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Original Article

Assessment of adipose tissue hormone levels after sleeve gastrectomy in rats with experimental metabolic syndrome

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Abstract

The increase in the number of patients with obesity causes significant interest of researchers and doctors in the study and analysis of physiological and pathological processes in adipose tissue. The aim of the work is to evaluate changes in the level of hormones in adipose tissue after sleeve gastrectomy. Changes in hormone levels were studied in 3 groups of 10 animals each: a control group with a normal weight, a control group with metabolic syndrome, and the main group with metabolic syndrome, which underwent sleeve gastrectomy. Three weeks after sleeve gastrectomy, weight loss was 13.7%, and after 6 weeks – 24.8%. The level of leptin in the control group was 22.08±2.61 ng/g, in the control group with metabolic syndrome – 34.01±3.01 ng/g, and in the main group 6 weeks after surgery, it was 26.92±2, 14 ng/h. When studying the level of adiponectin in the blood of rats, we found a statistically significant difference between the level of the control group with metabolic syndrome, which was 5.16±0.75 μg/ml, and the level in the control group – 7.01±0.87 μg/ml, in the main group 6 weeks after the operation – 6.32±0.57 μg/ml. The level of resistin in the control group was 12.14±1.73 μg/l, in the second group – 17.86±1.91 μg/l, in the main group – 15.06±1.68 μg/l. Sleeve gastrectomy creates hormonal changes that have a positive effect on the main mechanism of reducing the volume of adipose tissue, leading to the normalization of adipokine levels.

Keywords: insulin resistance, bariatric surgery, leptin, adiponectin, resistin.

Introduction

Metabolic syndrome (MS) is a complex of interrelated disorders of carbohydrate and lipid metabolism and, as a result, disorders of blood pressure and endothelial functions are caused by insulin resistance. Metabolic syndrome (MS) is confirmed in the presence of at least three of the following criteria: abdominal obesity, hypertension, hyperglycemia, increased triglyceride levels and low high-density lipoprotein cholesterol [1]. The number of obese people gradually increases by 10% every 10 years. According to the WHO, about 30% of the world's inhabitants are overweight, 16.8% are women and 14.9% are men [2].

There is a point of view that insulin resistance (IR) triggers the entire cascade of metabolic disturbances in MS. Abdominal adipose tissue plays an important role in the development and progression of IR and related metabolic disorders [3]. It has been known for several decades that adipose tissue is not only a source of energy

and thermal insulation but also the largest endocrine organ in the human body. Therefore, quantitative changes in adipose tissue, as well as the type of its distribution in the body, cause hormonal disorders, and *vice versa*; changes in hormonal status can affect fluctuations in body weight.

To date, about 100 adipokines are known. They are heterogeneous in their structure and the functions they perform. By having paracrine, autocrine and endocrine mechanisms of action, these hormone-like substances affect lipid metabolism, glucose homeostasis, blood coagulation processes, angiogenesis, bone tissue formation, inflammatory processes, tumor growth [4, 5].

The increase in the number of patients with obesity, type 2 diabetes, cardiovascular pathology, and cancer causes significant interest of researchers and doctors in the study and analysis of physiological and pathological processes in adipose tissue [6]. Considering the epidemic nature of the spread of MS, the development of new strategies with the use of effective and safe therapeutic agents capable of preventing the progression of metabolic disorders inherent in this symptom complex is an urgent issue of modern endocrinology.

The road to health for MS patients is long and thorny and begins with conservative measures. Changing lifestyle, eating habits, and increasing physical activity are the basis of conservative treatment. In some cases, drug therapy is added. However, the majority of patients, due to certain circumstances, cannot or do not want to radically change their lifestyle and have low adherence to treatment recommendations. As a result, no more than 10% of people with MS can achieve significant and, most importantly, sustainable results in the fight for weight loss and control of obesity-related diseases. An alternative to conservative treatment is bariatric surgery, which allows to achieve a stable result and is the most effective method of treating abdominal obesity and MS [7].

