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Associating serum ghrelin with some indicative markers of type 2 diabetic in healthy obese men

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ABSTRACT

Ghrelin is a gastrointestinal neuropeptide which plays an important role in appetite, food absorption and body weight regularization. In this study we intended to found serum ghrelin in relation to indicative markers of glucose levels in non-diabetic obese men. Forty eight obese sedentary males (age, 36 +/- 7 yr, BMI, 32.1 +/- 3.11 kg/m^2) were enrolled to the study by voluntarily. Blood samples were obtained after a 12-hour overnight fast for measuring serum ghrelin, glucose, insulin of studied subjects. Homeostasis model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA-BF) was calculated by fasting insulin and glucose. A Pearson method used for determine relation between serum ghrelin with glucose and the other diabetic determinatives. The data of person analysis showed that there was no significant relationship between ghrelin with glucose and other diabetic inductive variables such as insulin, insulin resistance and beta cell function. In conclusion we can say that fasting glucose and the other type 2 diabetic determinatives does not affect by serum ghrelin in healthy obese males.

Keywords: Obesity, Type 2 diabetic, Serum ghrelin

INTRODUCTION

Nowadays, obesity has been known as public health problem and is a cluster of related risk factors for metabolic syndrome and type 2 diabetes [1]. Obesity phenomenon is raised due to some genetic and environmental factors. Ghrelin is one of the circulating peptides which stimulates appetite and also regularizes energy balance, and has been recognized as one of the candidates of prevalence of obesity and type 2 diabetes [1]. This peptide hormone is a novel acylated 28-amino acid peptide which is mainly secreted by the stomach [2] and stimulates the growth hormone (GH) secretagogue-receptor (GHS-R) [2]. Plasma ghrelin levels rise progressively before meals and fall to a nadir within 1 hour after eating [3, 4]. Extensive studies have mentioned that ghrelin has a role in the development of metabolic syndrome and type 2 diabetes. [5]. Studies on obese diabetic patients have shown that blood ghrelin levels play important role in regulating insulin and glucose metabolism. [1].

These studies suggest that ghrelin reduces insulin secretion from beta cells in obesity-induced type 2 diabetic [6]. On the other hand, some studies on diabetic patients also suggest that the ghrelin concentration is similar in both obese and thin type 2 diabetic patients [7]. It has also been revealed that the acute ghrelin consumption increases plasma glucose levels by down regulation of insulin, which somehow notes the interaction of ghrelin, with blood insulin or glucose [1]. However, whether blood insulin or glucose along with serum ghrelin in obese or non-obese individuals, have stimulating or inhibitory roles towards one another has not been fully confirmed yet and there is no general consensus in this regard. So that, despite the findings above, in another study, increase of blood ghrelin levels due to its intravenous injection did not result in a significant change in glucose and fasting insulin levels [8]. This finding is

also supported by some other studies [9] and suggests no correlation between blood ghrelin levels and glucose and its determinant components.

Clinical studies support the role of ghrelin in regulating glucose metabolism a well as energy balance and insulin secretion in animal models [9], but the effect of ghrelin on insulin secretion and blood glucose levels in humans is not completely understood and most studies on humans are mostly related to determine the relationship between systemic ghrelin levels and mentioned variables in subjects with glucose disorders such as obese diabetic patients. The question is whether there is also a significant relationship between serum ghrelin levels and components determining blood glucose levels in non-diabetic obese healthy individuals. In this regard, some studies also state that the change in systematic ghrelin levels due to different glucose levels is related to the factors such as gender, obesity type and insulin resistance [10]. Thus, the present study is conducted to determine the relationship of baseline levels of serum ghrelin with insulin and fasting glucose as well as insulin resistance and beta cells function in obese male adults.

MATERIALS AND METHODS

The studied population of this correlation study are forty-eight none-diabetic middle-aged (36 ± 7 years mean ± standard deviation of mean (SDM)) obese (BMI=32.1±3.11 kg m−2) men that participated by voluntarily. All participants were informed verbally and in writing, as to the objectives of the experiments, together with the potential associated risks. All participants signed an informed consent document approved by the Human Research Ethics Committees of Azad University, Iran.

