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GHRELIN LEVEL CHANGES IN RATS WITH TYPE 2 DIABETES MELLITUS

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Doi:

ნათია ჩხეიძე, ელენე გიორგაძე, მარინა ნიკოლაიშვილი, ანა მალაზონია, ნიკოლოზ ურიდია, მარტა კლდიაშვილი

გრელინის რაოდენობრივი ცვლილებები ვირთაგვებში შაქრიანი დიაბეტი ტიპი 2-ის დროს

შ.პ.ს ენდოკრინოლოგიის ეროვნული ინსტიტუტი;
ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

რეზიუმე

კვლევის მიზანი: კვლევის მიზანს წარმოადგენდა გრელინის კონცენტრაციის განსაზღვრა შაქრიანი დიაბეტი ტიპი 2-ით დაავადებულ ექსპერიმენტულ ცხოველებში, სტრეპტოზოტოცინის მრავალჯერად დაბალი დოზებით ინექციამდე და ინექციის შემდეგ.

მასალა და მეთოდები: ექსპერიმენტი ჩატარდა ვისტარის მამრ ვირთაგვებზე. ცხოველები დაიყო ორ ჯგუფად; საკონტროლო და ექსპერიმენტულ ჯგუფად. საკონტროლო ჯგუფში ცხოველებს ჰქონდათ ნორმალური წონა. ექსპერიმენტული ჯგუფიდან ვირთაგვების წონის მიხედვით ცხოველები დაიყო სამ ქვეჯგუფად: ნორმალური წონის ჯგუფი (I ჯგუფი), ჭარბი წონის ჯგუფი (II ჯგუფი) და სიმსუქნის ჯგუფი (III ჯგუფი). გლუკოზის დონე შემოწმდა 4 კვირის შემდეგ სტრეპტოზოტოცინის მრავალჯერად დაბალი დოზებით ინექციის შემდეგ. ყველა ვირთაგვა, სისხლში უზმოდ გლუკოზის დონით > 14 მმოლ/ლ-ზე, მიჩნეულ იქნა შაქრიანი დიაბეტი ტიპი2-ით დაავადებულად და მათზე გაგრძელდა შემდგომი კვლევები. ყველა ვირთაგვასთან (საკონტროლო და ექსპერიმენტული ჯგუფი), გრელინის დონე შემოწმდა სტრეპტოზოტოცინის მრავალჯერად დაბალი დოზებით ინექციამდე და ინექციის შემდეგ.

შედეგები: გრელინის დონე უფრო მაღალი იყო ნორმალური წონის ვირთაგვებში, ვიდრე სიმსუქნით და ჭარბი წონით. ექსპერიმენტულ ჯგუფში, სტრეპტოზოტოცინის ინექციის შემდეგ (30 მგ/კგ - ორჯერ) გრელინის დონე პროპორციულად გაიზარდა სამივე ჯგუფში.

დასკვნა: ექსპერიმენტის დასაწყისში ვირთაგვებში T2DM-ის გარეშე, გრელინის დონე უფრო მაღალი იყო ნორმალური წონის მქონე ვირთაგვებში, ვიდრე სიმსუქნით და ჭარბი წონით, თუმცა, სტრეპტოზოტოცინის ინექციის შემდეგ (30 მგ/კგ- ორჯერ), გრელინის დონე გაიზარდა პროპორციულად სამივე ჯგუფში.

როგორც ბოლო კვლევებიდან ცნობილია, ფიზიოლოგიურ პირობებში გრელინი აფერხებს ინსულინის სეკრეციას. შაქრიანი დიაბეტი ტიპი 2-ის დროს ყველა ჯგუფში პროპორციულად იმატებს გრელინის დონე. შესაბამისად, შაქრიანი დიაბეტის დროს გრელინის დონის მატება კიდევ უფრო ამცირებს ენდოგენური ინსულინის სეკრეციას, რაც იწვევს გლუკოზის დონის მატებას და აუარესებს დაავადების გამოსავალს. აღნიშნული კვლევის შედეგი ადასტურებს გრელინის დამთრგუნველ ეფექტს ინსულინის სეკრეციაზე.