The aim of the work is to evaluate changes in the level of adipose tissue hormones after sleeve gastrectomy in rats with simulated MS.

Material and methods

Research on laboratory animals was carried out in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes dated March 18, 1986, Directives of the Council of Europe 2010/63/EU, Law of Ukraine "On the Protection of Animals from Cruelty". Animals in the amount of 30 sexually mature Wistar rats were kept in the conditions of an accredited vivarium on the basis of the I. Horbachevsky Ternopil National Medical University during the entire period of the experiment.

The animals were divided into 3 groups of 10 individuals. The control group included rats with a normal weight of 223.26±12.06 g; the second group – a control group with experimental MS, which included animals weighing 451.53±16.92 g; the third main group, which included animals with MS that underwent sleeve gastrectomy (SG) under ketamine anesthesia. The weight of males before surgery was 453.46±14.10 g. MS was modeled for 20 weeks by reaching the total amount of calories from 30% to 60% due to fats of animal origin (pork lard), bread – 6.0 g, pearl barley – 4 g, barley – 15 g, carrots 10 g were also added to the daily diet per individual. Lard was chopped, and sunflower seeds, peanuts and cake were added to improve the taste and attract animals [8]. MS was confirmed in the presence of three of the five signs inherent to it (abdominal obesity, hypertension, fasting hyperglycemia).

Determination of the resistin level was carried out using a quantitative sandwich enzyme immunoassay (ELISA kit for rat resistin, AD2 Ca., USA). The level of leptin and adiponectin in the blood was determined by the immunoenzymatic method using Roche Diagnostics GmbH Mannheim Mouse/Rat kits (Germany). Blood sampling to determine hormone levels in the main group was performed 6 weeks after SG. Postprandial hormone levels at 90 and 180 minutes were also assessed to determine how their levels were affected by food intake in normal-weight rats, MS animals and individuals after correction of MS by SG.

Before 36 hours of performing SG, the animals were transferred to liquid food with infant formula to free the gastrointestinal tract. Only water was given 8 hours before the operation. SG was performed under ketamine anesthesia at the rate of 5.0 mg per 100 g of rat weight, which was administered intraperitoneally. A laparotomy was performed in the projection of the white line of the abdomen. After visualization of the stomach, it was mobilized along the greater curvature with dissection of the ligamentous apparatus. The essence was to remove a significant part of the stomach (about 70%) with the formation of a so-called tube. Food and water were not given for the first 12 hours after SG, which were compensated by two subcutaneous injections of 10.0 ml of 0.9% NaCl. Over the next 48 hours, the animals were gradually transferred to a liquid diet with infant formula, which was fed for 5 days. Then,

for 1 week, they were gradually transferred to a normal diet with a high content of animal fats.

Results

All experimental animals of the main group tolerated SG well except for two; one animal died on the second day due to failure of the sutures, and another died on the fourth day due to impaired patency in the area of the formed new stomach. After 3 weeks, we noted a decrease in weight by 13.7%, and the weight was 391.26±13.21 g, and after 6 weeks – by 24.8%, which corresponded to a weight of 341.13±10.33 g. A decrease in weight was also evidenced by a visual decrease in the size of the animals; the rats were more mobile compared to the second group. There was also a decrease in the amount of food consumed by animals in the third group compared to the second.

The average level of leptin in the first group of animals was 22.08±2.61 ng/g; in the control group with MS, it was 1.5 times higher compared to the first group; in the main group 6 weeks after SG, the indicator was 21% lower than in the second group, but 1.2 times higher than in the first group. When studying the level of adiponectin in the blood of rats, we found a statistically significant difference between the level of the control group with MS, which was 16.3% lower than the level in the control group and 8.4% lower than in the main group. The level of resistin in the second group was 1.4 times higher than in the first group; in the main group, it was 15.7% lower compared to the second group (Table 1).

