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Original Research

Dynamics of antioxidant status and nitrogen oxide systems in rats with metabolic syndrome after bariatric surgeries

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Abstract

Background and aims: Metabolic syndrome still requires the development of new treatments. The aim of the study was to study the state of the nitric oxide system, lipid peroxidation and antioxidant system in the body of animals with metabolic syndrome, which has received surgical treatment of obesity by different methods. **Material and methods:** The studies were performed on 40 white male Wistar rats. Male rats of the main group (n = 8) were fed a high-fat diet (HFD) for 16 weeks, thus simulating the development of MS. After exposure to a high-fat diet for 16 weeks, rats in the HFD cohort were randomly assigned to non-surgical HFDs and HFDs who underwent appropriate surgery intervention. **Results:** Surgical treatment of obesity reduces the intensity of lipid peroxidation, nitrooxidative stress and improves antioxidant defense mechanisms in all studied groups of animals with simulated metabolic syndrome. However, only in the group of rats that underwent left gastric artery ligation surgery, the improvement of the pro- and anti-oxidant (the level of superoxide dismutase increased in 1.52 times; $p < 0.05$) system and the nitric oxide system (the level of NO-synthase decreased in 41.17%; $p < 0.05$) was statistically significant for all studied indicators.

Keywords: treatment of metabolic syndrome; nitro-oxidative stress; lipid peroxidation; bariatric surgery.

Background and aims

The prevalence of metabolic syndrome (MS) and its role as a predictor of cardiovascular diseases, determine the intensity of scientific research in key areas of pathogenesis, diagnosis, prevention and treatment of these symptoms complex. According to modern ideas, the development of insulin resistance of peripheral tissues is the basis for the formation of MS, but there is evidence that this pathology is a multi-organ failure [1]. In this consideration, there is a discussion about the mechanisms of formation of manifestations of MS [2]. It should be noted, that most of the studies consider aspects of the priority of one particular factor of pathogenesis over another. Information on the

relative contribution and interaction of different mechanisms of formation of both the MS itself and its individual components in the modern literature is limited. Many authors consider MS with concomitant dysfunction of visceral adipose tissue, which consists in impaired secretion of adipokines (leptin, adiponectin, etc.), hyperproduction of free fatty acids, anti-inflammatory cytokines, as the initiation of the pathogenesis of MS. According to another result, the pathogenesis of MS may also be autonomic dysfunction. In particular, hypertension in patients with MS is considered as hyperactivity of the sympathetic nervous system and renin-angiotensin-aldosterone system [3].

One of the aspects associated with the development of MS and attendant pathology is the excess of reactive oxygen species that initiate lipid peroxidation (LPO) processes and cause damage to various cellular components. There are many works devoted to the study of free radical oxidation in various pathological conditions [4]. However, results on the development of oxidative stress in MS are clearly insufficient, and this applies to studies of the activity of antioxidant enzymes. Another important aspect of research in the pathogenesis of MS is the study of the role of mediators of intercellular interaction, which include nitric oxide and its metabolites.

Also, it is important to find effective, low-cost and prognostically successful methods of treatment of MS [5]. The search for adequate methods of correction and treatment of complications in MS will lead to a reduction in morbidity and mortality from stroke and myocardial infarction, the number of patients with heart failure, the frequency of sudden death.

