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GHRELIN LEVEL CHANGES IN HUMANS WITH TYPE 2 DIABETES MELLITUS AND OBESITY

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გრელინის რაოდენობრივი ცვლილებები შაქრიანი დიაბეტი ტიპი 2-ით და სიმსუქნით დაავადებულ ადამიანებში

შ.პ.ს ენდოკრინოლოგიის ეროვნული ინსტიტუტი;
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რეზიუმე

კვლევის მიზანს წარმოადგენდა გრელინის სეკრეციის თავისებურებების შესწავლა ადამიანებში შაქრიანი დიაბეტი ტიპი 2-ით და კორელაციური კავშირების დადგენა გრელინის დონესა და მნიშვნელოვან მეტაბოლურ ფაქტორებს შორის შაქრიანი დიაბეტი ტიპი 2-ისა და სიმსუქნის დროს.

მასალები და მეთოდები: კვლევაში ჩართულ იქნა 30 პაციენტი შაქრიანი დიაბეტი ტიპი 2-ით და სიმსუქნით, აგრეთვე 10 მოხალისე საკონტროლი ჯგუფიდან. საკვლევი ჯგუფი სხეულის მასის ინდექსის მიხედვით დაიყო 3 ქვეჯგუფად: I ჯგუფი - ნორმალური წონის ან ჭარბი წონის მქონე პირები შდტ2-ით; II ჯგუფი - I ხარისხის სიმსუქნის მქონე პირები შდტ2-ით; III ჯგუფი - II ხარისხის სიმსუქნის მქონე პირები შდტ2-ით. განისაზღვრა პლაზმაში გრელინის კონცენტრაცია შდტ2-ისა და სიმსუქნის მქონე საკვლევ პირებში და ასევე საკონტროლო ჯგუფში. საკონტროლო ჯგუფში სუბიექტებს ჰქონდათ ნორმალური წონა და არ აღენიშნებოდათ შდტ2. კვლევის დროს ერთდროულად განისაზღვრა სისხლში პლაზმური გრელინის კონცენტრაცია, ლიპიდური ცვლა, HbA1c -ის დონე.

შედეგები: კვლევის შედეგად სტატისტიკურად მნიშვნელოვანი კორელაცია იქნა ნანახი გრელინის დონესა და სხეულის მასის ინდექსს, HBAlc-ს, HDL-სა და საერთო ქოლესტერინის დონეებს შორის.

დასკვნა: ჩატარებულ კვლევით დადასტურდა პლაზმური გრელინის ნორმალური დონის ცვლილება ადამიანებში შდტ2-ის და და სიმსუქნის სხვადასხვა ხარისხის დროს. რადგან პლაზმური გრელინის დონე იკლებს შდტ2-ისა და სიმსუქნის მქონე პირებში, სავარაუდოდ გრელინს აქვს დიაბეტოგენური მოქმედება, სხეულის წონაზე მისი ზემოქმედებისგან დამოუკიდებლად. ამავდროულად ექსპერიმენტულ მოდელებზე კვლევამ აჩვენა გლუკოზის ტოლერანტობის ტესტის გაუმჯობესება გრელინის სისტემის მოქმედების ბლოკადის შემდეგ. ამ აღმოჩენებმა ახალი აქტუალობა შესძინა გრელინს და გვაფიქრებინებს მის აქტიურ მონაწილეობაზე კვებით ქცევისა და ენერგეტიკული ჰომეოსტაზის რეგულირებაში. ერთად აღებული, ეს შედეგები მიუთითებს, რომ შესაძლოა არსებობდეს სისტემა გრელინის წარმომქმნელ უჯრედებში, რომელიც რეაგირებს მეტაბოლურ ცვლილებებზე შდტ2-ის და სიმსუქნის დროს. მოლეკულური სიგნალები, რომლებიც არეგულირებენ გრელინის სეკრეციას, კარგად არ არის ცნობილი. საჭიროა შემდგომი კვლევები, რათა უფრო დეტალურად იქნას შესწავლილი გრელინის რეცეპტორები, გადამტანები და ის მემბრანული არხები რომლებიც ლოკალიზებულია გრელინ წარმომქმნელ უჯრედებში.