INTRODUCTION. Ghrelin is a 28-amino-acid peptide predominantly secreted in the stomach and stimulates appetite and growth hormone (GH) release. The name ghrelin is based on 'ghre' a word root in Proto-Indo-European languages meaning 'grow' in reference to its ability to stimulate GH release. In 1976, Bowers and co-workers discovered opioid peptide derivatives that did not exhibit any opioid activity, but had weak GH-releasing activity, and were referred to as GH secretagogues (GHSs). GHSs act on the pituitary and hypothalamus to release GH, not through the GH-releasing hormone receptor (GHRH-R) but through an orphan receptor, the GHS-R. Synthetic GHSs and the GHS-R indicated that an unknown endogenous ligand for GHS-R should exist. In December 1999, Kojima et al. were the first to purify and identify ghrelin from rat stomach as the endogenous ligand for the GHS-R [1].

Based on its structure, it is a member of the motilin family of peptides. When administered peripherally or into the central nervous system, ghrelin stimulates the secretion of growth hormone, increases food intake, and causes weight gain [2]. Level of ghrelin, produced by the stomach, increases

during periods of fasting or under conditions associated with a negative energy balance, such as starvation or anorexia. In contrast, ghrelin levels are low after eating or with hyperglycemia [1,2]. There is growing evidence that ghrelin plays a central role in the neurohormonal regulation of food intake and energy homeostasis.

Ghrelin inhibits insulin release in mice, rats and humans. It has recently been shown that in healthy humans, ghrelin suppresses insulin secretion and elevates blood glucose in intravenous GTT (Glucose Tolerance Test). Conversely, GTT performed in mice showed that insulin responses were markedly enhanced and there were decreases in plasma glucose after simultaneous injection of a GHS-R antagonist [3].

Circulating plasma ghrelin levels decrease immediately after a meal. The meal-induced decrease of ghrelin levels is impaired in subjects with type 2 diabetes mellitus (T2DM), suggesting that the impaired suppression of circulating ghrelin during the meal intake may partly account for the glucose intolerance, as well as ongoing weight gain [4].

The majority of the publications addressing the relationship between ghrelin and insulin resistance and/or diabetic states suggest that a correlation between ghrelin and insulin resistance and/or diabetes mellitus might exist. There is a growing body of evidence indicating a suppressive role of ghrelin in the release of insulin from the pancreatic islets. Recently, Tong et al. (2010) suggested that circulating ghrelin suppresses glucose-stimulated insulin secretion and impairs glucose tolerance in healthy subjects. Their findings raised the possibility that endogenous ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve beta-cell function [5,8].

The aim of this research was to identify the ghrelin concentration in experimental animals with T2DM and different weight levels, compared to the control group (rats without T2DM, but with normal weight levels).

MATERIALS AND METHODS

Animal Care and Induction of a Type 2 Diabetic Rat Model

Experiments were performed on male Wistar rats, which were housed in standard polypropylene cages (three rats/cage) under a 12-hour /12-hour light/dark cycle, and an ambient temperature of 22–25°C. Type 2 diabetes was induced according to the method of Zhang et al. 2008 and Liu et al. 2013[6,7].

Animals were divided into two groups; control (N=11) and experimental group (N=33). In the control group animals had normal weight.

The rats in the control group and the normal weight group of experimental animals were fed a regular chow diet consisting of a total kcal value of 20 kJ/kg (5% fat, 52% carbohydrate, 20% protein), whereas some of the rats in the experimental group were placed on a high-fat diet (HFD) with a total kcal value of 40 kJ/kg (20% fat, 45% carbohydrate, 22% protein). Both groups were maintained on their diets for 8 weeks. At the beginning of the fourth week, animals from the experimental group were divided into three subgroups, based on the rats' weight, normal weight group (Group I), overweight group (Group II) and obesity group (Group III) and at the beginning of the experiment in all rats (control and experimental group), ghrelin levels were tested using a Rat Desacyl Ghrelin (dGHRL) ELISA Kit, which is based on sandwich enzyme-linked immuno-sorbent assay technology.