Exclusion Criteria: Participants were included if they had not been involved in regular physical activity/diet in the previous 6 months. Subjects were reported to be non-smokers, not currently taking supplements of any kind, and having no major health problems (i.e., diabetes, cardiovascular disease, etc.). All subjects had a body mass index (BMI) of upper than 30. Those with type 2 diabetic were excluded from the study. In addition, exclusion criteria included supplementations that alter carbohydrate or fat metabolism. Those that were on medications known to alter insulin sensitivity were excluded. Subjects were instructed to refrain from caffeine consumption and intense physical activity for 24 h before testing.

Anthropometric measurements: The weight and height of the participants were measured by the same person when the participant had thin clothes on and was wearing no shoes by using the standard hospital scales. The Body Mass index (BMI) was calculated using the formula body weight/height2 in terms of kg/m². Waist circumference was measured half distance between the lower border of the last rib and the upper border of the iliac crest at the end of a normal expiration, using a non-stretchable tape measure. The arterial systolic and diastolic blood pressures (BP) were calculated after they rested for 10 minutes with a mercury manometer with appropriate sleeves from the right and left arm, in sitting position on the condition that they had not eaten anything, had not taken any caffeine, had not smoked or exercised thirty minutes before the measurement, and then the averages were calculated.

Laboratory Analyses: Fasting blood samples were taken after an overnight fast to determine ghrelin, glucose, insulin, lipid profiles. For serum ghrelin measuring, blood samples were centrifuged at +4 degree centigrade for 10 minutes by 4500 rpm speed for serum separation. The intra-assay and inter-assay coefficient of variation of ghrelin (Biovendor, Austria) were 8.10% and 8.3% respectively. Glucose was determined by the oxidase method (Pars Azmoun, Tehran, Iran). Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured using the colorimetric enzymatic method by Kobas Auto-analyzer (German). Serum insulin was determined by ELISA method (Demedite, German). The Intra- assay coefficient of variation and sensitivity of the method were 2.6% and 2.88 µg/L, respectively. Insulin resistance index as well as beta cells function index was calculated using fasting insulin and glucose levels in each subject [11].

Statistical analysis: Statistical analysis was performed with the SPSS software version 16.0 using a Pearson correlation method to determine the relationship between ghrelin with glucose, insulin, insulin resistance and beta cells function. A p-value < 0.05 was considered to be statistically significant.

RESULTS

In the present study, the relationship between serum ghrelin levels and markers indicatives of type 2 diabetes was assessed in non-diabetic middle-aged obese men. The average body fat percentage of the study subjects was estimated as 30.2(%). The average body mass index of subjects was also 32.1 kg/m2. Measuring fasting blood glucose levels indicated that none of the subjects have type 2 diabetes. Mean and standard deviation of anthropometrical and biochemical variables of subjects are shown in Table 1. Findings from statistical spearman

correlation method showed that baseline serum ghrelin levels of the subjects is not related to fasting glucose concentrations (p = 0.263, r = 0.13). In other words, changes in serum ghrelin levels are independent of blood glucose of the subjects. Also, no significant relationship was observed between insulin resistance index and serum ghrelin in the subjects (p = 0.234, r = 0.19). Results showed that although the relationship between serum ghrelin levels and beta cells function index is reverse or in other words, an increase in serum ghrelin levels is associated with a reduction in beta cells function, this relationship is not statistically significant (p = 0.063, r = 0.26). On the other hand, no significant relationship was also observed between concentration of serum ghrelin and serum insulin which is of the other determinants of type 2 diabetes (p = 0.291, r = 0.33). This means that serum ghrelin levels do not affect insulin secreted by pancreatic beta cells.