Bariatric surgery is a clinically significant alternative to drug therapy in the treatment of MS, especially in combination with diabetes mellitus or cardio-vascular disfunction. After bariatric surgery, there is a decrease in excess weight by an average of 61.2%, and remission of diabetes mellitus is achieved in 76.8% of cases. Statistics show an increase in the number of bariatric surgeries performed in recent years [6]. The main criterion in assessing the effectiveness of bariatric surgery is the rate of reduction of excess body weight. However, bariatric surgery has a wide range of systemic effects that are insufficiently studied and therefore cannot be fully utilized. It is known that the metabolic effect of bariatric surgery is provided not only by the entry of nutrients into the body but also by influencing the level of secretion of hormones of the gastrointestinal tract. One of the most significant mechanisms of some bariatric surgeries is the incretin effect, which is to stimulates insulin production in response to oral glucose. However, in addition to metabolic action, gastrointestinal hormones have systemic pleiotropic effects. The systemic effects of glucagon-like peptide-1 and ghrelin hormones (ghrelin, obestatin) are the most studied [7]. According to recent researches which have been obtained on the pronounced protective effect of glucagon-like peptide-1 and ghrelin on beta cells of the islets of the pancreas, myocardium, brain, hepatocytes, and endothelium. In contrast to the systemic administration of hormone analogs after bariatric surgery, there is a steady change in the profile of secretion of gastrointestinal hormones, an endogenous depot of antidiabetic and a deficiency of diabetogenic hormones. At the same time, under the conditions of different types of bariatric interventions, the level of gastrointestinal hormones changes in different directions [8].

The currently available research on changes in the activity of lipoperoxidation processes, the state of antioxidant protection, the role of nitric oxide after various types of bariatric surgery in MS is fragmentary and contradictory, which makes it important to study these processes in detail after various types of bariatric interventions. The diversity of changes in MS in the body requires the development of new and improved known methods of treatment.

The aim of the study was to study the state of the nitric oxide system, LPO and antioxidant system in the body of experimental animals with simulated MS, which have received surgical treatment of obesity by different methods.

Material and Methods

Animals data and ethics statement

The studies were performed on 40 white male Wistar rats weighing 200–250 g (age 9–10

weeks), which were kept in standard vivarium conditions (air temperature: (22±2)°C, humidity – 30–60%, light / dark cycle: 12/12 hours). Male control rats (n=8) were fed a normal control diet. Male rats of the main group (n=8) were fed a high-fat diet (HFD) (over 60% energy from fats) for 16 weeks [9], thus simulating the development of MS. After exposure to a high-fat diet for 16 weeks, rats in the HFD cohort were randomly assigned to non-surgical HFDs and HFDs who underwent appropriate surgical intervention.

The experiment complied with the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and the European Union Directive 2010/10/63 EU on animal experiments. The Commission on Bioethics of I. Horbachevsky Ternopil National Medical University (Protocol No. 12 of November 4, 2020) did not find any violations of moral and ethical norms during this study.

Surgical procedures

Rats after HFD were divided into 3 groups according to the type of bariatric surgery: HFD sleeve-gastrectomy group (HFD SG) (n=8), HFD Roux-en-Y gastric bypass group (HFD Roux-en-Y GB) (n=8) and HFD left gastric artery ligation group (HFD LGAL) (n=8).

All obese rats fasted the night before surgery. Operations SG, Roux-en-Y GB, LGAL were performed in rats anesthetized with 4% intraperitoneal injection of sevoflurane. For the SG procedure, approximately 75–80% of the stomach was removed along the greater curvature from the antrum to the fundus using interrupted silk sutures 5-0.

During Roux-en-Y GB, a gastric pouch of approximately 20% of the total stomach volume was divided. Care was taken not to damage the neural and vascular supply (left gastric artery) to the esophagogastric junction and the pouch. Second, the jejunum was transected about 40 cm from the ileocecal valve (~40 mm from the ligament of Treitz), and the distal cut end was anastomosed to the gastric pouch. Third, the proximal cut end of the jejunum was anastomosed to the side of the lower jejunum (~25 mm from the ileocecal valve). This procedure resulted in an approximately 15-mm-long Roux limb, a 25-cm-long common limb, and a roughly 40-mm-long biliopancreatic limb. All anastomoses were performed using interrupted sutures 5-0 followed by abdominal closure using 3-0 silk and 5-0 prolene.

The LGAL procedure consisted of excision along the midline of 3–4 cm. The esophagus was then stripped of connective tissue 15–20 mm from the point of connection with the stomach; the left gastric artery was ligated with silk 3-0, and then cut.