During the fourth week, the rats in the experimental group were treated with Streptozotocin. Multiple low doses of STZ (30 mg/kg IP at weekly interval for 2 weeks) were injected into each rat intraperitoneally, which produced frank hyperglycemia in HFD-fed rats with a highly successful rate. HFD in combination with multiple low doses of STZ (30 mg/kg, twice injection at weekly interval) was considered to characterize the pathophysiology of type 2 diabetes. In normal weight group of experimental animals, a regular chow diet was continued before and after multiple low doses of STZ injection (30 mg/kg, twice injection at weekly interval). Blood glucose was tested using a blood glucose meter (Accu-Chek Performa; Roche Diagnostics.). At four weeks after the first injection, all rats with fasting blood glucose concentrations greater than 14 mmol/l were considered to be diabetic and were selected for further research. More that 14 mmol/l was also the rats blood glucose levels in the subgroup of experimental animals with normal weight, and these were fed a regular chow diet consisting of a total kcal value of 20 kJ/kg (5% fat, 52% carbohydrate, 20% protein).

RESULTS. During the experiment, ghrelin levels were measured at the beginning of the experiment and after Streptozotocin injections. Glucose levels were measured after Streptozotocin injection.

Ghrelin levels were higher in normal weight rats than in obese and overweight subjects (Table N1). In the experimental group, after multiple low doses of Streptozotocin injection (30 mg/kg- twice) Ghrelin levels increased proportionately in all three groups. (Table N1, Figure N1)

In the experimental group, after multiple low doses of Streptozotocin injection (30 mg/kg- twice) there were three subgroups of animals with T2DM (Table N2). Glucose levels were higher in obese rats, than in overweight and normal weight subjects.

Table N1. Ghrelin Levels (pg/ml) in experimental group

Ghrelin levels (pg/ml) before streptozotocin injection		
III group (obese)	II group (overweight)	I group (normal weight)
20.25±1.59	25.51±0.89	31.05±0.99
P<0,001	P<0,001	P<0,001
Ghrelin levels (pg/ml) in multiple low dose Streptozotocin induced (30 mg/kg- twice) Type 2 Diabetes rats		
III group (obese)	II group (overweight)	I group (normal weight)
28.05±1.03	35.33±1.31	45.5±1.46
P<0,001	P<0,001	P<0,001

Fugure N1. Ghrelin Level Changes (pg/ml) in rats before and after multiple low dose streptozotocin injection (30 mg/kg- twice)

