



The Effect of Low Doses of Radon on Ghrelin and Glucose Levels in Rats with Multiple Low-Dose Streptozotocin-induced Type 2 Diabetes Mellitus

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Abstract

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AIM: The aim of our research was to identify the ghrelin concentration in experimental animals with type 2 diabetes mellitus (T2DM) and to study the effect of radon hormesis balneotherapy using natural thermal waters of Tskaltubo spring, practically, its effect on ghrelin and glucose metabolism.

MATERIALS AND METHODS: To study the effect of radon in balneotherapy, group of experimental animals (multiple low doses streptozotocin-induced T2DM Wistar rats were used) went through the procedure of inhalation of radon by the Tskaltubo mineral water pool, once daily, during 10 days. In animals of the control groups, inhalation with radon was not used. The experimental group and the control group were brought from Tskaltubo to Tbilisi to the Ivane Beritashvili experimental biomedicine center, where the blood of the rats was analyzed.

RESULTS: After radon inhalation therapy with Tskaltubo mineral water, a normalization of the ghrelin levels was observed in the experimental group and despite the different body weight, the levels were approximately the same and close to those of the control group. In the experimental group, ghrelin level normalization was accompanied by glycemia normalization.

CONCLUSION: This research showed that Tskaltubo mineral water radon inhalation caused hormesis, which consequently decreased ghrelin levels in rodents with T2DM and obesity and the result was stable during 3 months. Ghrelin level stabilization positively influenced on glucose levels. The result of our experiment gives us a stimulus to continue future research to find more specific neurochemical mechanisms participating in radon hormesis processes and positively influencing on glucose levels and T2DM outcome.

Introduction

The increasing prevalence of type 2 diabetes mellitus (T2DM) and the consequent cardiovascular diseases has stimulated an active search for novel risk factors. The hormones regulating the energy balance are of special interest as potential risk factors for metabolic syndrome and type 2 diabetes. Ghrelin is a peptide hormone from the stomach with growth hormone releasing activity. It is also able to modify glucose and insulin metabolism, blood pressure levels, adipogenesis, and inflammatory processes in the experimental conditions.

Ghrelin is a 28-amino-acid peptide that is the natural ligand for the growth hormone secretagogue receptor (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 glucose tolerance test (GTT). 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 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].

The aim of this research was to identify the ghrelin concentration in the experimental animals with (T2DM) and different weight levels, compared to the control group (rats without T2DM, but with normal weight levels). As it is known that experimental animals with T2DM have increased levels of ghrelin, the main goal of this research was to find a treatment choice which could have an influence on increased ghrelin levels.

In recent years, there has been world-wide interest in studying natural substrates (traditional medicine) and their modern scientific-practical context. Such methods are characterized by fewer side effects, and therefore, chronic consumption is less harmful to humans. The method of treatment of natural water in the Tskaltubo region of Georgia is in this category.

Water from Tskaltubo spring is rich in chloride, hydrocarbonate, sulfate, magnesium, calcium, and sodium [4] and is characterized by stability and does not change according to time. Its radioactivity varies about 37 Bq/m³ [6], which is significantly lower than the permissible limit. It has passed baseline trials of experimental models with diabetes mellitus and the effectiveness of the study corresponds to the standards [7]. Based on these studies, the duration of each treatment procedure and treatment of water inhalation in Tskaltubo has been established. In experimental studies, the effectiveness of the methodology has been demonstrated in the case of diabetes, with the absence of side effects in improving blood biochemical data [8]. The results obtained from these experimental studies allow radon inhalation for treatment [9]. It is also very important that 3 h after inhalation, there is no trace of radon in the body. Due to the radon content, Tskaltubo water is used effectively for the treatment of pain, acute, and chronic inflammatory processes of the peripheral nervous system, subsequent bacterial or viral encephalitis of the brain, disorders of mental functions, disorders of depression, and behavioral disorders, and it accelerates the process of regeneration of nervous tissue and nervous fibers.

The aim of this experiment was to identify the influence of radon inhalation on ghrelin and glucose levels in experimental animals with T2DM. Radon inhalation was performed using Tskaltubo spring mineral water.

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-h/12-h light/dark cycle, and an ambient temperature of 22–25°C. Type 2 diabetes was induced according to the method of Zhang *et al.* and Liu *et al.* [10], [11].

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, and 20% protein), whereas some of the rats in the experimental group were placed on a high-fat diet Houston fire department (HFD) with a total kcal value of 40 kJ/kg (20% fat, 45% carbohydrate, and 22% protein). Both groups were maintained on their diets for 8 weeks. At the beginning of the 4th 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) enzyme-linked immunosorbent assay (ELISA) kit, which is based on sandwich enzyme-linked immunosorbent assay technology.