We obtained interesting results after analyzing changes in hormone levels 90 and 180 minutes after eating in three groups of animals. The level of leptin in the control group increased from 22.08±2.61 ng/g to 24.45±2.76 ng/g at 90 min and to 23.86±2.12 ng/g at 180 min. In the control group with MS, it decreased from 34.01±3.01 ng/g to 32.59±2.96 ng/g at 90 min, but at 180 min, it increased and was 35.36±3.17 ng/g. At 90 min, there was also an increase in the level from 26.92±2.12 ng/g to 28.12±2.62 ng/g in the main group, but it decreased at 180 min compared to 90 min and was 27.48±2.49 ng/h (Figure 1).

Regarding the level of adiponectin, there were statistically insignificant changes in the control group, where the level increased for 90 minutes from 7.01±0.87 μg/ml to 7.96±0.82 μg/ml, and for 180 minutes, it was 7.37± 0.76 μg/ml. In the control group with MS, similarly, like leptin, it decreased from 5.16±0.75 μg/ml to 4.81±0.67 μg/ml, and at 180 min, it was 5.31± 0.69 μg/ml. In the main group, it increased for 90 min from 6.32± 0.57 μg/ml to 6.73±0.68 μg/ml, with a decrease for 180 min to 6.21±0.62 μg/ml (Figure 2). As for the level of resistin, in the control group, it increased from 12.14± 1.73 μg/l to 13.01±1.79 μg/l at 90 min and to 12.51±1.74 μg/l at 180 min. In the control group with MS, it increased from 17.86±1.91 μg/l to 19.01±1.86 μg/l at 90 min and to 18.15±1.75 μg/l at 180 min. The increase also occurred in the main group of animals, where the level at 90 min was 16.01±1.73 μg/l and at 180 min – 15.21±1.69 μg/l against the level of 15.06±1.68 μg/l, who was fasting (Figure 3).

Discussion

Sleeve gastrectomy (SG) has gained increasing popularity in recent years as an independent bariatric intervention among surgeons around the world due to its high efficiency, which allows to achieve a sustainable reduction of excess body weight from 42.7% to 81.5% within 5 years with minimal side effects. SG is a variant of restrictive bariatric intervention based on the removal of most of the stomach in the longitudinal direction, placed in the area of the greater curvature with the preservation of the cardiac sphincter and pylorus and the formation of a narrow gastric tube with a volume of 60–150 ml, placed along the lesser curvature. In some cases, it is used as the first stage of technically

Table 1: Levels of adipose tissue hormones in different groups of animals.

Group of animals	Hormone levels		
	Leptin	Adiponectin	Resistin
Control group	22.08±2.61 ng/g	7.01±0.87 μg/ml	12.14±1.73 μg/l
Control group with MS	34.01±3.01 ng/g *	5.16±0.75 μg/ml *	17.86±1.91 μg/l *
Main group (6 weeks after SG)	26.92±2.14 ng/g **	6.32±0.57 μg/ml	15.06±1.68 μg/l

Note: * – $p<0.05$ in relation to the control group; ** – $p<0.05$ in relation to the control group with MS.

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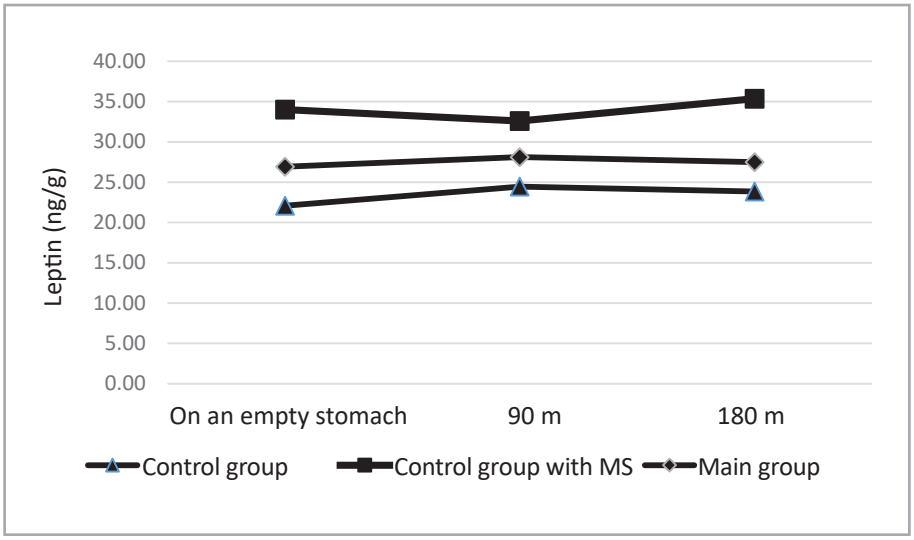

Figure 1: Changes in leptin levels after eating.