After surgery, rats were given subcutaneous injections of sterile normal saline immediately and a liquid diet for 3–5 days. At the end of the experiment, the animals were decontaminated 4 weeks after surgery by decapitation under thiopental anesthesia.

Laboratory data

Determination of the content of TBA-active products (TBA-AP) [10], level of ceruloplasmin (CP) [11], activity of catalase (CT) [12], total antioxidant activity of blood serum (TAA) [14], superoxide dismutase (SOD) activity [15] were performed using the photo spectrographic method. The principle of the method for determining the content of reduced glutathione (GSH) is that the interaction of 5,5'-dithiobis2-nitrobenzoic acid (Elman's reagent) with free SN groups of reduced glutathione forms a thionitrophenyl anion, the amount of which is directly proportional to the group content of S [13]. The concentration of reduced glutathione in serum was expressed in mmol/l. The total content of nitrates and nitrites was determined by the Griss method after the reduction of nitrates to nitrites with cadmium [16]. Calculations were performed according to the calibration schedule, using sodium nitrite as a standard. The content of nitrates and nitrites was expressed in mmol/l of blood serum. The total activity of NO-synthase (NOS) in blood serum was determined colorimetrically by the amount of nitrates and nitrites formed in the incubation medium [17].

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Statistical analysis

Statistical processing of the obtained research data was processed using the software Excel ("Microsoft", USA) and Statistica.10.1. (Statsoft, USA), by the method of variation statistics using the Mann-Whitney U-test and the Student's test. Changes at p <0.05 were considered statistically significant.

Results

In all animals with simulated MS (HFD cohort) we found a tendency to intensify LPO with a statistically higher level of TBA-AP (1.84 times) and decrease in antioxidant protection with a statistically lower level of SOD (2 times), GSH (1.9 times)), TAA (1.8 times) compared with intact animals. The level of CT and CP was compensatory increased by 2.3 and 2.1 times, respectively, compared with the control (Table 1).

After surgical treatment, lipoperoxidation decreased in all study groups. The level of TBA-AP in the HFD-LGAL group of animals decreased by 49.52% compared with the HFD group and was 1.2 times lower compared to the control (p<0.05). In animals of HFD-Roux-en-Y GB and HFD-SG groups, the level of TBA-AP also decreased compared with preoperative levels by 6.34% and 19.67%, respectively, however, this had no statistically significant significance (p>0.05).

Antioxidant status in the groups after surgical treatment has been improved in all study groups compared to preoperative parameters. Thus, the level of SOD and GSH significantly increased in the HFD-Roux-en-Y GB group and was higher by 11.11% and 4.92%, respectively, but this increase was not statistically significant (p>0.05). However, in the HFD-SG group the level of SOD and GSH was statistically significantly higher by 36.11% and 11.22%, respectively (p<0.05). In the HFD-LGAL group of animals, the level of SOD increased by 1.52 times, and the value of GSH – 1.48 times (p<0.05) compared with the HFD group, which was the best result.

TAA increased in all studied groups of surgical treatment of obesity due to the stress of antioxidant defense mechanisms, but only in HFD-SG group (increase by 21.34%) and in HFD-LGAL group (increase by 46.77%) it was statistically significant (p<0.05) compared with the HFD group. CP levels, on the other hand, were depleted and decreased in all study groups of surgical treatment (p<0.05) compared with HFD group of animals. Regarding CT, its level was also depleted in all groups of surgical treatment and was 1.61 times statistically significantly lower in

Table 1: Indicators of prooxidant-antioxidant system in blood plasma and liver of experimental rats (M±m).