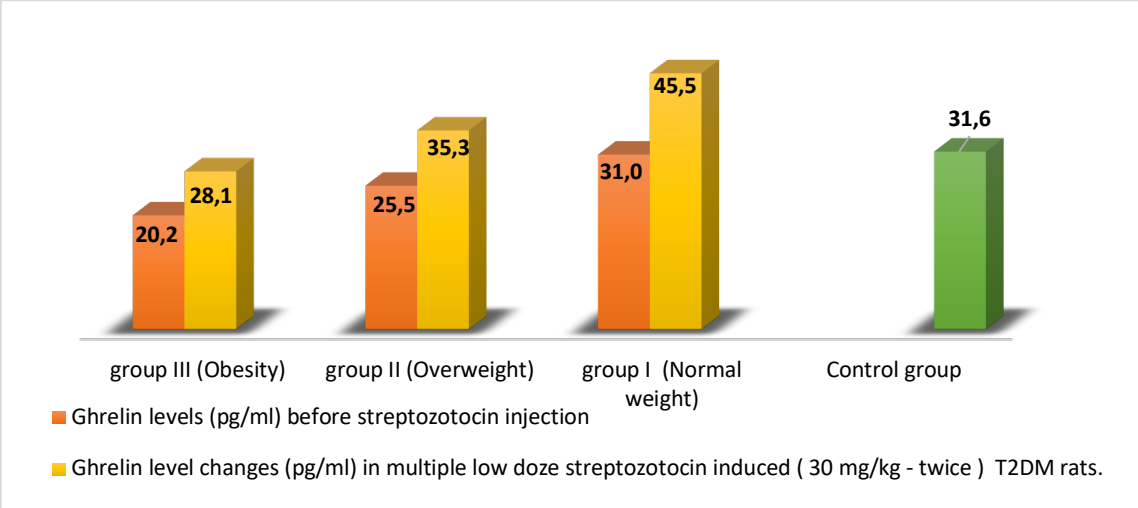
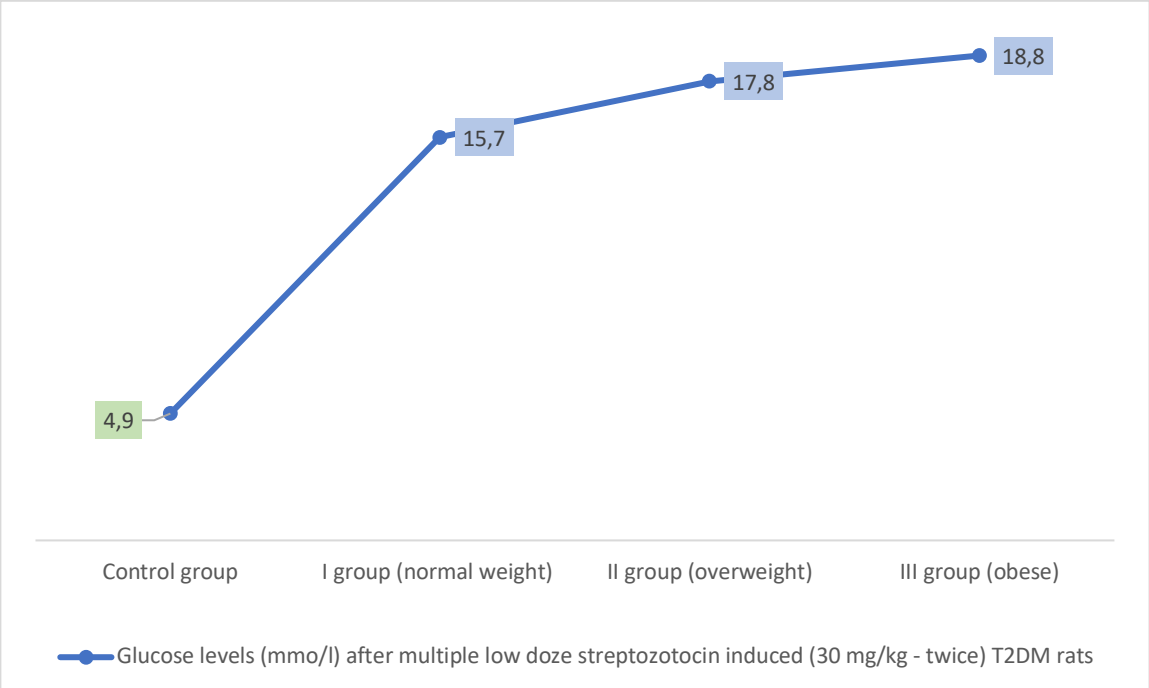


Table N2. Glucose level Changes in multiple low dose Streptozotocin induced (30 mg/kg- twice) Type 2 Diabetes rats T2DM rats

	Experimental group	Control group
group III (Obesity)	18.84±0.36**	4.93±0.36
group II (overweight)	17.84±0.3**	4.93±0.36
group I (normal weight)	15.65±0.43**	4.93±0.36

** P<0,001

Figure N3. glucose level Changes after multiple low dose Streptozotocin induced (30 mg/kg- twice) Type 2 Diabetes rats T2DM rats



DISCUSSION. Nowadays, it is known that the pathogenesis of type 2 diabetes mellitus involves not only a decrease of insulin secretion by the pancreatic beta cells, but also a number of metabolic disorders, which occur at the same time. In patients with T2DM, it is very important to eliminate obesity-induced lipotoxicity, which is primarily achieved by weight loss, and later, this significantly improves the outcome of diabetes, and helps to avoid various complications associated with T2DM. Because of its orexigenic, adipogenic and diabetigenic activities, ghrelin has emerged as an attractive target for the treatment of obesity and type 2 diabetes mellitus.