INTRODUCTION

Ghrelin is a 28-amino-acid peptide predominantly secreted in the stomach and stimulates appetite and growth hormone (GH) release. The GH-releasing effect of the ghrelin occurs through the direct effect of ghrelin on pituitary somatotroph cells, synergistic effect with GHRH and through stimulation of vagal afferents [1]. In high doses, ghrelin may also stimulate prolactin, corticotropin and cortisol secretion in humans [2].

Ghrelin is the only known orexigenic gut peptide. The pre-prandial elevation of ghrelin levels and its fall after meals led to the notion that ghrelin was a 'hunger' hormone responsible for meal initiation.

Ghrelin is involved in short-term regulation of food intake and long-term regulation of body weight through decreasing fat utilization [3]. The effect of ghrelin on feeding is mediated through the GHS-R1a, as indicated by the lack of its orexigenic effect in GHS-R knocked out mice [4]. GHS-R1a is highly expressed in hypothalamic cell populations that regulate feeding and bodyweight homeostasis [5].

The detection of ghrelin receptors on vagal afferent neurons in rat suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve [1].

Much effort has been directed at studying the actions of the endogenous ghrelin system on body weight, adiposity, and energy expenditure. Although endogenous ghrelin's actions on some of these processes remain ambiguous, its glucoregulatory actions have emerged as well-recognized features during extreme metabolic conditions, such as diabetes mellitus. The blood glucose–raising actions of ghrelin are beneficial during starvation-like conditions, defending against life-threatening falls in blood glucose, but they are seemingly detrimental in obese states and in certain forms of diabetes, contributing to hyperglycemia.

Some studies in rodents have demonstrated the acute blood glucose–raising actions of administered ghrelin [6]. Ghrelin administration also worsens glucose tolerance in rodents [7,8,9]. Increases in circulating ghrelin levels have consistently been noted to raise blood glucose and worsen glucose tolerance. Conversely, administration of GHSR or GOAT antagonists lowers fasting blood glucose and improves glucose tolerance in mice.

According to a number of studies on experimental animals, caloric restriction increases plasma ghrelin, whereas food ingestion causes a rapid fall in plasma ghrelin in healthy rodent models [10,11,12]. Oral administration of individual dietary macro-nutrients, including glucose, amino acids and fatty acids, each can suppress plasma ghrelin [10,13].

Glucose causes a rapid and profound suppression of plasma ghrelin in humans and rodents when administered by either parental or oral routes [14,15,16].

Reduction of plasma ghrelin by blood glucose in healthy subjects is likely a result of several factors that include direct inhibition of ghrelin secretion by glucose taken up by and metabolized within ghrelin cells, actions of gluco-regulatory hormones including insulin and glucagon that change with the administration of glucose, and potential attenuation of the sympathetic drive to ghrelin cells. It is likely that a direct effect of glucose on ghrelin cells figures substantially in the overall inhibitory effect of glucose on ghrelin secretion. The exact metabolic pathways and molecular mechanisms within the ghrelin cell responsible for modulating ghrelin secretion are unclear.

The suppression of ghrelin secretion with glucose administration is also likely mediated by changes to circulating levels of glucose-sensitive hormones, including insulin and glucagon. Experimental evidence suggests that insulin can act independently of glucose to affect plasma ghrelin and/or ghrelin secretion. Also, a bolus injection of insulin to induce hyperinsulinemia suppressed plasma ghrelin in rodents, regardless of whether individuals were maintained in hypoglycemic or normoglycemic ranges with concurrent glucose infusion [15,17]. Insulin can inhibit ghrelin secretion from mouse and neonatal rat gastric mucosal cell primary cultures when the glucose concentrations in the culture medium are in the hypoglycemic or normoglycemic range [18,19].