Indicator	Groups of animals				
	Control (n=8)	HFD (n=8)	HFD Roux-en-Y GB (n=8)	HFD SG (n=8)	HFD LGAL (n=8)
Blood plasma					
CP (mg/l)	302.1±14.9	634.5±25.2*	573.9±24.5*#	485.7±26.4*#	392.6±16.2*#
CT (mcat/l)	0.88±0.04	2.03±0.78*	1.76±0.60*	1.49±0.53*	1.26±0.46*#
GSH (mmol/l)	3.90±0.29	2.03±0.18*	2.13±0.17*	2.40±0.16*#	3.01±0.19*#
TAA (%)	59.43±4.09	32.65±2.36*	33.96±2.70*	39.62±3.01*#	47.92±2.90*#
TBA-AP (mkmol/l)	8.50±0.49	15.76±1.09*	14.82±1.55*	13.17±0.99*	10.54±1.07*#
Liver					
SOD (units/g)	0.77±0.04	0.36±0.03*	0.40±0.02*	0.49±0.03*#	0.55±0.02*#

Notes: *The probability of differences compared to findings in the control group;
#The probability of differences compared to findings in patients of HFD group.

comparison with HFD-LGAL group with preoperative level.

Nitric oxide metabolites in the HFD group of rats also increased compared to the control: the NOx content was 2.15 times higher (Figure 1), and the NO-synthase content was 2 times higher (Figure 2). This indicates the presence of nitro oxidative stress in rats with simulated MS.

After surgical treatment, the level of nitric oxide metabolites decreased in all study groups compared with preoperative parameters. Thus, the NOx level in the HFD-Roux-en-Y GB group decreased by 8.70% ($p>0.05$), in the HFD SG group – by 23.53%, in the HFD LGAL group – by 69.78% ($p<0.05$). The dynamics of NO-synthase after surgery were slightly different from the dynamics of NOx. In the HFD-Roux-en-Y GB group, the level of NO synthase was 19.25%, and in the HFD LGAL group – 41.17% statistically significantly lower compared to the preoperative index ($p<0.05$). However, in the HFD SG group, the value of NO synthase increased, but this increase was not statistically significant compared with the HFD group of animals ($p>0.05$).

Discussion

Results of our experimental research are consistent with the results of other authors, that in MS increases the formation of free radicals, which, being highly reactive unstable chemical compounds, damage the vascular wall [18]. Due to the increase in their concentration in excessive amounts, products of lipid peroxidation that are formed, have a cytotoxic effect on the membranes of erythrocytes and lysosomes of endothelial cells.

The end products of lipid peroxidation inhibit prostacyclin, causing platelet aggregation and thrombosis, and enlarge the synthesis of thromboxanes, which promote platelet adhesion to endothelial cells [19]. In addition, there is a diffuse generalized dysfunction of the vascular endothelium due to a sharp decrease in the synthesis of nitric oxide (NO).

Nitric oxide, as a basic factor of anti-atherogenesis, blocks the proliferation of smooth muscle cells, prevents the adhesion of monocytes and platelets, has antiplatelet properties and reduces the calcium content in platelets and smooth muscle. In addition, NO is involved in modulating the release of vasoactive mediators, blocking the oxidation of low-density lipoproteins, inhibits the expression of pro-inflammatory genes in the vascular wall. These considerations determine the exceptional relevance of the correction of carbohydrate metabolism disorders in obese patients in order to avoid atherosclerotic lesions of the vascular system [20].

Our results proved a more pronounced positive effect of LGAL surgery on the processes of lipoperoxidation in the postoperative period compared with the classic types of bariatric surgery – SG and Roux-en-Y GB. Thus, as a result of the last one, the content of TBA-AP tended to decrease, but we did not observe statistical significance. We observed a pronounced effect on the reduction of this indicator in the group of animals treated with LGAL on the background of MS ($p<0.05$). A similar trend was observed for hepatic NO synthase activity and nitrate and nitrite (NOx)

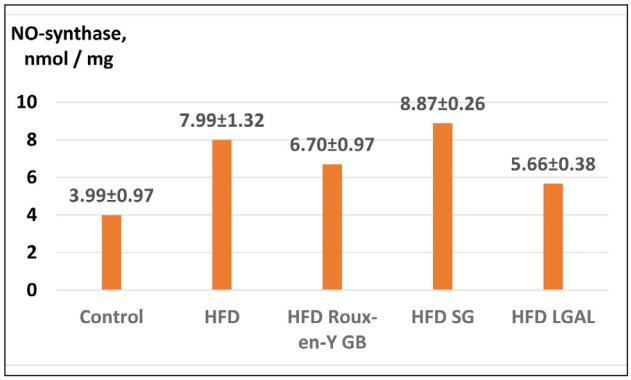

Figure 1: Indicators of NO-synthase activity in the liver of rats with simulated MS (M±m)/