During the 4th 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 experiment, an animals a regular chow diet were 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 4 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 than 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, and 20% protein).

All rats were brought from the Tbilisi Ivane Beritashvili experimental biomedicine center to the medical and rehabilitation center in Tskaltubo (Balneoresort Tskaltubo).

The group of experimental animals was exposed to the treatment by radon-containing water of Tskaltubo spring inhalation. Water temperature was 36°C humidity was 90% and Radon concentration was 37 Bq/m³ [7], [12].

The control group of animals was placed in the same conditions, but without the radon concentration in the water. None of the animals from the experimental group (n = 33) or the control group (n = 11) had physical contact with the mineral water, they were only subject to water vapor inhalation. Inhalation was obtained by nose, for 7–10 min, once a day, with high humidity conditions (about 90%). Due to the disease severity in the experimental group, the number of days was increased to 10 days. After the inhalation procedure, the rats were placed in a vivarium and were given their diet and water. The experimental group and the control group were brought from Tskaltubo to Tbilisi to the Ivane Beritashvili experimental biomedicine center, where the blood of the rats was analyzed, not only 10 days after inhalation, but also after 3 and 6 months. Glucose levels were tested by the electrochemical method, using a portable glycometer (Optium Xceed (Abbott, CSHA)). Ghrelin levels were tested using a Rat Desacyl Ghrelin (dGhRL) ELISA Kit, which is based on sandwich enzyme-linked immunosorbent assay technology.

Results

During the experiment, ghrelin levels were measured several times: At the beginning of the experiment, after streptozotocin injections and 10 days after Radon therapy. Glucose levels were measured after streptozotocin injection, 5 days after Radon therapy, 10 days after Radon therapy, and 3 months after Radon therapy

Ghrelin levels were higher in normal weight rats than in obese and overweight subjects (Table 1). In the experimental group, after multiple low doses of streptozotocin injection (30 mg/kg – twice) ghrelin levels increased proportionately in all three groups (Table 1, Figure 1).

Surprisingly, after radon inhalation therapy with Tskaltubo mineral water, a normalization of the ghrelin levels was observed in all groups, and despite the

Table 1: Ghrelin levels (pg/ml) in the experimental group

III Group (obese)	II Group (overweight)	I Group (normal weight)
Ghrelin levels (pg/ml) before streptozotocin injection		
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		
28.05 ± 1.03	35.33 ± 1.31	45.5 ± 1.46
p < 0.001	p < 0.001	p < 0.001
Ghrelin level changes (pg/ml) in multiple low-dose streptozotocin-induced (30 mg/kg – twice) type 2 diabetes rats after radon inhalation		
31.24 ± 1.14	30.69 ± 0.87	30.88 ± 0.75

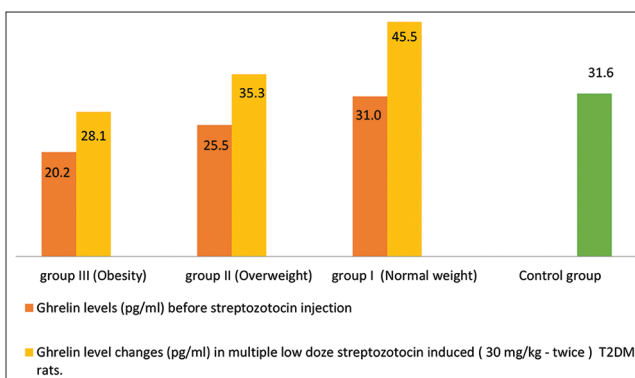


Figure 1: Ghrelin level changes (pg/ml) in rats before and after multiple low-dose streptozotocin injection (30 mg/kg – twice)

different body weight, the levels were approximately the same and close to those of the control group (Figure 2).

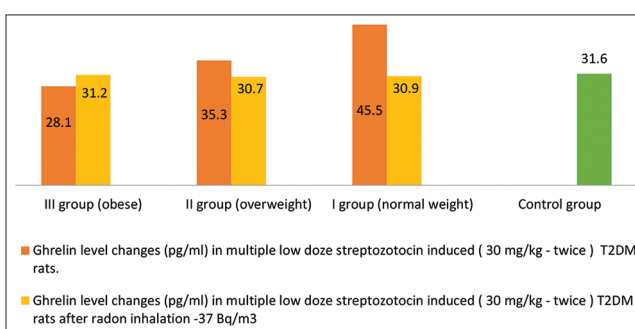


Figure 2: Ghrelin level changes before and after radon inhalation in multiple low-dose streptozotocin-induced (30 mg/kg – twice) type 2 diabetes rats

In the experimental group, after multiple low doses of streptozotocin injection (30 mg/kg – twice), there were three subgroups of animals with T2DM (Table 2). Glucose levels were higher in obese rats, than in overweight and normal weight subjects. Glucose levels were measured after 5 and 10 days of low-dose radon inhalation (Table 2). In all subjects in the experimental group, glucose levels decreased 5 days after radon inhalation, and 10 days after radon inhalation (Table 2).