Several studies support a model in which preprandial increases in plasma ghrelin prime intestinal L-cells to stimulate nutrient-induced enhancement of GLP-1 secretion [20]. Ghrelin- induced increases in GLP-1 secretion improve glucose tolerance in mice [20]. These studies suggest that the meal-induced increase in GLP-1 might mitigate ghrelin's postprandial actions in suppress insulin secretion from pancreatic b-cells. More broadly, these studies suggest that the overall effects of the ghrelin system on postprandial glucose turnover are determined by a complex interplay of several potential downstream effectors that include ghrelin and GLP-1, which have opposing actions on insulin secretion, food intake, and gastric emptying.

Ghrelin is now thought to play a significant role in the regulation of lipid storage in white adipose tissue (WAT). Although acute ghrelin exposure also induces GH secretion, the net effect of prolonged ghrelin exposure is increased fat mass. Ghrelin has been reported to enhance adipogenesis, augment fat storage enzyme activity, elevate triglyceride content and reduce fat utilization/lipolysis [21,22,23].

Evidence has demonstrated that the administration of peripheral ghrelin increases WAT mass in selective abdominal depots (retroperitoneal and inguinal) via a decrease in lipid export rather than a decrease in lipolysis per se. Thus, during periods of energy insufficiency, ghrelin may prevent lipid loss from responsive adipocytes thereby permitting depot-specific utilization of energy reserves. It was also found that ghrelin-induced lipid accumulation is not specific to WAT, as exogenous ghrelin markedly increased the number of lipid droplets in the livers of treated rats and mice, an effect mediated by direct activation of its receptor on hepatocytes. Ghrelin receptor antagonism or gene deletion significantly decreased obesity-associated hepatic steatosis by suppression of *de novo* lipogenesis [23,24].

As the experiments on animal models showed the significance of ghrelin changes during metabolic processes, the identification of ghrelin levels in diabetic patients with different degrees of obesity and their correlation with several metabolic parameters, was considered clinically important.

The aim of the research was to identify the plasma ghrelin concentration in humans with Type 2 Diabetes mellitus and obesity and to investigate a statistically significant relation between plasma ghrelin levels and other important metabolic factors in Type 2 Diabetes mellitus and obesity such as lipid panel, HbA1c and BMI.

SUBJECTS AND METHODS

Subjects. The study involved 30 volunteer patients from the National Institute of Endocrinology with Type 2 Diabetes Mellitus and 10 healthy volunteers from the control group. The following groups were studied: 10 healthy controls (5 men and 5 women; mean body mass index (BMI)-22.7±0.4 kg/m²); 10 patients with T2DM and normal weight or overweight (3 man and 7 women; BMI 24,9±1.9 kg/m²); 10 patients with T2DM and obesity Class I (5 men and 5 women; BMI-32.9± kg/m²); and 10 patients with T2DM and obesity Class II (4 men and 6 women; BMI-36.9±1.8 kg/m². Among the diabetic patients, all were treated with diet, exercise and oral hypoglycemic agents. All subjects were clinically stable at the time of the evaluation and had no evidence of gastrointestinal disease or cachectic states, such as cancer, thyroid disease, liver disease, or infection. Patients with renal dysfunction (eGFr < 90ml/min/1.73m²) and with acute cardiovascular diseases were excluded.

Protocols. Blood was collected at 08:00h after an overnight fast. Plasma HbA1c levels and Plasma Lipid Panel were measured using a BioSystems ABL 80 FLEX, BIO-RAD, HUMAN analyzer.

