11.87	101.89±16.07	0.988	107.70±13.74	0.537
	At 8th week	100.2±9.59	116±11.05	0.139	118±10.91	0.052
	At end	92.6±10.17	169.9±9.8	0.00	103±7.3	0.443
Food intake (gm/day)	Start of experiment	5±0	5.5±0.09	0.00	6.4±0.11	0.00
	At 8th week	6.46±0.13	7.17±0.24	0.00	7.15±0.16	0.00
	At end	6.52±0.16	7.17±0.22	0.00	6.85±0.21	0.004
Water intake (ml/day)	Start of experiment	5.21±0.17	5.31±0.11	0.00	6.54±0.17	0.00
	At 8th week	7.8±0.16	7.8±0.17	1.00	7.74±0.25	0.955
	At end	7.89±0.13	7.87±0.15	1.00	7.65±0.16	0.023

Table-2: Comparison of body weight, blood glucose levels, food and water intake in Group I, IV and V

Parameter	Time	Control Group I	Diabetic Group IV	*p*	Diabetic with ghrelin antagonist Group V	*p*
Weight of mice (Grams)	Start of experiment	28.1±0.27	27.91±0.38	0.692	28.07±0.38	0.997
	At 8th week	32.57±1.80	37.87±0.21	0.00	37.83±0.19	0.00
	At end	32.81±1.56	41.2±1.48	0.00	37.21±0.37	0.00
Blood glucose (mg/dl)	Start of experiment	97.2±11.87	105.90±0.93	0.702	109.67±17.25	0.482
	At 8th week	100.2±9.59	383.6±17.06	0.00	395.67±20.73	0.00
	At end	92.6±10.17	488.8±17.19	0.00	257.56±20.09	0.00
Food intake (gm/day)	Start of experiment	5±0	6.11±0.09	0.00	6.3±0.07	0.00
	At 8th week	6.46±0.13	7.04±0.21	0.00	7.11±0.18	0.00
	At end	6.52±0.16	7.06±1.78	0.00	6.84±0.18	0.004
Water intake (ml/day)	Start of experiment	5.21±0.17	6.54±0.17	0.00	6.54±0.18	0.00
	At 8th week	7.8±0.16	8±0.20	0.151	7.9±0.19	0.768
	At end	7.89±0.13	8.1±0.25	0.062	7.85±0.13	0.960

DISCUSSION

This study highlighted the association between ghrelin antagonist and food intake with obesity and type II diabetes. Ghrelin, a gastrointestinal hormone, is proven to have a strong influence on food intake. It causes hyperphagia, weight gain and increase in plasma glucose levels.[10]

Continuous weight gain among mice was seen during the experiment showing that the nutritional status of the animals was good. Obesity was confirmed by significant increase in weight gain among experimental groups as compared with the control. Diabetes type II and insulin resistance was also confirmed by fasting blood glucose levels.[12]

Ghrelin is a recently identified potential regulator of energy homeostasis. Previous studies show that it increases the appetite and thus cause weight gain and increase blood glucose levels. On the other hand, Ghrelin antagonist causes a reversal of these effects, causing a decrease in food intake and weight gain. Our results supported the previous studies in animal models where ghrelin antagonist decreased food intake in normal and obese mice. Our study showed that the ghrelin antagonist decreases the food intake by 3.21% among diabetic mice as compared to the mice that were not treated with the drug.

Our study is akin to a study by Dong J *et al*[15] who experimented on diabetic ghrelin knockout mice. Their results showed no increases in food intake in diabetic ghrelin knockout mice while the wild type obese mice showed hyperphagia, increased plasma ghrelin levels and increased in number of neuropeptide NPY. When these mice were treated with ghrelin antagonist (D-Lys3-GHRP-6) their food intake reduced by23%.[15]

Esler *et al*[16] have reported that daily oral administration of a GHS-R1a antagonist in diet of obese mice led to reduced food intake and weight loss (up to 15%) due to selective loss of fat mass. Pair-feeding experiments indicated that weight loss was largely a consequence of reduced food intake. Male C57BL/6 mice fed on high fat diet showed 28% reduction in food intake by 10th day of ghrelin antagonist administration. These findings are comparable to our study where the food intake was decreased by 4.5% in obese BLAB/c mice treated with ghrelin antagonist. The percentage decrease in our experiment was less, possibly due to reason that we use ghrelin antagonist for a period of 6 days rather than 10 days.[16]

In an another experimental study ghrelin antagonist was used IP at a dose of 200 ηmol/mouse for 7 days showed decreased food intake by 0.56 g/day as compared to saline treated group. In the same experiment it was seen that there was reduction in weight gain in ghrelin antagonist treated group as compared to saline treated group. The weight gain in ghrelin antagonist treated group was only 0.18 g/day as compared to saline treated group, in which the weight gain was 0.39 g/day. Our results were analogous to their findings as there was 0.32 g/day reduction in food intake and 5.9% reduction in weight gain in ghrelin antagonist treated group as compared to control group.[9]

We observed that ghrelin antagonist reduced the water intake in both obese and diabetic mice. These finding were similar to the results of another study done by Gomez *et al*[17] in which ghrelin antagonist when injected at a dose of 15 mg/Kg in male C57BL/6 mice showed reduction in water intake with a rapid tolerance. There was no further decrease in their animals with the same treatment.[17]

In a study[18] ghrelin at dose of 0.5 μg was injected to the animals. The food intake in them was increased but there was no significant change in water intake. The possible reason for that could be the difference in specie. Secondly the decreased water intake in our study could be ancillary to decreased food intake.

CONCLUSION

The study reveals the beneficial effect of ghrelin antagonist in decreasing food and water intake in obesity and type 2 diabetes mellitus, thus instigating weight reduction and improving glucose homeostasis.

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