Table 2: Glucose level changes in multiple low-dose streptozotocin-induced (30 mg/kg – twice) type 2 diabetes rats T2DM rats, before and after radon inhalation therapy

	Before radon inhalation	Radon inhalation day -5	Radon inhalation day -10	Control group
Group III (Obesity)	18.84 ± 0.36**	16.32 ± 0.65**	11.4 ± 0.73**	4.93 ± 0.36
Group II (overweight)	17.84 ± 0.3**	15.72 ± 0.9**	9.76 ± 0.83**	4.93 ± 0.36
Group I (normal weight)	15.65 ± 0.43**	14.34 ± 0.61**	9.53 ± 0.62**	4.93 ± 0.36

**p < 0.001

The blood samples of the rats were analyzed, not only 10 days after inhalation, but also after 3 months. After 3 months, the blood glucose and ghrelin tests showed the same picture for all animals

After radon inhalation therapy with Tskaltubo mineral water, a normalization of the ghrelin levels was observed in all experimental subgroups and despite the different body weight, the levels were approximately the same and close to those of the control group. In

the experimental group, ghrelin level normalization was accompanied by glycemia normalization (Figure 3).

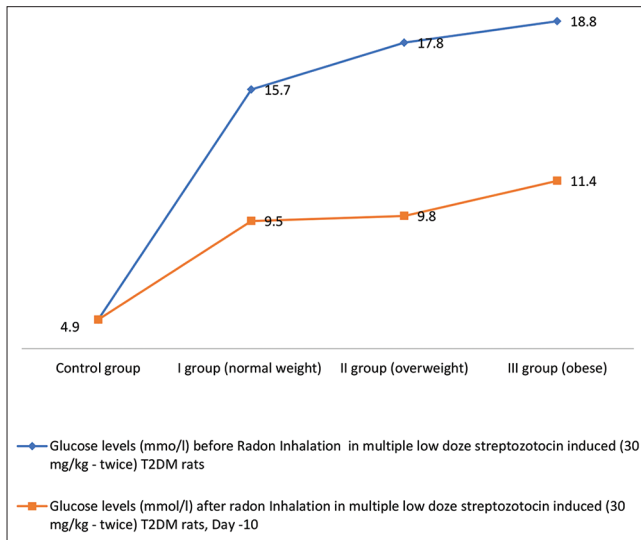


Figure 3: Glucose level changes in multiple low-dose streptozotocin-induced (30 mg/kg – twice) type 2 diabetes rats T2DM rats, before and 10 days after radon inhalation therapy

Discussion

T2DM is a multifactorial metabolic disease, resulting from both genetic and non-genetic (environmental) factors. Nowadays, it is known that the pathogenesis of the disease 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

Due to its orexigenic, adipogenic, and diabetogenic activities, ghrelin has emerged as an attractive target for the treatment of obesity and T2DM

The aim of our research was to identify the ghrelin concentration in the experimental animals with T2DM and to study the effect of radon hormesis balneotherapy, using the natural thermal waters of the Tskaltubo spring, on ghrelin and glucose metabolism. 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 weight gain and 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. After Tskaltubo mineral water inhalation, the hormesis of radon was responsible for the regulation of blood glucose and ghrelin levels in Wistar line rats with T2DM, and the result was stable over a 6-month period.

The result of our experiment gives us a stimulus to continue future research to find more specific neurochemical mechanisms participating in radon hormesis processes, and which positively influence glucose levels and T2DM outcomes. Therefore, Tskaltubo mineral water can be considered as a potential treatment for patients with T2DM and obesity.

Conclusion

According to this research, 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. After the treatment with radon therapy, the decrease of ghrelin levels, while very close to normal ranges, was accompanied by a noticeable decrease of hyperglycemia. This result is important, not only for future research to find specific neurochemical mechanisms of ghrelin and glucose metabolism but also for considering natural sources of Tskaltubo mineral water, which have fewer side effects, as a treatment option in type 2 diabetes and obesity.

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