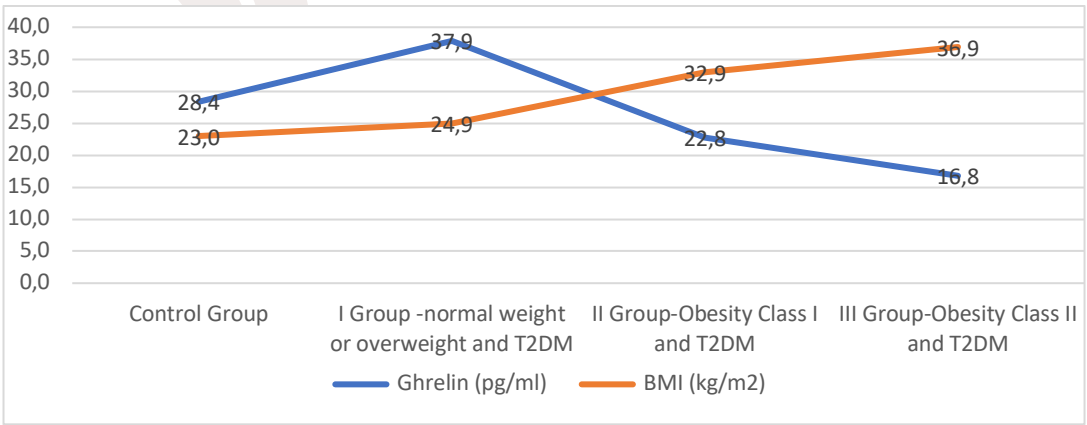
Ghrelin levels were tested using the human Ghrelin ELISA Kit, which is based on sandwich enzyme-linked immuno-sorbent assay technology and quantitates human Ghrelin in the serum and plasma.

Statistical analyses. Groups of data were compared using ANOVA test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

According to the results of this study, statistically significant negative correlations were observed between plasma ghrelin levels and BMI (Table 1.).

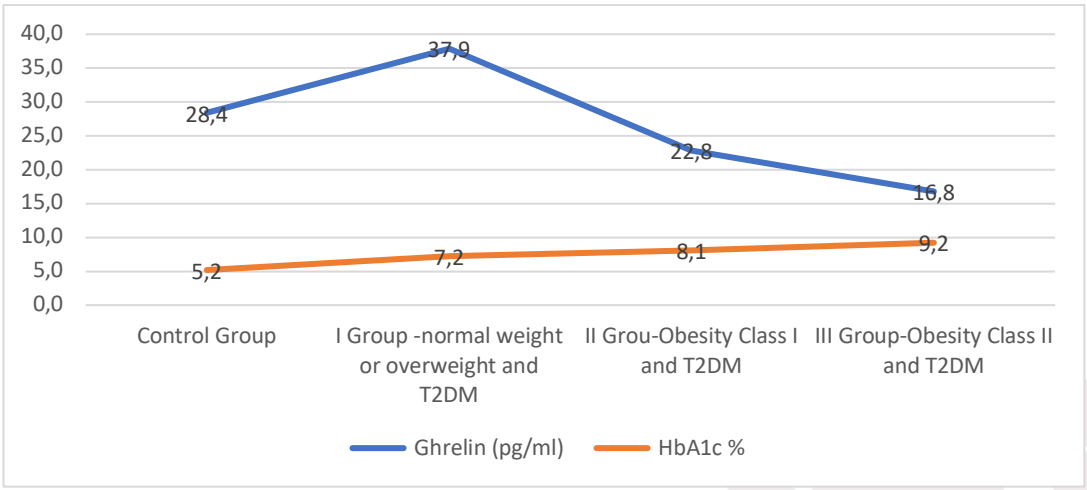
Table 1. Ghrelin level correlation with BMI



In the diabetic group, the decrease in ghrelin levels was accompanied by the increase of body mass index, compared to the control group.