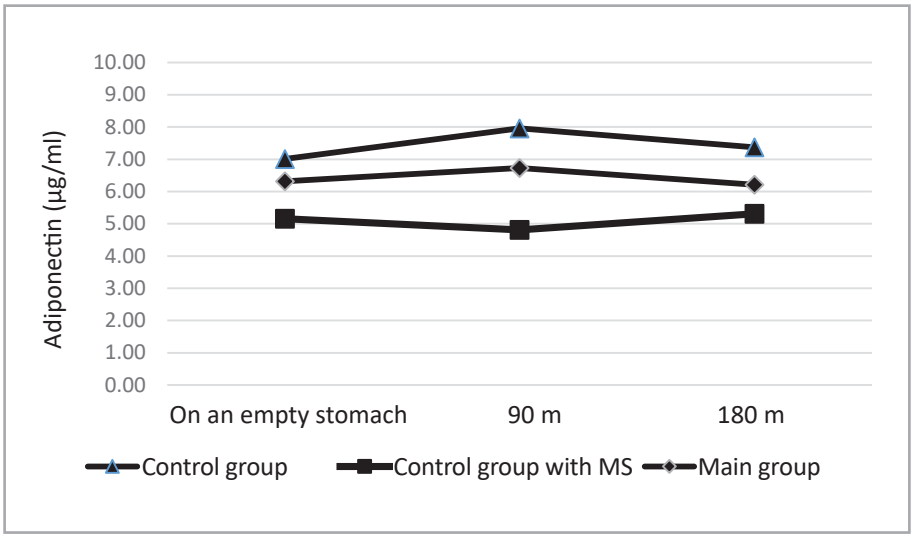

Figure 2: Changes in adiponectin levels after eating.

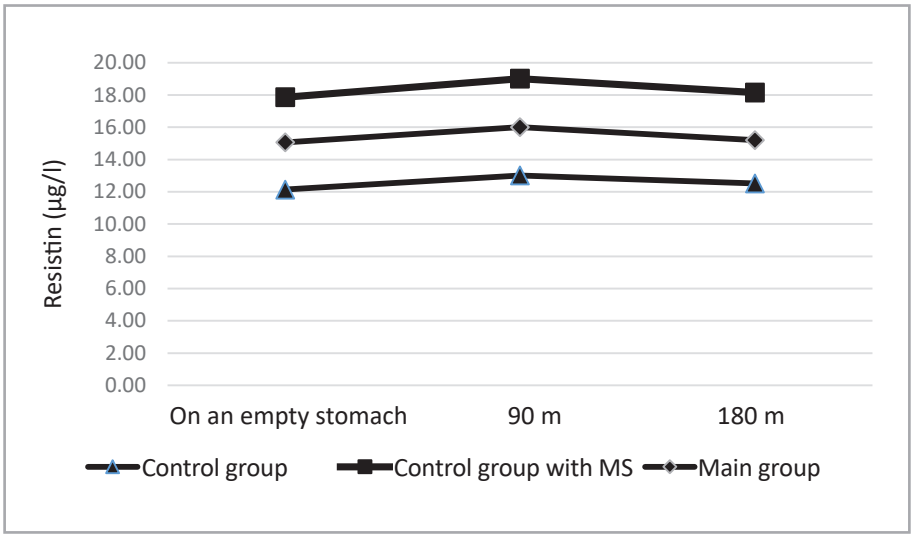

Figure 3: Changes in the level of resistin after eating.

complex biliopancreatic diversion with duodenal switch in patients with a body mass index (BMI) of more than 50 kg/m^2. According to the IFSO registry, the share of performing this type of surgical intervention in the general arsenal of bariatric operations in countries increased from 1–5% in 2008 to 45–72% in 2018 [7]. Due to the technical simplicity, the small number of complications and the absence of protein and electrolyte losses in the distant period, it favorably differs from other bariatric interventions and is a reasonable alternative to gastric bypass [7, 9].