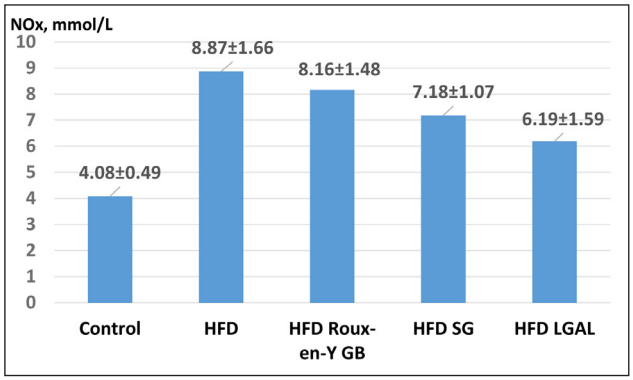

Figure 2: The content of nitrates and nitrites (NOx) in blood plasma of rats with simulated MS (M±m)

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levels in rat serum after LGAL metabolic syndrome correction compared to classic types of SG and Roux-en-Y GB bariatric surgeries.

Reducing the manifestations of enhanced free radical oxidation and nitro oxidative stress had a positive effect on the activity of enzymes of antioxidant protection. Thus, the activity of the acute phase antioxidant CP was statistically significantly reduced in animals of all operated groups, but we got the best effect after LGAL. According to other authors, the content of CP did not change or increase [21]. Such differences can be explained by the large compensatory capabilities of the antioxidant system in rats, a sign of which is the increase in the concentration of CP in simulated MS, which offsets the decrease in the specific activities of CP. Such contradictory research results in our case can be explained by multiple mechanisms regulating the expression of the CP gene, which involves estrogens, proinflammatory cytokines and thrombin, excess copper, iron deficiency and hypoxia, and finally insulin.

The concentration of GSH in animals with simulated MS before surgery was reduced, which may indicate the increased degradation and inhibition of synthesis. Reducing the intensity of GSH formation increases the possibility of further deepening of oxidative stress and the development of inflammatory reactions [22]. After surgery, the content of GSH tended to increase, and in the group of animals treated with LGAL almost reached the level of the control group of animals. The activity of another enzyme of antioxidant protection CT studied by us in animals with simulated MS increased, which can be explained by a compensatory increase in its activity in response to the enhanced activity of lipoperoxidation processes [23]. After surgery, the activity of the studied enzyme tended to decrease. Depletion of antioxidant protection (GSH, TAA and COD) in animals with simulated MS can be explained on the one hand by activation of lipoperoxidation processes caused by nitro oxidative stress, and on the other – by depletion of the pool of antioxidant enzymes in our experiment. This statement does not apply to the increase in the activity of CP and CT, which are considered acute and usually increase with inflammation, which is non-negative in MS.

According to our research, surgery reduces the intensity of lipid peroxidation, nitro oxidative stress, which prevents the entry into the bloodstream of under oxidized breakdown products. However, the condition and activity of the antioxidant blood system do not always objectively reflect changes in organs and systems. Pathophysiological changes in animals with simulated MS are caused by activation of LPO and depletion of antioxidant protection, and their severity increases with increasing time since the simulation of MS, and surgery improves the prognosis and postoperative mortality [24].

Conclusion

Surgical treatment of obesity reduces the intensity of LPO, nitro oxidative stress and improves antioxidant defense mechanisms in all studied groups of animals with simulated MS. However, only in the group of rats that underwent LGAL surgery, the improvement of the pro- and antioxidant system and the nitric oxide system was statistically significant for all studied indicators ($p<0.05$).

Conflict of Interest

The authors declare no conflict of interest.

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