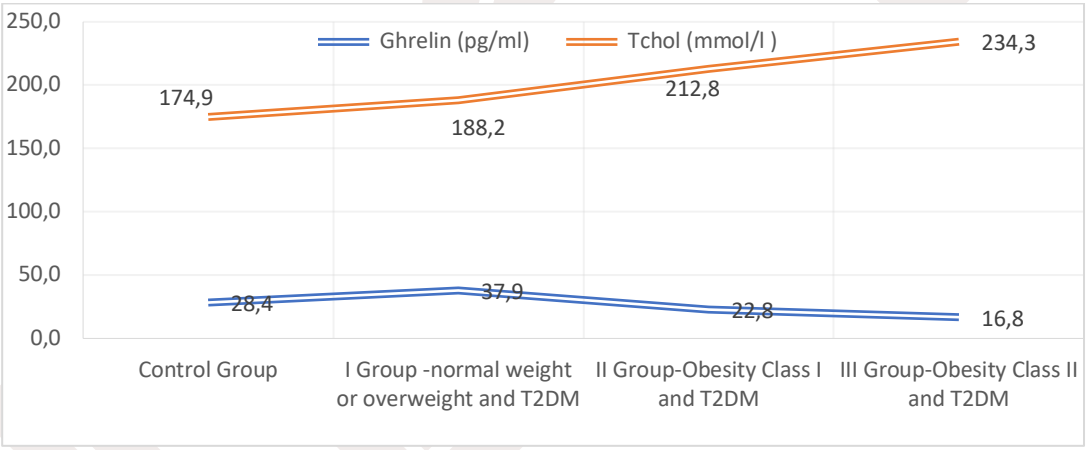
A statistically significant negative correlation was observed in the diabetic group between ghrelin levels and HbA1c (Table 2). An increase of HbA1c levels was accompanied by a decrease of plasma ghrelin concentrations in the diabetic group. But, surprisingly, in normal weight or overweight patients with t2dm, ghrelin levels were higher compared to the control group.

Table 2. Ghrelin level correlation with HbA1c.



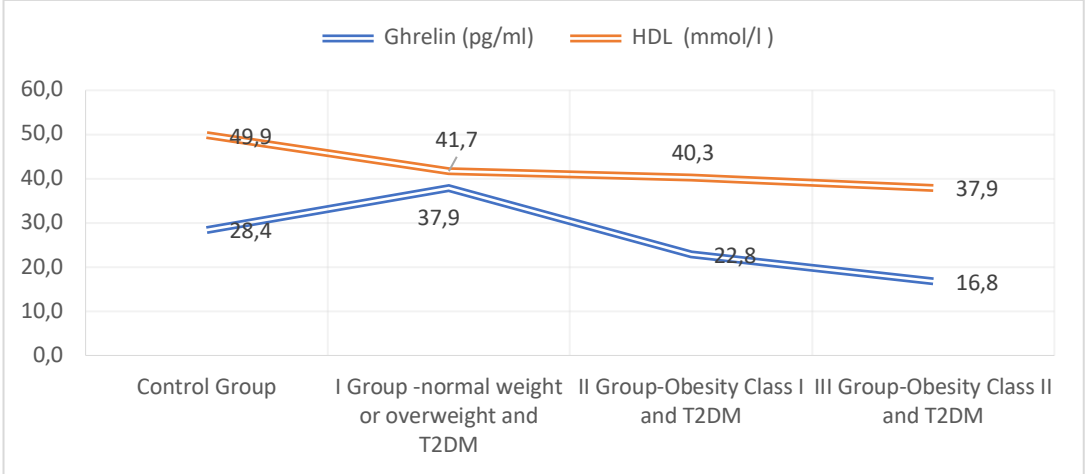
A decrease in ghrelin levels in all subgroups of diabetic patients was accompanied by a total cholesterol level increase compared to the control group (Table 3).

Table 3. Ghrelin level correlation with Total Cholesterol level.



A statistically significant positive correlation was found between ghrelin levels and HDL levels in all subgroups of diabetic patients compared to the control group (Table 4.).

Table 4. Ghrelin level correlation with HDL level.



DISCUSSION

The study showed that plasma ghrelin concentrations were lower in patients with T2DM and obesity compared with normal-weight control subjects. The study result showed that fasting plasma ghrelin concentration negatively correlates with percentage of HbA1c and fasting total cholesterol concentrations. It was also found that fasting plasma ghrelin concentrations in normal subjects and patients with obesity or T2DM correlated negatively with BMI within each group. Because experimental evidence suggests that insulin can act independently of glucose to affect plasma ghrelin and/or ghrelin secretion, and because of the fact that during T2DM and obesity insulin resistance / hyperinsulinemia is one of the common metabolic abnormalities, the probable reason for the decreased level of fasting ghrelin may be considered to be Hyperinsulinemia. But it remains to be seen whether insulin engagement and activation of insulin receptors expressed on ghrelin cells are necessary and sufficient for the ghrelin-suppressing actions of insulin. It also remains to be seen whether increased levels of insulin associated with obesity contribute to the obesity associated reduction in plasma ghrelin.