That is why our main goal was to study the changes in the circulating level of adipose tissue hormones, the excess of which is present in the metabolic syndrome. Their changes during weight loss under conditions of SGIn our study, a high level of leptin in the blood was found, which is explained by the increase in the mass of adipose tissue. As is known from the literature, this hormone is produced exclusively by adipocytes. Its main action is aimed at inhibiting food intake, manifested at the hypothalamus level, where it acts on the centers of hunger and satiety, binds to receptors and causes the activation of signals that inhibit food intake and increase energy expenditure. During the research, we did not observe a decrease in the daily volume of food consumed by the animals, which should occur when the hormone level increases due to obesity. We can assume that there is a compensatory resistance of the hypothalamus to leptin, contributing to further weight gain. This pattern is traced in the works of Hennige M. *et a*l. [10, 11].

Moreover, it is known that leptin level correlates with BMI and insulinemia in patients with type 2 diabetes [12]. Thus, in our study, rats with MS had a 1.5 times higher fasting leptin level than in the control group. A notable decrease in fasting leptin levels was observed only 6 weeks after SG, which is associated with the decrease in adipose tissue mass that occurs with weight loss. No significant changes in leptin levels were observed after eating in animals of all groups.

The concentration of adiponectin in blood plasma was inversely correlated with MS. That is, with an increased mass of adipose tissue, which occurs in abdominal obesity, one of the signs of MS, the level of this hormone in the blood of animals was 27% lower compared to a group of animals whose weight did not indicate obesity. A low level of adiponectin contributes to the development of insulin resistance, which increases the manifestations of MS. 6 weeks after SG, the main group rats had a 1.2-fold increase in the hormone level compared to the MS control group, which leads to a reduction in the manifestation of insulin resistance by stimulating tyrosine phosphorylation and enhancing the action of insulin in skeletal muscle and liver [6].

When researching the level of resistin in the blood, we noted a 1.4-fold increase in its level in the control group with MS, compared to the control group, which contributes to the development of insulin resistance by inhibiting the uptake of glucose by target tissues, to a greater extent by the liver, that is, this hormone acts insulin antagonist [6, 13]. The increased level of resistin can be considered as a prognostic marker of abdominal obesity and insulin resistance, stimulation of the mechanisms of inflammation, activation of the endothelium and proliferation of vascular smooth muscle cells, allows us to consider it also as a marker or etiological factor in the development of cardiovascular pathology [11, 13, 14]. Again, after conducting SG in the main group, a decrease in its concentration in the blood was observed by 16%, which theoretically leads to increased sensitivity to insulin and decreased insulin resistance.

This type of restrictive surgery can be considered a method of correction of type 2 diabetes, as indicated by the changes in hormone levels that are demonstrated in our study. We understand that it is not possible to judge the treatment of diabetes by changes in adipose tissue hormone levels, so this motivates us to study the effect of SG on the body even more deeply, in particular in type 2 diabetes.

Conclusion

As our research showed, adipose tissue is an active metabolic and endocrine organ that plays a key role in the development of metabolic syndrome, capable of secreting various biologically active substances that play a significant role in the body's homeostasis and the development of diseases. The simulated metabolic syndrome was accompanied by a high level of leptin, resistin in the blood and a decrease in the level of adiponectin, which affects the severity of insulin resistance and triggers the mechanism of concomitant diseases. Sleeve gastrectomy creates hormonal changes that have a positive effect on the main mechanism of reducing the volume of adipose tissue, which leads to the normalization of adipokine levels.

Conflict of interest

The authors declare no conflict of interest.

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