The aim of our research was to identify the ghrelin concentration in experimental animals with Type 2 Diabetes Mellitus (T2DM). At the beginning of our experiment, in subjects without T2DM, ghrelin levels were higher in normal weight rats than in obese and overweight subjects, but after multiple low doses of streptozotocin injection (30 mg/kg- twice), ghrelin levels increased proportionately in all three groups. As is known from recent research, ghrelin inhibits insulin secretion, and in the situation where there is an already high insulin resistance, in type 2 diabetes, ghrelin levels increase with diabetes compared to a control group, and it potentially decreases the effectiveness of endogenous insulin. It has also been observed that an increase of ghrelin is accompanied by increased glucose levels. This confirms ghrelin's suppressive effect on insulin secretion.

CONCLUSION. During Type 2 diabetes and obesity, ghrelin levels increase, which in turn suppress the endogenous insulin effect on already impaired glucose metabolism and this promotes hyperglycemia. This result is important, for future research to find specific neurochemical mechanisms of ghrelin and glucose metabolism to consider new treatment option targeting on ghrelin level normalization. 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.

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НАТИЯ ЧХЕИДЗЕ, ЕЛЕНЕ ГИОРГАДЗЕ, МАРИНА НИКОЛАИШВИЛИ, АННА МАЛАЗОНИЯ, НИКОЛОЗ УРИДИЯ, МАРТА КЛДИАШВИЛИ

ИЗМЕНЕНИЯ УРОВНЯ ГРЕЛИНА У КРЫС С САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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РЕЗЮМЕ

Эксперименты были проведены на самцах крыс Вистар. Животные были разделены на две группы: контрольная и экспериментальная группа. В контрольной группе животные имели нормальный вес. Животные из экспериментальной группы были разделены на три подгруппы, основываясь на весе крыс: нормальный вес (группа I), группа с избыточным весом (группа II) и группу ожирения (группа III). Уровни глюкозы проверяли через 4 недели после нескольких низких доз инъекции STZ. Все крысы с концентрациями глюкозы в крови натощак, более 14 ммоль/л, считались диабетическими и были отобраны для дальнейших исследований. У всех крыс (контрольная и экспериментальная группа) уровни грелина были протестированы до и после нескольких низких доз инъекции STZ.

Уровни грелина были выше у крыс с нормальным весом, чем у пациентов с ожирением и с избыточным весом. В экспериментальной группе после множественных низких доз инъекции стрептозотоцина (30 мг/кг-два раза) уровни грелина увеличились пропорционально во всех трех группах.

NATIA CHKHEIDZE, ELENE GIORGADZE, MARINA NIKOLAISHVILI, ANA MALAZONIA, NIKOLOZ URIDIA, MARTA KLDIASHVILI

GHRELIN LEVEL CHANGES IN RATS WITH TYPE 2 DIABETES MELLITUS

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SUMMARY

Purpose: The aim of our research was to identify the ghrelin concentration in experimental animals before and after low doses of Streptozotoccin injection.

Materials and methods: Experiments were performed on male Wistar rats. Animals were divided into two groups; control and experimental group. In the control group animals had normal weight. Animals from the experimental group were divided into three subgroups, based on the rats weight: normal weight group (Group I), overweight group (Group II) and obesity group (Group III). Glucose levels were tested 4 weeks after multiple low doses of STZ injection. All rats with fasting blood glucose concentrations

greater than 14 mmol/l were considered to be diabetic and were selected for further research. In all rats (control and experimental group), ghrelin levels were tested before and after multiple low doses of STZ injection.

Results: Ghrelin levels were higher in normal weight rats than in obese and overweight subjects. In the experimental group, after multiple low doses of Streptozotocin injection (30 mg/kg- twice) Ghrelin levels increased proportionately in all three groups.

Conclusion: At the beginning of our experiment, in subjects without T2DM, ghrelin levels were higher in normal weight rats than in obese and overweight subjects, but after multiple low doses of streptozotocin injection (30 mg/kg- twice), ghrelin levels increased proportionately in all three groups. As is known from recent research, ghrelin inhibits insulin secretion, and in the situation where there is an already high insulin resistance, in type 2 diabetes, ghrelin levels increase with diabetes compared to a control group, and it potentially decreases the effectiveness of endogenous insulin. It has also been observed that an increase of ghrelin is accompanied by increased glucose levels. Results of the experiment confirms ghrelin's suppressive effect on insulin secretion.

Keywords: T2DM; Ghrelin; Glucose levels; STZ