During T2DM, the levels of incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are frequently found to be insufficient. For this reason, the decreased concentration of ghrelin in the study group may be connected to the decreased levels of GLP-1 during diabetes as several studies support a model in which preprandial increases in plasma ghrelin stimulate nutrient-induced enhancement of GLP-1 secretion in rodents.

According to recent studies on rodents, ghrelin is now thought to play a significant role in the regulation of lipid storage in white adipose tissue, adipogenesis and lipolysis. Identification of ghrelin's negative correlation with total cholesterol levels and positive correlation with HDL levels gives a stimulus to continue future research to find more specific biochemical mechanisms between ghrelin and lipid metabolism.

In this study plasma GH levels were not investigated, but basal plasma ghrelin concentration probably correlates with that of GH.

CONCLUSSION

The present study demonstrates plasma ghrelin changes in humans with Type 2 Diabetes Mellitus (T2DM) and different classes of obesity. As plasma ghrelin levels are lower in individuals with T2DM and obesity, ghrelin appears to have diabetogenic actions, independent of its action on body weight. Research on experimental models showed an improvement of the glucose tolerance test after a blockade of ghrelin system action. These findings shed new light upon the involvement of the novel gastrointestinal peptide, ghrelin, in the regulation of feeding behavior and energy homeostasis.

Ghrelin exerts many physiological roles and is regulated by several factors. Understanding the mechanisms of ghrelin regulation by modifying its secretion, acylation and degradation will provide a better therapeutic benefit of ghrelin, ghrelin mimetics, inverse agonists and ghrelin antagonists. Taken together, these results indicate that there may be a system in ghrelin-producing cells that responds to metabolic changes during T2DM and obesity. Molecular signals that regulate ghrelin secretion are not well known. Further investigation is needed to define the receptors, transporters, and/or channels expressed in ghrelin-producing cells.

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SUMMARY

Purpose: The aim of the research was to identify the plasma ghrelin concentration in humans with Type 2 Diabetes mellitus and obesity and to investigate a statistically significant relation between plasma ghrelin levels and other important metabolic factors in Type 2 Diabetes mellitus and obesity pathogenesis.

Materials and methods: In this study, to investigate the possible involvement of ghrelin in the regulation of metabolic balance, plasma ghrelin concentrations were measured in patients with type 2 diabetes mellitus(T2DM) and obesity. Subjects were divided into two groups; control and experimental group. In the control group subjects had normal weight without T2DM. Subjects from the experimental group were divided into three subgroups, based on their weight: Group I- normal weight or overweight patients with T2DM; Group II – obesity I degree patients with T2DM, and Group III- obesity II degree patients with T2DM. Plasma Ghrelin concentration, lipid panel, HbA1c level were measured at the same time.

Results: A statistically significant correlation was found between ghrelin levels and BMI, HBAlc, HDL and Total Cholesterol levels.

Conclusion: The present study demonstrates plasma ghrelin changes in humans with Type 2 Diabetes Mellitus (T2DM) and different classes of obesity. As plasma ghrelin levels are lower in individuals with T2DM and obesity, ghrelin appears to have diabetogenic actions, independent of its effects on body weight. Research on experimental models showed an improvement of glucose tolerance test after a blockade of ghrelin system action. These findings shed new light upon the involvement of the novel gastrointestinal peptide, ghrelin, in the regulation of feeding behavior and energy homeostasis.

Taken together, these results indicate that there may be a system in ghrelin-producing cells that responds to metabolic changes during T2DM and obesity. Molecular signals that regulate ghrelin secretion are not well known. Further investigation is needed to define the receptors, transporters, and/or channels expressed in ghrelin-producing cells.

Keywords: T2DM; Obesity; Ghrelin; Total Cholesterol; HDL; BMI