Table 1: Mean and standard deviation of the descriptive anthropometric and biochemical features of the studied subjects

Variable	Mean	SD	Range
Age (years)	36	7	33 – 43
Weight (kg)	98	11	92 – 111
Height (cm)	175	7	168 – 181
Abdominal circumference (cm)	104	16	96 – 128
Hip circumference (cm)	105	14	98 – 125
Waist to hip circumference ratio (WHO)	0.99	0.08	0.95 – 1.o7
Body mass index (kg/m^2)	32.01	3.11	29.6 – 34.6
Body fat percentage (%)	32	5	29 – 36
Fasting blood glucose (mg/dl)	100	14	89 – 114
Insulin (µIU/ml)	8.44	2.67	5.68 – 11.o1
Ghrelin (µg /ml)	66	23	47 – 88
Systolic blood pressure (mmHg)	12.78	2.14	11.05 – 14.43
Diastolic blood pressure (mmHg)	8.83	0.98	8.11 – 10.01
Cholesterol (mg/dl)	188	44	142 – 213
Triglyceride (mg/dl)	168	53	133 – 234
LDL (mg/dl)	111	59	99 - 176
HDL(mg/dl)	44	6	41 – 51
WBC	6648	418	5800 – 7631
RBC	5.11	0,89	4.18 – 6.12
HB	15.12	2.34	13.63 – 17.32
HCT	46.64	6.14	40.21 – 50.42
PLT	314356	67342	278321- 333543
LYMPH%	39.44	5.21	34.21 – 43.12

DISCUSSION

Ghrelin, leptin and adiponectin are three hormones which are frequently related with metabolism, obesity and appetite [12]. The mechanisms by which ghrelin regulates insulin secretion or glucose levels is not fully understood. Ghrelin supplementing increases obesity prevalence due to its appetizing feature [13] but the specific mechanisms responsible for these observations are not obvious. The findings of the present study regarding interaction between ghrelin and glucose in obese healthy people are unlike observations on obese diabetic patients. So that, this study showed that there is no significant relationship between serum ghrelin and fasting glucose in health obese subjects. In other words, in this population, changes in blood glucose concentrations are independent of changes in systemic ghrelin levels. Hence, it seem that changes in ghrelin levels of obese subjects is more related with other components of obesity prevalence such as hunger stimulation and appetite increase rather than with glucose metabolism. In this regard, some other studies did not observed significant correlation between ghrelin and blood glucose levels [8, 9]. Our study also showed that a serum ghrelin level is not related with insulin resistance in these subjects. It seems that one of the reasons for the lack of relationship between glucose and serum ghrelin is rooted in lack of relationship between ghrelin and serum insulin. Because, decrease or increase in insulin secretion is one of the major causes of the changes in blood glucose. In this regard, no significant relationship was observed between ghrelin and serum insulin levels in these subjects.

There is Limited information regarding the relationship of ghrelin effect on beta cells function in human populations. Despite the conducted studies, the role of ghrelin in insulin secretion and glucose homeostasis is not completely recognized yet. Although some studies have revealed that the ghrelin injection leads to insulin secretion inhibition [14, 15, 16], and have somehow mentioned the role of ghrelin in beta cells dysfunction. Some other has reported the increase of insulin secretion from pancreatic beta cells subsequent with ghrelin injection [17, 18, and 19]. But Contrary to these results, our study has not indicated any significant correlation between ghrelin levels and beta cell function, and suggests that beta cells function in healthy obese people is independent of changes in systemic ghrelin levels of these subjects. These findings are different with the results of other studies on diabetic patients in which beta cells dysfunction is one of the effective components in increase of blood glucose in them. In this regard, the

findings of another study did not show a significant correlation between ghrelin and insulin levels either [20]. Altogether, although the absence of control groups of diabetic patients group is one of the limitations of this study, comparing the findings of this study on healthy individuals regarding the relationship of serum ghrelin levels with blood glucose and other components determining glucose levels is different with the results of other studies on diabetic patients. It seems that the role of ghrelin in obese non-diabetics affect energy balance more due to appetite increase and food absorption and interaction between ghrelin and glucose levels and other effective components appears in diabetic patients.

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